# THE SILENT ASSASSIN: EMPIRICAL FORMULAS, MOLAR MASSES, BIOSYNTHESIS REACTIONS, ENTHALPIES, ENTROPIES AND GIBBS ENERGIES OF BIOSYNTHESIS AND GIBBS ENERGIES OF BINDING OF COXSACKIEVIRUSES A AND B

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*Coxsackievirus B represents a nightmare for a large number of medical staff. Due to exposure to Coxsackievirus in closed spaces (ambulances and waiting rooms), infections by Coxsackievirus B are a common occurrence. This paper for the first time reports chemical and thermodynamic properties of Coxsackieviruses A and B, and offers a mechanistic model of Coxsackievirus-host interaction. The driving force of the interaction at the membrane (antigen-receptor binding) is Gibbs energy of binding. The driving force of virus-host interaction in the cytoplasm is Gibbs energy of biosynthesis. This paper analyzes the mechanism of hijacking of cell metabolic machinery of susceptible cells.*
Key words*: Biothermodynamics; Bioenergetics; Virus-host interaction; Infectivity; Pathogenicity; Virus multiplication; Permissiveness; Susceptibility; Microorganism; Infection*

## 1. Introduction

Since the times of formation of medicine as a science, the times of Hippocrates, medicine has made efforts to study the interactions of microorganisms with their human host [1]. However, microorganism-host interactions need not always result in disease [2]. During the past millennia, medicine and biology have given their contribution to research on virus-host interactions [3]. During the last century, microbiology and virology have joined the effort to identify microorganisms and develop models of pathogenesis of microorganism-host interactions. For several decades, these hard efforts were joined by chemists and biochemists, who made great efforts to characterize microorganisms and processes they perform [4-10].

It seems obvious that, except for a biological system, a virus represents a chemical system [11-14]. However, before 2019, the empirical formula was known only for the poliovirus [11,12]. After 2019, empirical formulas were reported for all major variants of SARS-CoV-2 [15-26], Ebola virus [27], Mpox [28], West Nile virus [29] and bacteriophages [30]. Chemical and thermodynamic

properties were also reported for other classes of organisms, including bacteria [4,5,31-34], fungi [5,31,32,34], algae [31,35], plants [36-38], insects [39], fish [40] and human tissues [41], as well as for biological macromolecules [4,32,42]. The well-known biological processes performed by viruses represent chemical processes (reactions) [24,43-45]. Virus-host interactions begin at the cell membrane through the antigen-receptor binding reaction, which represents a chemical reaction similar to protein-ligand interactions [21,22,24,25,27,46-55]. After attachment and entry of a virus into the host cell, there is an interaction within the host cell. The process of virus multiplication represents a chemical reaction of polymerization of nucleotides into nucleic acid and amino acids into proteins [44,56-59]. Chemical reactions are competitive. The host cell and virus compete for the cell metabolic machinery and resources (amino acids and nucleotides) [26,44]. The outcome of the competition is determined by the driving force for the appropriate reaction [26,44]. The driving force for chemical reactions is Gibbs energy [8,9,60,61]. The driving force for the described biological processes of virus-host interactions are Gibbs energy of binding and Gibbs energy of biosynthesis [21,24,29,44]. Before 2019, biothermodynamic properties of virus particles and processes performed by viruses have not been reported.

Even though during the mid-19th century, Clausius has formulated the scientific framework and developed powerful tools for analysis of systems, his work found its first applications in engineering [62-66]. A century after Clausius, the great potential offered by thermodynamics was applied for analysis of biological systems and processes in the papers of Morowitz [6,7], Battley [4,5], von Stockar [8-10,61] and Hansen [67-69]. Biothermodynamics has found many applications in research in life sciences, biomedical sciences and bioprocess development [70-73].

Coxsackievirus B is a significant cause of myocarditis and cardiomyopathy [74]. Pathogenesis of Coxsackie myocarditis is not fully studied [74]. The cause might be direct cytopathic effects of the virus or pathologic immune response or autoimmunity triggered by viral infection [74]. One hypothesis states that virus persistence is directly associated with pathology. So Coxsackievirus B slowly replicates and is capable to establish a low grade infection in the heart. This is why it is important to determine the driving force for replication of Coxsackieviruses, estimate the rate of replication and determine the role of damage of myocardial cells in development of myocarditis and the accompanying myopathy. Cell tropism defines receptor usage and thus contributes to cell entry. Cell entry results in virus-host interaction: infection. Coxsackievirus B uses Coxsackievirus and adenovirus receptor (CAR).

The driving force for Coxsackievirus antigen-myocardial cell receptor interaction is Gibbs energy of binding [75]. Equations of nonequilibrium thermodynamics show that the antigen-receptor binding rate is proportional to the driving force – Gibbs energy of binding, through the binding phenomenological equation

$$r_B = -\frac{L_B}{T}\Delta_B G \tag{1}$$

where $r_B$ is the binding rate, $L_B$ binding phenomenological coefficient, $T$ temperature and $\Delta_B G$ Gibbs energy of binding [21,22,44].

Biosynthesis phenomenological equation, which belongs to nonequilibrium thermodynamics, shows that virus multiplication rate (rate of polymerization of nucleotides and amino acids into nucleic acid and proteins, respectively) is also proportional to the driving force for the biosynthesis reaction

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs} G \tag{2}$$

where $r_{bs}$ is the biosynthesis rate, $L_{bs}$ biosynthesis phenomenological coefficient and $\Delta_{bs}G$ Gibbs energy of biosynthesis [21,29,44].

The aim of this paper is to determine empirical formulas and thermodynamic properties of virus particles of Coxsackieviruses A and B, as well as thermodynamic properties of virus-host interactions at the membrane (antigen-receptor binding) and in the cytoplasm (virus multiplication).

## 2. Methods

### 2.1. Data sources

Genetic sequences of Coxsackieviruses A6, A9, A10, A24, B3 and B4 were taken from the NCBI database [76,77]. Protein sequences of the analyzed Coxsackieviruses were taken from the NCBI database [76,77] and Protein Data Bank in Europe [76-82]. Virus morphology data was taken from [83,84]. Dissociation equilibrium constants of the analyzed viruses were taken from [75,85-87]. Empirical formulas, enthalpies of formation, molar entropies and Gibbs energies of formation of the myocard and pancreas tissues were taken from [41].

Genetic sequences of Coxsackieviruses A6, A9, A10, A24, B3 and B4 were taken from the NCBI database [76,77]. Genetic sequence of Coxsackievirus A6 is available under the accession number KR815992.1 [88]. Genetic sequence of Coxsackievirus A9 is available under the accession number D00627.1 [89]. Genetic sequence of Coxsackievirus A10 is available under the accession number MH118035.1 [90]. Genetic sequence of Coxsackievirus A24 is available under the accession number JN228097.1 [91]. Genetic sequence of Coxsackievirus B3 is available under the accession number NC_038307.1 [92]. Genetic sequence of Coxsackievirus B4 is available under the accession number DQ480420.1 [93].

Sequences of structural proteins (VP1, VP2, VP3 and VP4) of the analyzed Coxsackieviruses were taken from the NCBI database [76,77] and Protein Data Bank in Europe (PDBe database) [78-82]. The sequences of the structural proteins of Coxsackievirus A6 can be found at the PDBe database under the accession number 7qw9 [94,95]. The sequences of the structural proteins of Coxsackievirus A9 can be found at the PDBe database under the accession number 8at5 [96,97]. The sequences of the structural proteins of Coxsackievirus A10 can be found at the PDBe database under the accession number 6smg [98,99]. The sequences of the structural proteins of Coxsackievirus A24 can be found at the PDBe database under the accession number 4q4x [100,101]. The sequences of the structural proteins of Coxsackievirus B3 can be found at the PDBe database under the accession number 7vxh [75,102]. The sequences of the structural proteins of Coxsackievirus B4 can be found at the NCBI database under the accession numbers AAB22445.2 for VP1 [103], AAB22446.1 for VP2 [104], 6ZCK_C for VP3 [105] and BAE06045.1 for VP4 [106]. Virus morphology data was taken from [83,84]. The virus particles contain 60 copies of VP1, 60 copies of VP2, 60 copies of VP3 and 60 copies of VP4 [83,84].

Dissociation equilibrium constants, $K_d$, of Coxsackieviruses A9, A10 and B3 were taken from the literature. Dissociation equilibrium constants of Coxsackievirus B3 strains CG was taken from [85]. Dissociation equilibrium constants of Coxsackievirus B3 strains CVB3E (VP3-234E), CVB3D (VP3-234D), CVB3V (VP3-234V), CVB3N (VP3-234N) and CVB3Q (VP3-234Q) were taken from [75]. Dissociation equilibrium constant of Coxsackievirus A10 was taken from [87]. Dissociation equilibrium constant of Coxsackievirus A9 was taken from [86]. The result from the two-state reaction

model from [86] was taken, since it is in better agreement with the two-in-one attachment and uncoating mechanism from [87]. All the dissociation equilibrium constants were measured with surface plasmon resonance [75,85-87].

## 2.2. Empirical and Chemical formulas

Empirical and chemical formulas of the analyzed Coxsackieviruses were determined with the atom counting method, as described in [107]. Atom counting method is a computational method for determination of empirical and chemical formulas of macromolecules (e.g. nucleic acids and proteins) and macromolecular assemblies (e.g. virus particles), based on their sequences and morphology [107].
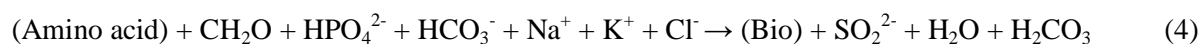
## 2.3. Thermodynamic properties of virus particles

Thermodynamic properties of the analyzed viruses were determined with the Battley approach, as described in [4,5,108]. Based on the empirical formulas, enthalpies were determined with the Patel-Erickson model [5,108,109] and entropies were determined with the Battley model [4]. Enthalpies and entropies were combined to find Gibbs energies.

## 2.4. Biosynthesis reactions and thermodynamic properties of biosynthesis

Biosynthesis reactions of the analyzed viruses and their host tissues (myocard and pancreas) were formulated based on their empirical formulas. Biosynthesis reactions are macrochemical equations that explain how nutrients are converted into new live matter [5,8-10]. The general biosynthesis reaction for virus particles has the form

$$(\text{Amino acid}) + O_2 + HPO_4^{2-} + HCO_3^- \rightarrow (\text{Bio}) + SO_4^{2-} + H_2O + H_2CO_3 \qquad (3)$$
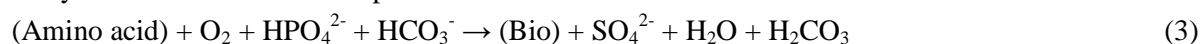
where (Amino acid) represents amino acids with the empirical formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ and (Bio) is the empirical formula of live matter [20-22,44]. The general biosynthesis reaction of the host tissues is

$$(\text{Amino acid}) + CH_2O + HPO_4^{2-} + HCO_3^- + Na^+ + K^+ + Cl^- \rightarrow (\text{Bio}) + SO_2^{2-} + H_2O + H_2CO_3 \qquad (4)$$

where the sources of sodium, potassium and chlorine are the $Na^+$, $K^+$ and $Cl^-$ ions, respectively [8,9,22,29]. Thermodynamic properties of biosynthesis were found based on the biosynthesis reactions and thermodynamic properties of virus particles, with the Hess's law [110].

## 2.5. Gibbs energies of binding

Gibbs energies of antigen-receptor binding of the analyzed viruses were determined based on their dissociation equilibrium constants. Antigen-receptor binding represents a chemical process similar to the protein-ligand interaction [44,46,55]. Antigen-receptor binding can be described by the antigen-receptor binding reaction, which is characterized with a dissociation equilibrium constant, binding equilibrium constant and Gibbs energy of binding [44,55]. Gibbs energy of binding was determined based on binding equilibrium constants, which were determined based on dissociation equilibrium constants, as described in [44,55].

## 3. Results and discussion

## 3.1. Chemical properties of Coxsackievirus particles

Coxsackieviruses belong to the *Picornaviridae* family, together with the poliovirus and rhinovirus. The poliovirus represents a macromolecular assembly with the chemical formula

$C_{332652}H_{492388}O_{131196}N_{98245}P_{7501}S_{2340}$ [11,12]. In this research, chemical formulas of Coxsackieviruses were found for the first time (Table 1): Coxsackievirus A6 $C_{327191}H_{479825}O_{131111}N_{96958}P_{7621}S_{1740}$, Coxsackievirus A9 $C_{326864}H_{480998}O_{129705}N_{97840}P_{7452}S_{2940}$, Coxsackievirus A10 $C_{324672}H_{477018}O_{129357}N_{96790}P_{7550}S_{1920}$, Coxsackievirus A24 $C_{333816}H_{490814}O_{131604}N_{97464}P_{7460}S_{2460}$, Coxsackievirus B3 $C_{321267}H_{470809}O_{127845}N_{95408}P_{7399}S_{2640}$ and Coxsackievirus B4 $C_{319785}H_{469297}O_{128295}N_{95600}P_{7395}S_{3120}$. The chemical formulas of the analyzed Coxsackieviruses are in very good agreement with that of the poliovirus $C_{332652}H_{492388}O_{131196}N_{98245}P_{7501}S_{2340}$, which was both calculated and determined experimentally [11,12]. The reason for this is that Coxsackieviruses and the poliovirus belong to *Picornaviridae* and have a similar morphology [111]. On the other hand, the chemical formula of the West Nile virus is $C_{1.54\times10^6}H_{2.71\times10^6}O_{4.01\times10^5}N_{2.26\times10^5}P_{3.03\times10^4}S_{5.76\times10^3}$ [29]. The West Nile virus particle contains many more atoms than the Picornaviruses, due to its larger size and presence of a lipid envelope.

Empirical formulas have been determined for the analyzed Coxsackieviruses (Table 2): Coxsackievirus A6 $CH_{1.4665}O_{0.4007}N_{0.2963}P_{0.023292}S_{0.005318}$, Coxsackievirus A9 $CH_{1.4716}O_{0.3968}N_{0.2993}P_{0.022798}S_{0.008995}$, Coxsackievirus A10 $CH_{1.4692}O_{0.3984}N_{0.2981}P_{0.023254}S_{0.005914}$, Coxsackievirus A24 $CH_{1.4703}O_{0.3942}N_{0.2920}P_{0.022348}S_{0.007369}$, Coxsackievirus B3 $CH_{1.4655}O_{0.3979}N_{0.2970}P_{0.023031}S_{0.008217}$ and Coxsackievirus B4 $CH_{1.4675}O_{0.4012}N_{0.2990}P_{0.023125}S_{0.009757}$. Empirical formulas are also available in the literature for other viruses: SARS-CoV-2 Hu-1 wild type $CH_{1.6390}O_{0.2851}N_{0.2301}P_{0.0065}S_{0.0038}$ [21], SARS-CoV-2 EG.5 Eris variant $CH_{1.639011}O_{0.284146}N_{0.230034}P_{0.006444}S_{0.003765}$ [17], West Nile virus $CH_{1.7651}O_{0.2609}N_{0.1469}P_{0.019712}S_{0.003745}$ [29], poliovirus $CH_{1.4802}O_{0.3944}N_{0.2953}P_{0.0225}S_{0.0070}$ [11,12] etc. It can be seen that SARS-CoV-2 and West Nile virus contain a higher number of H atoms (1.6 or 1.7), and lower number of O atoms (0.26 or 0.28) and N atoms (0.14 or 0.23) in the empirical formula. On the other hand, the Coxsackieviruses and poliovirus have a lower number of H atoms (1.4), and higher number of O atoms (0.39 or 0.40) and N atoms (0.29). The reason for this are differences in virus morphology: SARS-CoV-2 and West Nile virus are enveloped viruses, while the Coxsackieviruses and poliovirus lack an envelope [111]. The viral envelope consists of a lipid bilayer, which contains phospholipid molecules with large hydrocarbon chains. Due to the presence of the hydrocarbon chains, the hydrogen content is higher in the enveloped viruses. On the other hand, the non-enveloped viruses lack lipids and contain more protein. Proteins consist of amino acid residues, which contain the peptide (amide) bond (R-CO-NH-R'). The peptide bonds contain O and N atoms, which make the O and N content in non-enveloped viruses higher. Finally, it can be seen that every virus has a specific empirical formula different than those of other viruses. This means that the empirical formula can be used to identify the virus. This is in agreement with the conclusions of Degueldre, who suggested identification of virus particles with single particle ICP-MS [16].

**Table 1: Chemical formulas of entire virus particles, nucleic acids and structural proteins of Coxsackieviruses. The chemical formulas have the general form $C_{mC}H_{mH}O_{mO}N_{mN}P_{mP}S_{mS}$, where $m_C$, $m_H$, $m_O$, $m_N$, $m_P$ and $m_S$ are the numbers of C, H, O, N, P and S atoms in the chemical formula.**

| Virus | Particle | $m_C$ | $m_H$ | $m_O$ | $m_N$ | $m_P$ | $m_S$ | $M_r(tot)$ (Da) |
|---|---|---|---|---|---|---|---|---|
| Coxsackievirus A6 | Virus particle | 327191 | 479825 | 131111 | 96958 | 7621 | 1740 | 8161029 |
| | Nucleic acid | 72491 | 89525 | 53171 | 28738 | 7621 | | 2450175 |
| | VP1 | 1474 | 2263 | 459 | 413 | | 12 | 33498 |
| | VP2 | 1266 | 1919 | 372 | 331 | | 8 | 27984 |
| | VP3 | 1191 | 1837 | 358 | 307 | | 9 | 26472 |

| | Particle | $n_C$ | $n_H$ | $n_O$ | $n_N$ | $n_P$ | $n_S$ | $M_r$ |
|---|---|---|---|---|---|---|---|---|
| | VP4 | 314 | 486 | 110 | 86 | | 0 | 7226 |
| Coxsackievirus A9 | Virus particle | 326864 | 480998 | 129705 | 97840 | 7452 | 2940 | 8181386 |
| | Nucleic acid | 71024 | 87638 | 51825 | 28480 | 7452 | | 2400270 |
| | VP1 | 1493 | 2303 | 452 | 423 | | 13 | 33826 |
| | VP2 | 1280 | 1945 | 388 | 341 | | 17 | 28863 |
| | VP3 | 1173 | 1808 | 350 | 302 | | 18 | 26318 |
| | VP4 | 318 | 500 | 108 | 90 | | 1 | 7344 |
| Coxsackievirus A10 | Virus particle | 324672 | 477018 | 129357 | 96790 | 7550 | 1920 | 8101101 |
| | Nucleic acid | 71892 | 88758 | 52557 | 28690 | 7550 | | 2429512 |
| | VP1 | 1465 | 2264 | 441 | 410 | | 15 | 33157 |
| | VP2 | 1252 | 1889 | 373 | 329 | | 6 | 27709 |
| | VP3 | 1176 | 1819 | 355 | 303 | | 10 | 26202 |
| | VP4 | 320 | 499 | 111 | 93 | | 1 | 7457 |
| Coxsackievirus A24 | Virus particle | 333816 | 490814 | 131604 | 97464 | 7460 | 2460 | 8284752 |
| | Nucleic acid | 71076 | 87674 | 51864 | 28404 | 7460 | | 2400738 |
| | VP1 | 1538 | 2354 | 469 | 412 | | 7 | 34344 |
| | VP2 | 1333 | 2019 | 403 | 351 | | 12 | 29794 |
| | VP3 | 1192 | 1846 | 348 | 302 | | 20 | 26616 |
| | VP4 | 316 | 500 | 109 | 86 | | 2 | 7312 |
| Coxsackievirus B3 | Virus particle | 321267 | 470809 | 127845 | 95408 | 7399 | 2640 | 8028808 |
| | Nucleic acid | 70527 | 87049 | 51405 | 28328 | 7399 | | 2383217 |
| | VP1 | 1408 | 2160 | 425 | 390 | | 8 | 31607 |
| | VP2 | 1273 | 1951 | 389 | 341 | | 17 | 28801 |
| | VP3 | 1175 | 1777 | 352 | 295 | | 17 | 26212 |
| | VP4 | 323 | 508 | 108 | 92 | | 2 | 7472 |
| Coxsackievirus B4 | Virus particle | 319785 | 469297 | 128295 | 95600 | 7395 | 3120 | 8034640 |
| | Nucleic acid | 70485 | 86977 | 51435 | 28280 | 7395 | | 2382323 |
| | VP1 | 1396 | 2155 | 431 | 393 | | 14 | 31788 |
| | VP2 | 1254 | 1896 | 390 | 334 | | 19 | 28499 |
| | VP3 | 1182 | 1812 | 350 | 304 | | 17 | 26426 |
| | VP4 | 323 | 509 | 110 | 91 | | 2 | 7491 |

Molar masses of entire Coxsackievirus particles were determined (Table 1): 8.161 MDa for Coxsackievirus A6, 8.181 MDa for Coxsackievirus A9, 8.101 MDa for Coxsackievirus A10, 8.285 MDa for Coxsackievirus A24, 8.029 MDa for Coxsackievirus B3 and 8.035 MDa for Coxsackievirus B4. These values are in very good agreement with the molar mass of Picornavirus particles of 8 MDa reported in the literature [83]. Molar masses of Coxsackie A viruses (8.1-8.2) are slightly higher than those of Coxsackie B viruses (8.0). On the other hand, the molar mass of a West Nile virus particle is 31.9 MDa [29], which is consistent with the much larger size of West Nile virus particles [111].

**Table 2: Empirical formulas of virus particles, nucleic acids and structural proteins of Coxsackieviruses. The empirical formulas have the general form $C_{n_C}H_{n_H}O_{n_O}N_{n_N}P_{n_P}S_{n_S}$, where $n_C$, $n_H$, $n_O$, $n_N$, $n_P$ and $n_S$ are the numbers of C, H, O, N, P and S atoms in the empirical formula, respectively, which are given in this table.**

| Virus | Particle | $n_C$ | $n_H$ | $n_O$ | $n_N$ | $n_P$ | $n_S$ | $M_r$ (g/C-mol) |
|---|---|---|---|---|---|---|---|---|
| Coxsackievirus A6 | Virus particle | 1 | 1.4665 | 0.4007 | 0.2963 | 0.023292 | 0.005318 | 24.94 |
| | Nucleic acid | 1 | 1.2350 | 0.7335 | 0.3964 | 0.105130 | | 33.80 |
| | VP1 | 1 | 1.5353 | 0.3114 | 0.2802 | | 0.008141 | 22.73 |
| | VP2 | 1 | 1.5158 | 0.2938 | 0.2615 | | 0.006319 | 22.10 |
| | VP3 | 1 | 1.5424 | 0.3006 | 0.2578 | | 0.007557 | 22.23 |
| | VP4 | 1 | 1.5478 | 0.3503 | 0.2739 | | 0 | 23.01 |
| Coxsackievirus A9 | Virus particle | 1 | 1.4716 | 0.3968 | 0.2993 | 0.022798 | 0.008995 | 25.03 |
| | Nucleic acid | 1 | 1.2339 | 0.7297 | 0.4010 | 0.104922 | | 33.80 |
| | VP1 | 1 | 1.5425 | 0.3027 | 0.2833 | | 0.008707 | 22.66 |
| | VP2 | 1 | 1.5195 | 0.3031 | 0.2664 | | 0.013281 | 22.55 |
| | VP3 | 1 | 1.5413 | 0.2984 | 0.2575 | | 0.015345 | 22.44 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | VP4 | 1 | 1.5723 | 0.3396 | 0.2830 | | 0.003145 | 23.09 |
| Coxsackievirus A10 | Virus particle | 1 | 1.4692 | 0.3984 | 0.2981 | 0.023254 | 0.005914 | 24.95 |
| | Nucleic acid | 1 | 1.2346 | 0.7311 | 0.3991 | 0.105019 | | 33.79 |
| | VP1 | 1 | 1.5454 | 0.3010 | 0.2799 | | 0.010239 | 22.63 |
| | VP2 | 1 | 1.5088 | 0.2979 | 0.2628 | | 0.004792 | 22.13 |
| | VP3 | 1 | 1.5468 | 0.3019 | 0.2577 | | 0.008503 | 22.28 |
| | VP4 | 1 | 1.5594 | 0.3469 | 0.2906 | | 0.003125 | 23.30 |
| Coxsackievirus A24 | Virus particle | 1 | 1.4703 | 0.3942 | 0.2920 | 0.022348 | 0.007369 | 24.82 |
| | Nucleic acid | 1 | 1.2335 | 0.7297 | 0.3996 | 0.104958 | | 33.78 |
| | VP1 | 1 | 1.5306 | 0.3049 | 0.2679 | | 0.004551 | 22.33 |
| | VP2 | 1 | 1.5146 | 0.3023 | 0.2633 | | 0.009002 | 22.35 |
| | VP3 | 1 | 1.5487 | 0.2919 | 0.2534 | | 0.016779 | 22.33 |
| | VP4 | 1 | 1.5823 | 0.3449 | 0.2722 | | 0.006329 | 23.14 |
| Coxsackievirus B3 | Virus particle | 1 | 1.4655 | 0.3979 | 0.2970 | 0.023031 | 0.008217 | 24.99 |
| | Nucleic acid | 1 | 1.2343 | 0.7289 | 0.4017 | 0.104910 | | 33.79 |
| | VP1 | 1 | 1.5341 | 0.3018 | 0.2770 | | 0.005682 | 22.45 |
| | VP2 | 1 | 1.5326 | 0.3056 | 0.2679 | | 0.013354 | 22.62 |
| | VP3 | 1 | 1.5123 | 0.2996 | 0.2511 | | 0.014468 | 22.31 |
| | VP4 | 1 | 1.5728 | 0.3344 | 0.2848 | | 0.006192 | 23.13 |
| Coxsackievirus B4 | Virus particle | 1 | 1.4675 | 0.4012 | 0.2990 | 0.023125 | 0.009757 | 25.13 |
| | Nucleic acid | 1 | 1.2340 | 0.7297 | 0.4012 | 0.104916 | | 33.80 |
| | VP1 | 1 | 1.5437 | 0.3087 | 0.2815 | | 0.010029 | 22.77 |
| | VP2 | 1 | 1.5120 | 0.3110 | 0.2663 | | 0.015152 | 22.73 |
| | VP3 | 1 | 1.5330 | 0.2961 | 0.2572 | | 0.014382 | 22.36 |
| | VP4 | 1 | 1.5759 | 0.3406 | 0.2817 | | 0.006192 | 23.19 |

### 3.2. Biothermodynamic properties of Coxsackievirus particles

Thermodynamic properties of Coxsackievirus particles were determined (Table 3). As can be seen from Table 3, for all the Coxsackievirus particles, nucleic acids and proteins, enthalpies of formation are negative. This means that the total energy content of the virus particles is lower than that of their constituent elements. The reason for this is Thornton's rule, according to which energy is released when electrons are passed from less electronegative atoms (C, H, P, S) to more electronegative atoms (O, N) [108,109]. Virus live matter consists of macromolecules like nucleic acids and proteins, where atoms of different elements are bond by chemical bonds. Electrons in the bonds are attracted more to nuclei of the more electronegative elements like O and N, away from less electronegative nuclei of C, H, P and S [110]. During this process, energy is released due to greater attraction of electrons to nuclei of more electronegative elements [110]. This means that the total energy content of live matter is lower than that of its constituent elements, where there are no polar bonds.

Molar entropies of all the Coxsackievirus particles, nucleic acids and proteins are positive (Table 3). This can be explained by the third law of thermodynamics, which states that no substance can have a lower entropy than that of a perfect crystal at absolute zero, which is zero entropy [60,66,110].

Gibbs energies of formation of all the Coxsackievirus particles, nucleic acids and proteins are negative (Table 3). This is due to their negative enthalpies of formation, since Gibbs energy is determined by enthalpy and entropy: $G = H - TS$ [60,66,110]. Gibbs energy represents the maximum useful energy content that is stored in a system [60,66,110]. The negative Gibbs energies of formation imply that Coxsackeivirus particles and their constituent macromolecules have lower useful energy content than their constituent elements.

Gibbs energies of formation have been reported in the literature for other viruses as well. Gibbs energy of formation of SARS-CoV-2 EG.5 Eris virus particles is -24.64 kJ/C-mol [17], while that of the West Nile virus particles is -26.47 kJ/C-mol [29]. We see that Gibbs energies of formation of SARS-CoV-2 and West Nile virus particles are less negative than those of Coxsackievirus particles (-44 to -46 kJ/C-mol) (Table 3). The less negative (greater) Gibbs energy of formation of SARS-CoV-2 and West Nile virus particles implies that they have a greater useful energy content. The greater useful energy content can be explained by virus morphology. SARS-CoV-2 and West Nile virus particles contain an envelope made of a lipid bilayer. The lipids in the envelope are molecules with a high useful energy content [60]. The high useful energy content in the envelope lipids makes the total energy content of the enveloped SARS-CoV-2 and West Nile virus particles greater than that of the non-enveloped Coxsackievirus particles.

Table 3 shows that Gibbs energies of formation of Coxsackievirus particles are between -44 and -46 kJ/C-mol. Gibbs energies of formation of nucleic acids of Coxsackieviruses are between -121 and -122 kJ/C-mol, while those of the structural proteins are between -19 and -35 kJ/C-mol. This means that Gibbs energies of formation of Coxsackievirus particles are between those of their nucleic acids and proteins. The explanation for this is that the Coxsackieviruses consist of nucleic acids and proteins [111]. Moreover, the useful energy content is indicated by the Gibbs energy. The structural proteins have the highest usable energy content, indicated by the least negative Gibbs energy, and are followed by virus particles and then nucleic acids. The order of Gibbs energies of formation can be explained by the order of enthalpies of formation, since Gibbs energy is determined by enthalpy and entropy as described above.
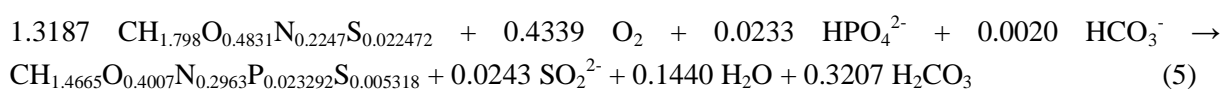
**Table 3: Thermodynamic properties of live matter of virus particles, nucleic acids and structural proteins of Coxsackieviruses: standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, $S_m^0$, and standard Gibbs energy of formation, $\Delta_f G^0$.**

| Virus | Particle | $\Delta_f H^0$ (kJ/C-mol) | $S_m^0$ (J/C-mol K) | $\Delta_f G^0$ (kJ/C-mol) |
|---|---|---|---|---|
| Coxsackievirus A6 | Virus particle | -87.75 | 32.16 | -46.07 |
| | Nucleic acid | -171.87 | 38.10 | -122.49 |
| | VP1 | -64.74 | 30.83 | -24.78 |
| | VP2 | -60.72 | 29.91 | -21.95 |
| | VP3 | -62.73 | 30.31 | -23.44 |
| | VP4 | -76.00 | 31.57 | -35.08 |
| Coxsackievirus A9 | Virus particle | -85.95 | 32.21 | -44.19 |
| | Nucleic acid | -170.95 | 38.09 | -121.57 |
| | VP1 | -62.90 | 30.81 | -22.96 |
| | VP2 | -61.01 | 30.26 | -21.78 |
| | VP3 | -60.09 | 30.29 | -20.83 |
| | VP4 | -73.55 | 31.85 | -32.27 |
| Coxsackievirus A10 | Virus particle | -87.16 | 32.18 | -45.45 |
| | Nucleic acid | -171.30 | 38.09 | -121.92 |
| | VP1 | -62.19 | 30.76 | -22.32 |
| | VP2 | -61.81 | 29.92 | -23.04 |
| | VP3 | -62.89 | 30.39 | -23.50 |
| | VP4 | -74.76 | 31.96 | -33.32 |
| Coxsackievirus A24 | Virus particle | -85.69 | 32.00 | -44.21 |
| | Nucleic acid | -170.95 | 38.07 | -121.61 |
| | VP1 | -64.13 | 30.41 | -24.72 |
| | VP2 | -61.84 | 30.11 | -22.81 |
| | VP3 | -58.50 | 30.19 | -19.37 |
| | VP4 | -74.18 | 31.89 | -32.84 |

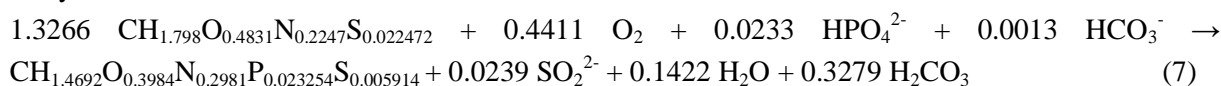| Coxsackievirus B3 | Virus particle | -86.26 | 32.12 | -44.63 |
|---|---|---|---|---|
| | Nucleic acid | -170.78 | 38.09 | -121.40 |
| | VP1 | -63.25 | 30.56 | -23.64 |
| | VP2 | -61.95 | 30.50 | -22.42 |
| | VP3 | -59.67 | 29.84 | -20.99 |
| | VP4 | -71.57 | 31.80 | -30.35 |
| Coxsackievirus B4 | Virus particle | -86.65 | 32.25 | -44.85 |
| | Nucleic acid | -170.96 | 38.10 | -121.58 |
| | VP1 | -63.91 | 30.92 | -23.83 |
| | VP2 | -62.01 | 30.33 | -22.70 |
| | VP3 | -59.58 | 30.14 | -20.52 |
| | VP4 | -73.04 | 31.90 | -31.69 |

### 3.3. Biosynthesis of Coxsackievirus particles

Based on the empirical formulas, biosynthesis reactions were formulated, which are given in Table 4. The biosynthesis reactions show how nutrients are converted into new virus particles in virus multiplication, using the host cell metabolic machinery. The biosynthesis reaction of Coxsackievirus A6 is
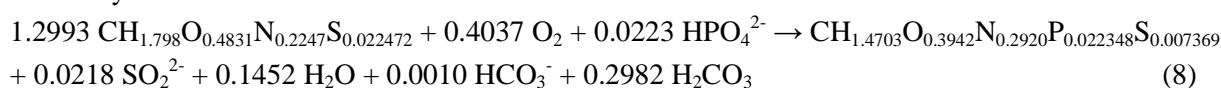
$$1.3187\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4339\ O_2 + 0.0233\ HPO_4^{2-} + 0.0020\ HCO_3^- \rightarrow$$
$$CH_{1.4665}O_{0.4007}N_{0.2963}P_{0.023292}S_{0.005318} + 0.0243\ SO_2^{2-} + 0.1440\ H_2O + 0.3207\ H_2CO_3 \tag{5}$$

where $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ is the empirical formula of amino acids and $CH_{1.4665}O_{0.4007}N_{0.2963}P_{0.023292}S_{0.005318}$ is the empirical formula of Coxsackievirus A6 particles. The biosynthesis reaction of Coxsackievirus A9 is

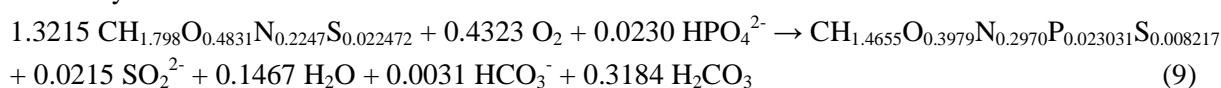$$1.3320\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4423\ O_2 + 0.0228\ HPO_4^{2-} \rightarrow CH_{1.4716}O_{0.3968}N_{0.2993}P_{0.022798}S_{0.008995}$$
$$+ 0.0209\ SO_2^{2-} + 0.1428\ H_2O + 0.0037\ HCO_3^- + 0.3283\ H_2CO_3 \tag{6}$$
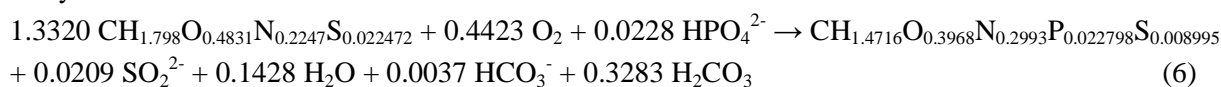
where $CH_{1.4716}O_{0.3968}N_{0.2993}P_{0.022798}S_{0.008995}$ is the empirical formula of Coxsackievirus A9 particles. The biosynthesis reaction of Coxsackievirus A10 is

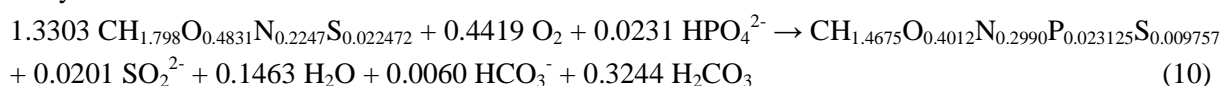$$1.3266\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4411\ O_2 + 0.0233\ HPO_4^{2-} + 0.0013\ HCO_3^- \rightarrow$$
$$CH_{1.4692}O_{0.3984}N_{0.2981}P_{0.023254}S_{0.005914} + 0.0239\ SO_2^{2-} + 0.1422\ H_2O + 0.3279\ H_2CO_3 \tag{7}$$

where $CH_{1.4692}O_{0.3984}N_{0.2981}P_{0.023254}S_{0.005914}$ is the empirical formula of Coxsackievirus A10 particles. The biosynthesis reaction of Coxsackievirus A24 is

$$1.2993\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4037\ O_2 + 0.0223\ HPO_4^{2-} \rightarrow CH_{1.4703}O_{0.3942}N_{0.2920}P_{0.022348}S_{0.007369}$$
$$+ 0.0218\ SO_2^{2-} + 0.1452\ H_2O + 0.0010\ HCO_3^- + 0.2982\ H_2CO_3 \tag{8}$$

where $CH_{1.4703}O_{0.3942}N_{0.2920}P_{0.022348}S_{0.007369}$ is the empirical formula of Coxsackievirus A24 particles. The biosynthesis reaction of Coxsackievirus B3 is
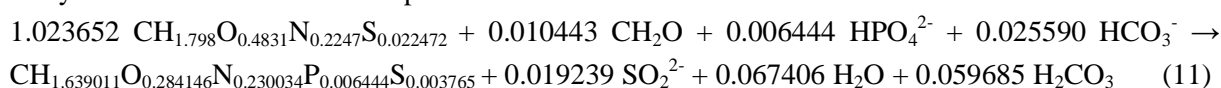
$$1.3215\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4323\ O_2 + 0.0230\ HPO_4^{2-} \rightarrow CH_{1.4655}O_{0.3979}N_{0.2970}P_{0.023031}S_{0.008217}$$
$$+ 0.0215\ SO_2^{2-} + 0.1467\ H_2O + 0.0031\ HCO_3^- + 0.3184\ H_2CO_3 \tag{9}$$

where $CH_{1.4655}O_{0.3979}N_{0.2970}P_{0.023031}S_{0.008217}$ is the empirical formula of Coxsackievirus B3 particles. The biosynthesis reaction of Coxsackievirus B4 is
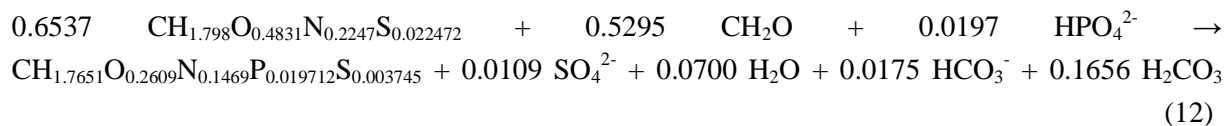
$$1.3303\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4419\ O_2 + 0.0231\ HPO_4^{2-} \rightarrow CH_{1.4675}O_{0.4012}N_{0.2990}P_{0.023125}S_{0.009757}$$
$$+ 0.0201\ SO_2^{2-} + 0.1463\ H_2O + 0.0060\ HCO_3^- + 0.3244\ H_2CO_3 \tag{10}$$

where $CH_{1.4675}O_{0.4012}N_{0.2990}P_{0.023125}S_{0.009757}$ is the empirical formula of Coxsackievirus B4 particles.

Biosynthesis reactions of other viruses can be found in the literature. For example, the biosynthesis reaction of the virus particle of the Omicron EG.5 Eris variant of SARS-CoV-2 is

$$1.023652\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.010443\ CH_2O + 0.006444\ HPO_4^{2-} + 0.025590\ HCO_3^- \rightarrow$$
$$CH_{1.639011}O_{0.284146}N_{0.230034}P_{0.006444}S_{0.003765} + 0.019239\ SO_2^{2-} + 0.067406\ H_2O + 0.059685\ H_2CO_3 \tag{11}$$

where $CH_2O$ represents carbohydrates and $CH_{1.639011}O_{0.284146}N_{0.230034}P_{0.006444}S_{0.003765}$ is the empirical formula of the virus particle of the EG.5 variant [17]. Another example is the biosynthesis reaction of the virus particle of the West Nile virus

$$0.6537 \quad CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} \quad + \quad 0.5295 \quad CH_2O \quad + \quad 0.0197 \quad HPO_4^{2-} \quad \rightarrow$$
$$CH_{1.7651}O_{0.2609}N_{0.1469}P_{0.019712}S_{0.003745} + 0.0109\ SO_4^{2-} + 0.0700\ H_2O + 0.0175\ HCO_3^- + 0.1656\ H_2CO_3$$
$$(12)$$

where $CH_{1.7651}O_{0.2609}N_{0.1469}P_{0.019712}S_{0.003745}$ is the empirical formula of the West Nile virus particles [29].

The biosynthesis reactions of virus particles of SARS-CoV-2 EG.5 Eris variant and West Nile virus require less amino acids (0.65-1.02 moles of amino acids for 1 synthetized mole of live matter) than those of the Coxsackieviruses (1.30-1.33). On the other hand, biosynthesis of virus particles of SARS-CoV-2 EG.5 Eris variant and West Nile virus requires carbohydrates ($CH_2O$), which are not needed to produce virus particles of Coxsackieviruses. The reason for the differences is biosynthesis reactions are differences in virus morphology. SARS-CoV-2 and West Nile virus are enveloped viruses, while the Coxsackieviruses lack an envelope [111]. The envelope contains lipids, which are molecules with a high energy content [60]. This means that biosynthesis of the envelope lipids requires additional energy. This extra energy comes from the carbohydrates, which are required as an additional energy source for the biosynthesis of SARS-CoV-2 and West Nile virus particles. Due to lower energy requirements for biosynthesis, carbohydrates are not needed for biosynthesis of Coxsackieviruses. On the other hand, the Coxsackieviruses lack a lipid envelope and have a higher protein content [111]. Due to the higher protein content, more amino acids are required for biosynthesis of the Coxsackievirus particles than for the SARS-CoV-2 and West Nile virus particles.

**Table 4: Biosynthesis stoichiometries of the Coxsackievirus particles, nucleic acids and structural proteins. The general biosynthesis reaction has the form (Amino acid) + $O_2$ + $HPO_4^{2-}$ + $HCO_3^- \rightarrow$ (Bio) + $SO_2^{2-}$ + $H_2O$ + $H_2CO_3$.**
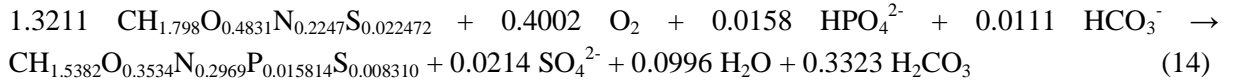
| Virus | Particle | Reactants | | | | → | Products | | | | |
| | | Amino acid | $O_2$ | $HPO_4^{2-}$ | $HCO_3^-$ | | Bio | $SO_4^{2-}$ | $H_2O$ | $HCO_3^-$ | $H_2CO_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Coxsackievirus A6 | Virus particle | 1.3187 | 0.4339 | 0.0233 | 0.0020 | → | 1 | 0.0243 | 0.1440 | 0.0000 | 0.3207 |
| | Nucleic acid | 1.7641 | 1.1169 | 0.1051 | 0.0000 | → | 1 | 0.0396 | 0.3222 | 0.1310 | 0.6332 |
| | VP1 | 1.2468 | 0.3077 | 0.0000 | 0.0398 | → | 1 | 0.0199 | 0.0864 | 0.0000 | 0.2866 |
| | VP2 | 1.1635 | 0.2030 | 0.0000 | 0.0397 | → | 1 | 0.0198 | 0.1046 | 0.0000 | 0.2031 |
| | VP3 | 1.1471 | 0.1775 | 0.0000 | 0.0364 | → | 1 | 0.0182 | 0.0946 | 0.0000 | 0.1835 |
| | VP4 | 1.2188 | 0.3014 | 0.0000 | 0.0548 | → | 1 | 0.0274 | 0.0755 | 0.0000 | 0.2736 |
| Coxsackievirus A9 | Virus particle | 1.3320 | 0.4423 | 0.0228 | 0.0000 | → | 1 | 0.0209 | 0.1428 | 0.0037 | 0.3283 |
| | Nucleic acid | 1.7844 | 1.1407 | 0.1049 | 0.0000 | → | 1 | 0.0401 | 0.3199 | 0.1296 | 0.6548 |
| | VP1 | 1.2608 | 0.3180 | 0.0000 | 0.0392 | → | 1 | 0.0196 | 0.0816 | 0.0000 | 0.3000 |
| | VP2 | 1.1855 | 0.2237 | 0.0000 | 0.0267 | → | 1 | 0.0134 | 0.1070 | 0.0000 | 0.2122 |
| | VP3 | 1.1457 | 0.1633 | 0.0000 | 0.0208 | → | 1 | 0.0104 | 0.1031 | 0.0000 | 0.1665 |
| | VP4 | 1.2594 | 0.3357 | 0.0000 | 0.0503 | → | 1 | 0.0252 | 0.0613 | 0.0000 | 0.3097 |
| Coxsackievirus A10 | Virus particle | 1.3266 | 0.4411 | 0.0233 | 0.0013 | → | 1 | 0.0239 | 0.1422 | 0.0000 | 0.3279 |
| | Nucleic acid | 1.7759 | 1.1305 | 0.1050 | 0.0000 | → | 1 | 0.0399 | 0.3207 | 0.1302 | 0.6456 |
| | VP1 | 1.2454 | 0.2951 | 0.0000 | 0.0355 | → | 1 | 0.0177 | 0.0836 | 0.0000 | 0.2809 |
| | VP2 | 1.1694 | 0.2164 | 0.0000 | 0.0430 | → | 1 | 0.0215 | 0.1059 | 0.0000 | 0.2123 |
| | VP3 | 1.1466 | 0.1750 | 0.0000 | 0.0345 | → | 1 | 0.0173 | 0.0934 | 0.0000 | 0.1811 |
| | VP4 | 1.2933 | 0.3846 | 0.0000 | 0.0519 | → | 1 | 0.0259 | 0.0636 | 0.0000 | 0.3452 |
| Coxsackievirus A24 | Virus particle | 1.2993 | 0.4037 | 0.0223 | 0.0000 | → | 1 | 0.0218 | 0.1452 | 0.0010 | 0.2982 |
| | Nucleic acid | 1.7783 | 1.1332 | 0.1050 | 0.0000 | → | 1 | 0.0400 | 0.3209 | 0.1300 | 0.6484 |
| | VP1 | 1.1921 | 0.2430 | 0.0000 | 0.0445 | → | 1 | 0.0222 | 0.0919 | 0.0000 | 0.2365 |
| | VP2 | 1.1718 | 0.2138 | 0.0000 | 0.0347 | → | 1 | 0.0173 | 0.1069 | 0.0000 | 0.2064 |

| | | | | | | | → | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VP3 | 1.1274 | 0.1334 | 0.0000 | 0.0171 | → | 1 | 0.0086 | 0.1031 | 0.0000 | 0.1445 |
| | VP4 | 1.2111 | 0.2710 | 0.0000 | 0.0418 | → | 1 | 0.0209 | 0.0655 | 0.0000 | 0.2528 |
| Coxsackievirus B3 | Virus particle | 1.3215 | 0.4323 | 0.0230 | 0.0000 | → | 1 | 0.0215 | 0.1467 | 0.0031 | 0.3184 |
| | Nucleic acid | 1.7874 | 1.1439 | 0.1049 | 0.0000 | → | 1 | 0.0402 | 0.3193 | 0.1295 | 0.6579 |
| | VP1 | 1.2326 | 0.2892 | 0.0000 | 0.0440 | → | 1 | 0.0220 | 0.0863 | 0.0000 | 0.2766 |
| | VP2 | 1.1920 | 0.2296 | 0.0000 | 0.0269 | → | 1 | 0.0134 | 0.0997 | 0.0000 | 0.2189 |
| | VP3 | 1.1172 | 0.1371 | 0.0000 | 0.0213 | → | 1 | 0.0106 | 0.1202 | 0.0000 | 0.1385 |
| | VP4 | 1.2675 | 0.3384 | 0.0000 | 0.0446 | → | 1 | 0.0223 | 0.0632 | 0.0000 | 0.3121 |
| Coxsackievirus B4 | Virus particle | 1.3303 | 0.4419 | 0.0231 | 0.0000 | → | 1 | 0.0201 | 0.1463 | 0.0060 | 0.3244 |
| | Nucleic acid | 1.7854 | 1.1420 | 0.1049 | 0.0000 | → | 1 | 0.0401 | 0.3197 | 0.1296 | 0.6558 |
| | VP1 | 1.2528 | 0.3088 | 0.0000 | 0.0362 | → | 1 | 0.0181 | 0.0833 | 0.0000 | 0.2890 |
| | VP2 | 1.1852 | 0.2264 | 0.0000 | 0.0230 | → | 1 | 0.0115 | 0.1127 | 0.0000 | 0.2082 |
| | VP3 | 1.1445 | 0.1642 | 0.0000 | 0.0227 | → | 1 | 0.0113 | 0.1064 | 0.0000 | 0.1672 |
| | VP4 | 1.2537 | 0.3236 | 0.0000 | 0.0440 | → | 1 | 0.0220 | 0.0633 | 0.0000 | 0.2977 |

The analysis above is confirmed by the biosynthesis reactions of SARS-CoV-2 and West Nile virus without the lipid envelope. The biosynthesis reaction of the SARS-CoV-2 EG.5 Eris nucleocapsid, which does not contain lipids, is

$$1.390330 \ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.492496 \ O_2 + 0.006015 \ HPO_4^{2-} + 0.043760 \ HCO_3^- \rightarrow$$
$$CH_{1.570926}O_{0.343147}N_{0.312434}P_{0.006015}S_{0.003349} + 0.027895 \ SO_2^{2-} + 0.055069 \ H_2O + 0.434090 \ H_2CO_3 \qquad (13)$$
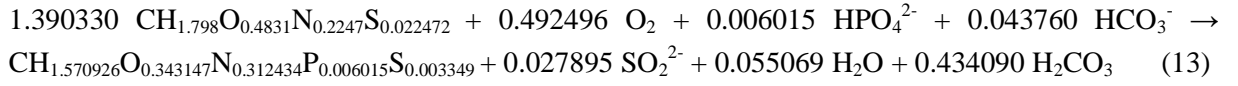
where $CH_{1.570926}O_{0.343147}N_{0.312434}P_{0.006015}S_{0.003349}$ is the empirical formula of the nucleocapsid of the EG.5 variant [17]. The biosynthesis reaction of the West Nile virus nucleic acid and structural proteins is

$$1.3211 \ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.4002 \ O_2 + 0.0158 \ HPO_4^{2-} + 0.0111 \ HCO_3^- \rightarrow$$
$$CH_{1.5382}O_{0.3534}N_{0.2969}P_{0.015814}S_{0.008310} + 0.0214 \ SO_4^{2-} + 0.0996 \ H_2O + 0.3323 \ H_2CO_3 \qquad (14)$$

where $CH_{1.5382}O_{0.3534}N_{0.2969}P_{0.015814}S_{0.008310}$ is the empirical formula of the West Nile virus nucleic acid and structural proteins [20]. We see that these biosynthesis reactions lack carbohydrates and need more amino acids, like those of the Coxsackieviruses. Therefore, the biosynthesis reactions of viruses show how virus morphology determines the building blocks needed for production of virus particles.
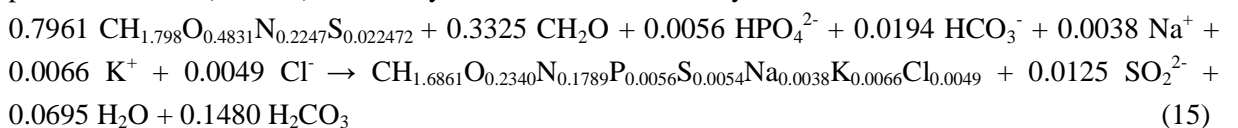
**Table 5: Biosynthesis stoichiometries of the myocard and pancreas tissues. The general biosynthesis reaction has form: (Amino acid) + $CH_2O$ + $O_2$ + $HPO_4^{2-}$ + $HCO_3^-$ + $Na^+$+ $K^+$+ $Cl^-$ → (Bio) + $SO_4^{2-}$ + $H_2O$ + $H_2CO_3$. (Amino acid) denotes the empirical formula of amino acids and (Bio) denotes the empirical formula of live matter.**

| Tissue | Reactants | | | | | | | | → | Products | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amino acid | $CH_2O$ | $O_2$ | $HPO_4^{2-}$ | $HCO_3^-$ | $Na^+$ | $K^+$ | $Cl^-$ | → | Bio | $SO_4^{2-}$ | $H_2O$ | $H_2CO_3$ |
| Myocard | 0.7961 | 0.3325 | 0.0000 | 0.0056 | 0.0194 | 0.0038 | 0.0066 | 0.0049 | → | 1 | 0.0125 | 0.0695 | 0.1480 |
| Pancreas | 1.0719 | 0.0000 | 0.0330 | 0.0044 | 0.0413 | 0.0117 | 0.0069 | 0.0000 | → | 1 | 0.0157 | 0.0400 | 0.1131 |

**Table 6: Thermodynamic properties of biosynthesis of myocard and pancreas tissues: standard enthalpy of biosynthesis, $\Delta_{bs}H^0$ , standard entropy of biosynthesis, $\Delta_{bs}S^0$ , and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$ .**

| Tissue | $\Delta_{bs}H^0$ (kJ/C-mol) | $\Delta_{bs}S^0$ (J/C-mol K) | $\Delta_{bs}G^0$ (kJ/C-mol) |
|---|---|---|---|
| Myocard | -11.32 | 18.73 | -16.64 |
| Pancreas | -18.09 | 4.71 | -19.54 |

Biosynthesis reactions were also formulated in this research for the host myocard and pancreas tissues (Table 5). The biosynthesis reaction of the myocard tissue is

$$0.7961 \ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.3325 \ CH_2O + 0.0056 \ HPO_4^{2-} + 0.0194 \ HCO_3^- + 0.0038 \ Na^+ +$$
$$0.0066 \ K^+ + 0.0049 \ Cl^- \rightarrow CH_{1.6861}O_{0.2340}N_{0.1789}P_{0.0056}S_{0.0054}Na_{0.0038}K_{0.0066}Cl_{0.0049} + 0.0125 \ SO_2^{2-} +$$
$$0.0695 \ H_2O + 0.1480 \ H_2CO_3 \qquad (15)$$

where $CH_{1.6861}O_{0.2340}N_{0.1789}P_{0.0056}S_{0.0054}Na_{0.0038}K_{0.0066}Cl_{0.0049}$ is the empirical formula of the myocard tissue. The myocard tissue is characterized by standard enthalpy of biosynthesis of $\Delta_{bs}H^0$ = -11.32

kJ/C-mol, standard entropy of biosynthesis of $\Delta_{bs}S^0$ = 18.73 J/C-mol K and standard Gibbs energy of biosynthesis of $\Delta_{bs}G^0$ = -16.64 kJ/C-mol (Table 6). The biosynthesis reaction of the pancreas tissue is

$1.0719\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.0330\ O_2 + 0.0044\ HPO_4^{2-} + 0.0413\ HCO_3^- + 0.0117\ Na^+ + 0.0069\ K^+ \rightarrow CH_{1.6861}O_{0.2340}N_{0.1789}P_{0.0056}S_{0.0054}Na_{0.0038}K_{0.0066}Cl_{0.0049} + 0.0157\ SO_2^{2-} + 0.0400\ H_2O + 0.1131\ H_2CO_3$ (16)

where $CH_{1.6663}O_{0.2830}N_{0.2409}P_{0.0044}S_{0.0084}Na_{0.0117}K_{0.0069}$ is the empirical formula of the pancreas tissue. The pancreas tissue is characterized by standard enthalpy of biosynthesis of $\Delta_{bs}H^0$ = -18.09 kJ/C-mol, standard entropy of biosynthesis of $\Delta_{bs}S^0$ = 4.71 J/C-mol K and standard Gibbs energy of biosynthesis of $\Delta_{bs}G^0$ = -19.54 kJ/C-mol (Table 6).
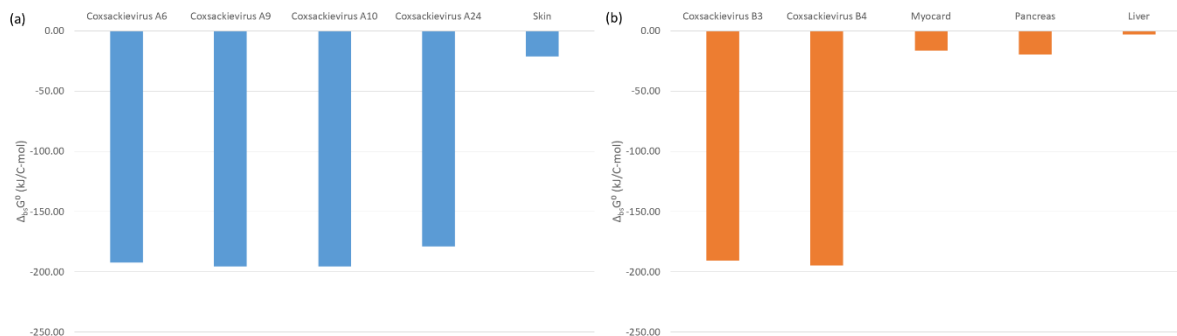


**Figure 1: Gibbs energies of biosynthesis, $\Delta_{bs}G^0$ , of Coxsackieviruses and their host tissues. (a) Gibbs energies of biosynthesis of Coxsackieviruses A and skin tissue. (b) Gibbs energies of biosynthesis of Coxsackieviruses B, and myocard, pancreas and liver tissues.**

**Table 7: Thermodynamic properties of biosynthesis of virus particles, nucleic acids and structural proteins of Coxsackieviruses: standard enthalpy of biosynthesis, $\Delta_{bs}H^0$ , standard entropy of biosynthesis, $\Delta_{bs}S^0$ , and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$ .**

| Virus | Particle | $\Delta_{bs}H^0$ (kJ/C-mol) | $\Delta_{bs}S^0$ (J/C-mol K) | $\Delta_{bs}G^0$ (kJ/C-mol) |
|---|---|---|---|---|
| Coxsackievirus A6 | Virus particle | -202.32 | -34.60 | -192.27 |
| | Nucleic acid | -508.36 | -102.24 | -479.12 |
| | VP1 | -146.64 | -21.19 | -140.30 |
| | VP2 | -98.39 | -11.96 | -94.81 |
| | VP3 | -86.33 | -9.85 | -83.38 |
| | VP4 | -145.11 | -22.56 | -138.36 |
| Coxsackievirus A9 | Virus particle | -205.58 | -34.97 | -195.41 |
| | Nucleic acid | -519.49 | -104.09 | -489.69 |
| | VP1 | -151.45 | -21.60 | -144.99 |
| | VP2 | -106.44 | -13.71 | -102.34 |
| | VP3 | -78.08 | -8.22 | -75.62 |
| | VP4 | -160.62 | -24.64 | -153.25 |
| Coxsackievirus A10 | Virus particle | -205.57 | -35.06 | -195.38 |
| | Nucleic acid | -514.72 | -103.28 | -485.16 |
| | VP1 | -140.47 | -19.52 | -134.62 |
| | VP2 | -104.88 | -13.37 | -100.88 |
| | VP3 | -84.98 | -9.62 | -82.10 |
| | VP4 | -183.24 | -29.12 | -174.53 |
| Coxsackievirus A24 | Virus particle | -188.01 | -31.75 | -178.80 |
| | Nucleic acid | -516.01 | -103.46 | -486.39 |
| | VP1 | -117.35 | -15.72 | -112.65 |
| | VP2 | -102.76 | -13.10 | -98.84 |
| | VP3 | -63.99 | -5.34 | -62.38 |
| | VP4 | -129.86 | -19.25 | -124.10 |
| Coxsackievirus B3 | Virus particle | -201.00 | -34.24 | -191.05 |

| | | | | |
|---|---|---|---|---|
| | Nucleic acid | -521.00 | -104.32 | -491.13 |
| | VP1 | -138.66 | -19.37 | -132.86 |
| | VP2 | -109.23 | -14.21 | -104.98 |
| | VP3 | -65.93 | -6.35 | -64.02 |
| | VP4 | -161.29 | -24.50 | -153.96 |
| Coxsackievirus B4 | Virus particle | -205.11 | -35.09 | -194.91 |
| | Nucleic acid | -520.09 | -104.20 | -490.25 |
| | VP1 | -146.82 | -21.01 | -140.53 |
| | VP2 | -107.18 | -14.24 | -102.92 |
| | VP3 | -78.69 | -8.31 | -76.20 |
| | VP4 | -154.36 | -23.50 | -147.33 |

**3.4. Biothermodynamic analysis of virus-host interactions of Coxsackieviruses**

Coxsackievirus A tends to infect skin and mucus membranes. Thus, it can cause herpangina, conjunctivitis, and hand foot and mouth disease [112]. Host cells are skin and mucus tissues. They are susceptible to Coxsackievirus A, with the basic reproduction number of about 2.5 [113]. After entry into host cells (virus-host interaction at the membrane) there is competition with host cells for the metabolic machinery and resources (amino acids, nucleotides etc.). Since virus multiplication is a chemical process, like biosynthesis of proteins needed for reparation of host cells, there is competition that is driven by the driving force – Gibbs energy of biosynthesis. Gibbs energy of biosynthesis for skin is -21.29 kJ/C-mol [17]. Gibbs energy of biosynthesis of Coxsackie A viruses is: -192.27 kJ/C-mol Coxsackievirus A6, -195.41 kJ/C-mol for Coxsackievirus A9, -195.38 for Coxsackievirus A10 and -178.80 kJ/C-mol for Coxsackievirus A24 (Table 7). Gibbs energy of biosynthesis of Coxsackie A viruses is more negative than Gibbs energy of biosynthesis of host cell building blocks (Figure 1a). This means that in the competition, the winner is biosynthesis of virus components – virus multiplication. This is why the virus can hijack the metabolism of the susceptible cell. It multiplies within the cell, damages it and causes the diseases mentioned above.

Coxsackie B viruses tend to infect cells of heart, pleura, pancreas and liver. After entry into a susceptible host cell, there is competition for the cell metabolic machinery, like in the case of Coxsackie A viruses. Gibbs energy of biosynthesis of the host tissues are: myocard -16.64 kJ/C-mol, pancreas -19.54 kJ/C-mol and liver -3.10 kJ/C-mol [20]. Gibbs energies of biosynthesis of Coxsackie B viruses are: -191.05 kJ/C-mol for Coxsackievirus B3 and -194.91 kJ/C-mol for Coxsackievirus B4 (Table 7). The driving force of biosynthesis of virus particles is greater and thus the virus can hijack the host cell metabolic machinery and multiply (Figure 1b).

By hijacking the host cell metabolic machinery, the virus can damage the host cell in two ways: (a) by inhibiting the normal host metabolism and thereby preventing reparative processes inside the cell and (b) by its presence inside the cell and exit from it, leading to its damage. In that way virus multiplication leads to initiation of the inflammatory process: myocarditis, pericarditis and hepatitis. There is a possibility that if cells of Langerhans islets are susceptible to Coxsackievirus B, then the virus is able to cause pancreatitis and development of insulin-dependent diabetes. Even though data are not available on thermodynamic properties of cells of Langerhans islets, Gibbs energy of the pancreas indicates this possibility. In that case, development of a vaccine for Coxsackie B viruses should prevent development of insulin dependent diabetes.

As was shown above the permissiveness, during the interaction of a susceptible host with the virus, is high for the Coxsackieviruses. Permissiveness is a consequence of the virus-host interaction in the cytoplasm. It is led by Gibbs energy of biosynthesis. However, susceptibility is a consequence

of the virus-host interaction at the membrane. It is led by a driving force – Gibbs energy of binding. Gibbs energy of binding of Coxsackieviruses was reported for the first time in this research: -51.77 kJ/mol for binding of Coxsackievirus A9 particle to host cell $a_V b_6$ receptor, -42.10 kJ/mol for binding of Coxsackievirus A10 to KRM1 receptor, -29.72 kJ/mol for Coxsackievirus B3 CVB3E strain to CAR receptor etc. (Table 8). In general, Gibbs energy of binding of Coxsackieviruses is on the same order of magnitude as those of different variants of SARS-CoV-2. Gibbs energy of binding of XBB.1.5 Kraken variant of SARS-CoV-2 is -48.34 kJ/mol, while that of the Omicron BA.2.75 Centaurus variant is -49.91 kJ/mol [21]. Due to similar values of Gibbs energies of binding, we can conclude that the antigen-receptor binding rates are similar. Thus, the rate of entry of viruses into host cells is similar. This means that infectivity is very similar in the cases of Coxsackieviruses and SARS-CoV-2.

**Table 8: Thermodynamic properties of antigen-receptor binding of Coxsackieviruses: dissociation equilibrium constant, $K_d$, binding equilibrium constant, $K_B$, and standard Gibbs energy of binding, $\Delta_B G^0$ . The $K_d$ data were taken from [75,85-87].**

| Virus | Strain | Proteins | $K_d$ (M) | $K_B$ (M$^{-1}$) | $\Delta_B G^0$ (kJ/mol) |
|---|---|---|---|---|---|
| Coxsackievirus A9 | | $a_V b_6$ with virus particle | 8.50E-10 | 1.18E+09 | -51.77 |
| Coxsackievirus A10 | | KRM1 with virus particle | 4.21E-08 | 2.38E+07 | -42.10 |
| Coxsackievirus B3 | CVB3N (VP3-234N) | CAR with virus particle | 3.4E-05 | 2.94E+04 | -25.50 |
| Coxsackievirus B3 | CVB3V (VP3-234V) | CAR with virus particle | 2.0E-05 | 5.00E+04 | -26.82 |
| Coxsackievirus B3 | CVB3D (VP3-234D) | CAR with virus particle | 1.5E-05 | 6.67E+04 | -27.53 |
| Coxsackievirus B3 | CVB3E (VP3-234E) | CAR with virus particle | 6.20E-06 | 1.61E+05 | -29.72 |
| Coxsackievirus B3 | CVB3Q (VP3-234Q) | CAR with virus particle | 3.16E-06 | 3.16E+05 | -31.39 |
| Coxsackievirus B3 | CG strain | CAR with virus particle | 2.45E-07 | 4.08E+06 | -37.73 |
| Coxsackievirus B3 | CG strain | Dimeric CAR with virus particle | 3.84E-10 | 2.60E+09 | -53.74 |

## 4. Conclusions

Empirical formulas of Coxsackieviruses are similar to those of other viruses from the *Picornaviridae* family. However, since Coxsackieviruses lack an envelope their empirical formulas are different than those of viruses with an envelope, due to lower content of lipids and higher content of proteins.

Gibbs energies of binding of Coxsackieviruses are on the same order of magnitude as those of SARS-CoV-2 variants. This means that rate of entry of viruses into host cells and infectivity are similar.

Gibbs energy of biosynthesis enables Coxsackieviruses to hijack host cell metabolic machinery and resources, leading to damage of host cells and development of the inflammatory process in susceptible tissues.

## Acknowledgements

## Nomenclature

$L_B$ (mol² K/ kJ s) - binding phenomenological coefficient

$L_{bs}$ (C-mol² K/ kJ s) - biosynthesis phenomenological coefficient

$m_X$ (/) – number of atoms of element X in molecular formula

$M_r$ (g/C-mol) – empirical formula molar mass

$M_r(tot)$ (g/mol) – entire particle molar mass

$n_X$ (/) – number of atoms of element X in empirical formula

$r_B$ (mol/s) - the binding rate

$r_{bs}$ (C-mol/s) - biosynthesis rate

$S_m^0$ (J/C-mol K) - standard molar entropy

$T$ (K) - temperature

$\Delta_B G$ (kJ/mol) - Gibbs energy of binding

$\Delta_{bs} G$ (kJ/C-mol) - Gibbs energy of biosynthesis

$\Delta_{bs} G^0$ (kJ/C-mol) – standard Gibbs energy of biosynthesis

$\Delta_{bs} H^0$ (kJ/C-mol) - standard enthalpy of biosynthesis

$\Delta_{bs} S^0$ (J/C-mol K) - standard entropy of biosynthesis

$\Delta_f G^0$ (kJ/C-mol) - standard Gibbs energy of formation

$\Delta_f H^0$ (kJ/C-mol) - standard enthalpy of formation


**Author statement**

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