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Le Chatelier's principle and metabolism: Biothermodynamic analysis of the metabolic pathway for synthesis of glucagon

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Abstract: Glucagon is the main catabolic hormone in the human organism. Glucagon has been well studied from the aspect of life and biomedical sciences. However, no analysis of glucagon based on chemical thermodynamics can be found in the literature. The approach of biothermodynamics would allow to study the driving force of glucagon production, as well as provide an understanding of the process from the aspect of the fundamental laws of nature. This research reports an analysis of glucagon with the methodology of biothermodynamics. Based on the protein sequences, chemical and thermodynamic characterization of glucagon, proglucagon, preproglucagon and related peptides is performed, with the atom counting method and Patel-Erickson-Battley model. Reactions of translation at the ribosomes and post-translational processing are formulated and their driving force (Gibbs energy change) is calculated. The process of translation at the ribosomes that produces preproglucagon is studied from the aspect of chemical thermodynamics. Based on Gibbs energy, an analysis is performed of the metabolic pathway for production of glucagon. The role of Le Chatelier's principle in coupling of the reactions in the metabolic pathway is studied. Finally, a discussion is made of applications of the biothermodynamic methodology in omics research for determination of feasibility of metabolic pathways.

Keywords: enthalpy; entropy; Gibbs energy; mechanistic model; omics; post-translational processing.

INTRODUCTION

Glucagon is produced in the alpha cells of the islets of Langerhans in the endocrine part of the pancreas. The physiological effects of glucagon are opposite to those of insulin. Glucagon and insulin control the blood glucose level. Insulin is the main anabolic hormone in the organism, which stimulates absorption of

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glucose into cells, where it can be used for anabolic processes or as an energy source. Glucagon is the main catabolic hormone in the organism. Glucagon increases the concentration of glucose in the blood, by stimulation of glycogenolysis and gluconeogenesis. Glucose is stored in the liver in form of the polysaccharide glycogen. Glucagon stimulates liver cells to degrade glycogen and produce glucose, which is released into the blood. This process is called glycogenolysis. Also, glucagon inhibits formation of glycogen by glycogenesis in liver cells. Glycogen represents the short-term energy storage of the organism. When it becomes depleted, glucagon promotes production of glucose in liver and kidney cells through gluconeogenesis. Gluconeogenesis is a metabolic process in which glucose is produced from substances that are not carbohydrates, like lactate, glycerol, alanine or glutamine. Also, glucagon inhibits glucose degradation by glycolysis in liver cells. Moreover, glucagon stimulates lipolysis in the adipose tissue and liver. Lipolysis leads to release of fatty acids into the blood, which can be used in catabolism to provide energy.

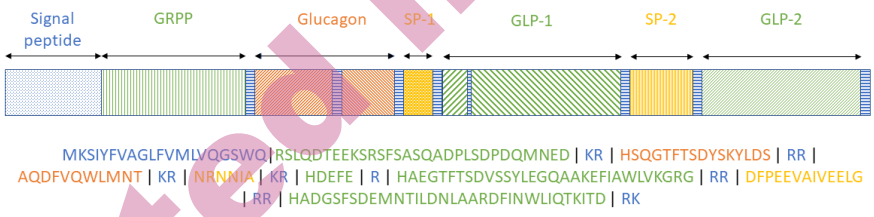

Figure 1: Domains of preproglucagon. Preproglucagon consists of seven domains: signal peptide, glicentin-related pancreatic peptide (GRPP), glucagon, hexapeptide or spacer peptide 1 (SP-1), glucagon-like peptide 1 (GLP-1), spacer peptide 2 (SP-2) and glucagon-like peptide 2 (GLP-2). Each domain consists of one or more segments, which are marked by patterns and colors. The lower part of the figure shows the amino acid sequence of preproglucagon. The vertical lines | separate the segments shown in the upper part of the figure by patterns and colors.

Glucagon is a polypeptide hormone that consists of 29 amino acid residues. It is produced by translation at the ribosomes based on the mRNA transcribed from the human glucagon gene.[1] The translation process produces the precursor molecule preproglucagon,[1] which is schematically presented in Figure 1. Preproglucagon consists of seven domains: signal peptide, glicentin-related pancreatic peptide (GRPP), glucagon, hexapeptide or spacer peptide 1 (SP-1), glucagon-like peptide 1 (GLP-1), spacer peptide 2 (SP-2) and glucagon-like peptide 2 (GLP-2).[2] The domains are connected by Lys-Arg (KR), Arg-Arg (RR) or Arg-Lys (RK) dibasic sites.[2]

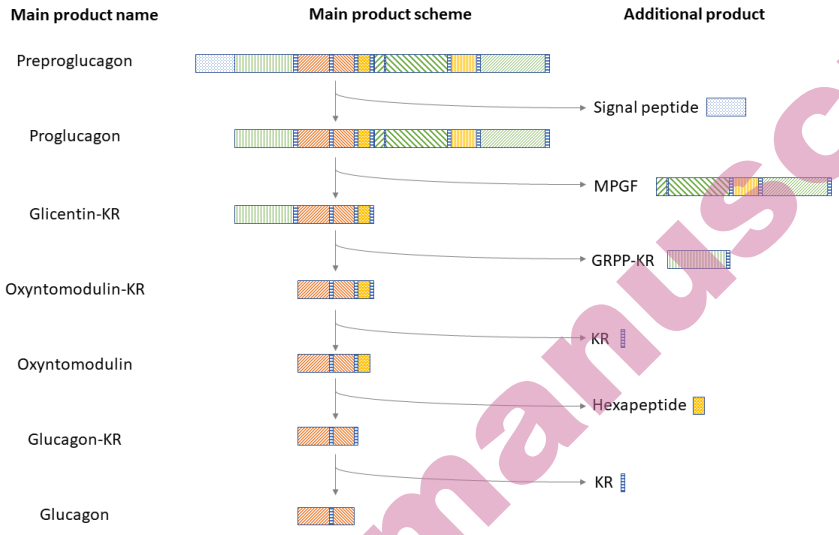

Figure 2: Metabolic pathway for production of glucagon from preproglucagon. After production at the ribosomes, preproglucagon is targeted to the reticulum by the signal peptide. At the reticulum, preproglucagon is transformed into glucagon, which is excreted into the blood. The figure presents the chemical reactions that transform preproglucagon into glucagon. The process is catalyzed by a phoromone convertase enzyme.

After production at the ribosomes, preproglucagon is converted into glucagon by post-translational processing, as shown in Figure 2. The signal peptide domain (Figure 1) targets preproglucagon to the endoplasmic reticulum for excretion.[3,4] Inside the endoplasmic reticulum the signal peptide is cleaved to produce proglucagon.[5] After that, proglucagon is cleaved by a phoromone convertase enzyme (PC2) to produce glicentin-KR (glicentin with a Lys-Arg extension at the C-terminus) and major proglucagon fragment (MPGF).[2,6,7] Then glicentin-KR is cleaved to produce oxyntomodulin-KR (oxyntomodulin with a Lys-Arg extension at the C-terminus).[2] Oxyntomodulin is then transformed into glucagon-KR (glucagon with a Lys-Arg extension at the C-terminus).[2] After that, glucagon-KR is converted into glucagon.[2] Finally, glucagon is excreted from the cells into the blood.[8]

Glucagon has been extensively studied from the aspect of life and biomedical sciences. However, chemical and thermodynamic analysis of glucagon cannot be found in the literature. Biothermodynamics applies the methodology of chemical and nonequilibrium thermodynamics to study biological structures and processes.[31,36] Biothermodynamic analysis has been applied to study microbial growth,[37,38] metabolism[39,40] and interactions,[41-43] as well as multicellular organisms.[35,44,45] Moreover, biothermodynamic methodology has been applied to biological molecules and metabolic reactions.[23,24,46,47]

Organisms are open nonequilibrium thermodynamic systems.[36,48] This is why the methodology of nonequilibrium thermodynamics is very important in analysis of organisms.[36,48] A cell is an open thermodynamic system out of equilibrium, which means that it exchanges matter and energy with its environment.[36,51] The cell takes nutrients from the environment.[35,36,51] A part of the nutrients is degraded into simple products in catabolism to provide energy.[35,36,51] The rest of the nutrients is used in anabolic processes to produce more complex products.[35,36,51] For example, amino acids are taken by the cells of the pancreas as nutrients, which are transformed by anabolic processes into glucagon. To perform metabolic processes, a cell dissipates usable (Gibbs) energy released by degradation of nutrients in catabolism.[35,36,51] For example, during the process of translation amino acids are activated by hydrolysis of ATP, which is obtained from catabolism.[32-34] The activated amino acids are in the form of aminoacyl-tRNAs, which react at the ribosomes to produce polypeptides.[32-34]

Le Chatelier's principle states that when a system at equilibrium is subjected to an external perturbation, the composition of the system adjusts to minimize its effect.[14,50] For example, if there is a chemical reaction in the system, removal of products will make the reaction proceed towards formation of more products. Le Chatelier's principle has be extended to systems under nonequilbrium conditions.[48-50]

Biothermodynamic models allow calculation of thermodynamic properties of biological matter, based on chemical composition.[31,52,53] Biothermodynamic models include the Patel-Erickson-Battley,[25-27] Roels,[53] Sandler-Orbey,[54] Hurst-Harrison[30,31] models etc. Biothermodynamic models have been extensively used in analysis of viruses, cellular microorganisms and multicellular organisms.[35,44,52,53,55] They give results for enthalpy and entropy in good agreement with experiment.[56-58] Moreover, biothermodynamic models have been applied in analysis of macromolecules and macromolecular assemblies.[23,24,58,59]

The aim of this paper is to perform a chemical and thermodynamic characterization of human glucagon, proglucagon and preproglucagon, as well as to make a biothermodynamic analysis of metabolic synthesis of glucagon. Empirical formulas and thermodynamic properties of glucagon, proglucagon and preproglucagon will be determined with the atom counting method and Patel-Erickson-Battley model. Moreover, the chemical reaction of translation will be formulated, in which preproglucagon is synthetized from amino acids at the ribosomes. Thermodynamic properties of the translation reaction will be calculated. Thermodynamic properties will also be calculated for the chemical reactions in the post-translational processing of preproglucagon into glucagon. Furthermore, the driving force for biosynthesis of glucagon will be determined. Based on the determined thermodynamic properties, an analysis will be made of

the metabolic pathway for production of glucagon in the alpha cells of the pancreas.

METHODS

Data sources

The amino acid sequences of human glucagon, proglucagon, preproglucagon and related peptides were taken from.[2,9-12] The amino acid sequence of preproglucagon can be found under the UniProt access number P01275 (section *Sequence*)[9] and NCBI access number NP_002045.1 (Ref. 11). The amino acid sequence of glucagon can be found under the UniProt access number P01275 (section *PTM/Processing*),[9] PubChem CID 16132283 (Ref. 10) and in references 2 and 12. The amino acid sequences of proglucagon and related peptides can be found under the UniProt access number P01275 (section *PTM/Processing*)[9] and in reference 2. More information can be found in the Supplementary Material (Table S.I).

Thermodynamic properties of ATP, AMP, Pi and amino acids were taken from.[13-21]

Atom counting method

Molecular formulas, empirical formulas and molar masses of glucagon, proglucagon and preproglucagon and related peptides were calculated with the atom counting method, based on amino acid sequences, as described in references 22 and 23. Atom counting method is a computational approach for calculation of chemical properties of macromolecules and macromolecular assemblies.[22,23] The input are genetic sequences, proteins sequences and morphology.[22,23] The program goes along the nucleic acid and protein sequences and adds atoms that come from the monomer residues to find molecular formulas.[22,23] Empirical formulas are obtained when numbers of atoms of all constituent elements are divided by the number of carbon atoms.[22,23]

Patel-Erickson-Battley model

Enthalpy, entropy and Gibbs energy of glucagon, proglucagon, preproglucagon and related peptides were calculated with the Patel-Erickson-Battley model, based on molecular and empirical formulas, as described in references 24-27. Empirical formulas were used to calculate the degree of reduction, E, with the equation

$$E = 4n_C + n_H - 2n_O - 0\,n_N + 5n_P + 6n_S \quad (1)$$

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, respectively, in the empirical formula.[25,27] Based on E, standard enthalpy of combustion, $\Delta_C H^o$, was calculated with the Patel-Erickson equation[25,27]

$$\Delta_C H^0(bio) = -111.14\ \frac{kJ}{C-mol} \cdot E \quad (2)$$

$\Delta_C H^o$ was used to find standard enthalpy of formation, $\Delta_f H^o$, with Hess's law[23,25]

$$\Delta_f H^0(bio) = n_C\ \Delta_f H^0(CO_2) + \frac{n_H}{2}\Delta_f H^0(H_2O) + \frac{n_P}{4}\Delta_f H^0(P_4O_{10}) + n_S\ \Delta_f H^0(SO_3) - \Delta_C H^0(bio) \quad (3)$$

Standard molar entropy, S^o_m, was calculated with the Battley equation

$$S^0_m(bio) = 0.187\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (4)$$

where $S^o_m(J)$ is standard molar entropy of element J, a_J number of atoms of element J in its standard state elemental form, and n_J the number of atoms of element J in the empirical

formula of live matter.[26,28] The summation is over all J elements that form the analyzed macromolecules.[26,28] Standard entropy of formation, $\Delta_f S^o$, was calculated with the modified Battley equation[26,28]

$$\Delta_f S^0(bio) = -0.813 \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (5)$$

Standard Gibbs energy of formation, $\Delta_f G^o$, was calculated with the equation

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\Delta_f S^0(bio) \quad (6)$$

where T is temperature.[14,29]

Hurst-Harrison model

Heat capacities of glucagon, proglucagon, preproglucagon and related peptides were calculated with the Hurst-Harrison model, based on molecular and empirical formulas, as described in references 30 and 31. The Hurst-Harrison model calculates standard molar heat capacity at constant pressure, $C^o_{p,m}$, with the equation

$$C^0_{p,m} = \sum_J C_J n_J \quad (7)$$

where n_J is the number of atoms of element J in the empirical formula and C_J is the contribution of element J to heat capacity.[30,31]

Translation reaction

Translation is a chemical process in which amino acids activated by ATP hydrolysis polymerize into a polypeptide.[32-34] Amino acids are activated by ATP hydrolysis to AMP and Pi ($H_2PO_4^-$) and are loaded onto tRNAs to form aminoacyl-tRNAs.[32-34] The aminoacyl-tRNAs react at the ribosomes to extend the growing polypeptide chain and the tRNAs are regenerated.[32-34] For each peptide bond formed, an ATP molecule is hydrolyzed to AMP. Therefore, the overall reaction of translation is

$$x \text{ (Amino acids)} + (x\text{-}1) \text{ ATP} + (x\text{-}1) \text{ } H_2O \rightarrow \text{(Polypeptide)} + (x\text{-}1) \text{ AMP} + 2(x\text{-}1) \text{ Pi} \quad (8)$$

where x is the number of amino acids in the polypeptide.

Reaction thermodynamic properties at room temperature

Changes in thermodynamic properties during chemical reactions in the metabolic pathway of production of glucagon were calculated with the methodology of thermochemistry, as described in references 14 and 23. The determined reaction thermodynamic properties include: standard reaction enthalpy at 25°C, $\Delta_r H^o$, standard reaction entropy at 25°C, $\Delta_r S^o$, standard reaction Gibbs energy at 25°C, $\Delta_r G^o$, standard reaction heat capacity at constant pressure at 25°C, $\Delta_r C_p{}^o$. They were calculated with Hess's law

$$\Delta_r H^0 = \sum_{products} \nu \, \Delta_f H^0 - \sum_{reactants} \nu \, \Delta_f H^0 \quad (9)$$

$$\Delta_r S^0 = \sum_{products} \nu \, S^o_m - \sum_{reactants} \nu \; S^o_m \quad (10)$$

$$\Delta_r G^0 = \sum_{products} \nu \, \Delta_f G^0 - \sum_{reactants} \nu \, \Delta_f G^0 \quad (11)$$

$$\Delta_r C^0_p = \sum_{products} \nu \, C^o_{p,m} - \sum_{reactants} \nu \; C^o_{p,m} \quad (12)$$

where ν represents a stoichiometric coefficient.[14,29]

Reaction thermodynamic properties at physiological temperature

Changes in thermodynamic properties at the physiological temperature (37°C) were calculated as described in references 14, 29 and 35. Standard reaction enthalpy at 37°C, $\Delta_r H'$, at physiological temperature was calculated with Kirchhoff's law

$$\Delta_r H' = \Delta_r H^0 + \Delta_r C_p^0 (T_2 - T_1) \quad (13)$$

where $\Delta_r H^0$ is the standard reaction enthalpy at room temperature, $\Delta_r C_p{}^0$ standard reaction heat capacity at constant pressure, T_2 is the physiological temperature (37°C) and T_1 is room temperature (25°C).[14,29,35] Standard reaction entropy at 37°C, $\Delta_r S'$, was calculated with the equation

$$\Delta_r S' = \Delta_r S^0 + \Delta_r C_p^0 \ln \frac{T_2}{T_1} \quad (14)$$

where $\Delta_r S^0$ is the standard reaction entropy at room temperature.[14,29,35] Standard reaction Gibbs energy at 37°C, $\Delta_r G'$, was calculated with the equation[14,29,35]

$$\Delta_r G' = \Delta_r H' - T\Delta_r S' \quad (15)$$

RESULTS AND DISCUSSION

Glucagon is the antagonist of insulin. Both represent peptide hormones. Chemical and thermodynamic properties of insulin are available in reference 23. Chemical and thermodynamic properties of glucagon have not been reported. The dynamics of synthesis of glucagon depends on the driving force of the biosynthesis process and occurs in the alpha cells on the pancreas. The driving force of the biosynthesis reactions of glucagon is Gibbs energy. Therefore, to understand the physiology and pathophysiology of regulation of the blood glucose level, it is necessary to know the chemical and thermodynamic parameters that characterize and regulate insulin and glucagon. The empirical formula of glucagon is available in reference 10.

Glucagon is produced by translation at the ribosomes in the form of preproglucagon, the structure of which is shown in Figure 1. Preproglucagon is transformed into glucagon in a series of reactions, which are presented in Figure 2. Chemical and thermodynamic properties of glucagon and related peptides are presented in Tables I and II, and in the Supplementary Material (Tables S.II an S.III). Thermodynamic properties of the chemical reactions of production of preproglucagon at the ribosomes and post-translational processing into glucagon are shown in Tables III and IV, and in the Supplementary Material (Table S.IV).

Chemical and thermodynamic properties of glucagon

Based on protein sequences, chemical and thermodynamic properties of glucagon, proglucagon, preproglucagon and related peptides were calculated with the atom counting method, Patel-Erickson-Battley model and Hurst-Harrison model. Table I presents molecular formulas and molar masses. Tables II presents thermodynamic properties of entire molecules. For empirical formulas and thermodynamic properties per C-mole please see the Supplementary Material (Tables S.II and S.III).

Table I: Molecular formulas and molar masses of glucagon, proglucagon, preproglucagon and related peptides. The molecular formulas have the general form $C_{mC}H_{mH}O_{mO}N_{mN}S_{mS}$, where m_C, m_H, m_O, m_N and m_S represent the numbers of carbon, hydrogen, oxygen, nitrogen and sulfur atoms in the molecular formula, respectively. The table also gives the molecular formula of the entire molecules, *Mr*(*tot*), in daltons.

Name	m_C	m_H	m_O	m_N	m_S	Mr(tot) (Da)
Preproglucagon	914	1414	287	268	5	20909
Proglucagon	804	1250	262	244	3	18622
GRPP	136	215	58	41	1	3384
GRPP-KR	148	239	60	47	1	3669
Glicentin-KR	352	556	121	114	2	8385
Glicentin	340	532	119	108	2	8101
Oxyntomodulin-KR	204	319	62	67	1	4734
Oxyntomodulin	192	295	60	61	1	4450
Glucagon-KR	165	249	51	49	1	3767
Glucagon	153	225	49	43	1	3483
Mini-glucagon	61	89	18	15	1	1352
Hexapeptide	27	48	10	12	0	701
GLI9000	301	462	108	90	2	7133
MPGF	452	696	142	130	1	10255
GLP-1	186	275	59	51	0	4169
t-GLP-1	151	228	47	40	0	3356
GLP-2	171	266	56	48	1	3922
Signal peptide	110	166	26	24	2	2305
KR	12	26	3	6	0	302

Standard enthalpies of formation, $\Delta_f H^o$, of all the analyzed molecules are negative. The reason for the negative standard enthalpies of formation is energy released by the attraction of valence electrons of less electronegative C, H and S by more electronegative O and N atoms. Standard molar entropies, $S_m{}^o$, and standard molar heat capacities at constant pressure, $C^o_{p,m}$, are positive for all the analyzed molecules, due to the third law of thermodynamics. Standard Gibbs energies of formation, $\Delta_f G^o$, are negative for all the analyzed molecules. The negative $\Delta_f G^o$ values originate from the negative standard enthalpies of formation, $\Delta_f H^o$, through the equation $G = H - TS$.

Standard entropies of formation, $\Delta_f S^o$, are negative for all the analyzed molecules. $\Delta_f S^o$ is the entropy change of the hypothetical reaction where the analyzed molecules are formed from elements in their standard state: $C + H_2 + O_2 + N_2 + S \rightarrow$ (molecule). The reason for the negative $\Delta_f S^o$ values is formation of compact and ordered live matter from simple gaseous molecules like H_2, O_2 and N_2. Standard enthalpies of combustion, $\Delta_C H^o$, are negative for all the analyzed molecules. The reason for the negative $\Delta_C H^o$ values is energy released by the acceptance of valence electrons of less electronegative elements, (C, H and S) by oxygen during the combustion process.

Table II: Thermodynamic properties of entire molecules of glucagon, proglucagon, preproglucagon and related peptides. Symbols: standard enthalpy of formation, $\Delta_f H^o$, standard molar entropy, $S_m{}^o$, standard Gibbs energy of formation, $\Delta_f G^o$, standard molar heat capacity at constant pressure, $C^o{}_{p,m}$, standard entropy of formation, $\Delta_f S^o$, standard enthalpy of combustion, $\Delta_C H^o$. The properties in this table are for the entire molecules and are given per mole (of molecules).

Name	$\Delta_f H^o$ (kJ/mol)	$S^o{}_m$ (J/mol K)	$\Delta_f G^o$ (kJ/mol)	$C^o{}_{p,m}$ (J/mol K)	$\Delta_f S^o$ (kJ/mol)	$\Delta_C H^o$ (kJ/mol)
Preproglucagon	-60708.91	28555.26	-23694.58	29578.96	-124146.67	-503019.64
Proglucagon	-56098.55	25516.57	-23023.07	26331.24	-110935.70	-440114.4
GRPP	-12509.95	4620.18	-6521.10	4665.5	-20086.68	-72129.86
GRPP-KR	-13104.51	5071.65	-6530.45	5116.9	-22049.48	-79687.38
Glicentin-KR	-26050.94	11531.54	-11103.35	11821.54	-50134.47	-192716.76
Glicentin	-25456.38	11080.08	-11094.00	11370.14	-48171.68	-185159.24
Oxyntomodulin-KR	-13232.27	6503.51	-4802.18	6733.18	-28274.63	-113029.38
Oxyntomodulin	-12637.71	6052.05	-4792.83	6281.78	-26311.83	-105471.86
Glucagon-KR	-10553.89	5074.55	-3976.07	5294.33	-22062.08	-90356.82
Glucagon	-9959.33	4623.08	-3966.72	4842.93	-20099.29	-82799.3
Mini-glucagon	-3443.85	1770.30	-1149.12	1872.15	-7696.55	-33675.42
Hexapeptide	-2369.65	1021.11	-1046.05	1015.99	-4439.39	-15115.04
GLI9000	-22778.00	9651.12	-10267.88	9931.29	-41959.13	-162486.68
MPGF	-30333.44	14028.65	-12149.01	14538.24	-60990.86	-247397.64
GLP-1	-12357.35	5597.16	-5102.11	5852.06	-24334.18	-100137.14
t-GLP-1	-9983.31	4559.58	-4073.03	4748.41	-19823.18	-82021.32
GLP-2	-11899.16	5366.44	-4942.99	5536.55	-23331.10	-93802.16
Signal peptide	-4896.19	3082.31	-900.80	3276.26	-13400.61	-62905.24
KR	-880.39	495.09	-238.64	479.94	-2152.43	-7557.52

Biothermodynamic analysis of translation at the ribosomes

Figure 3 and Table III show an analysis of changes in thermodynamic properties (enthalpy, entropy, Gibbs energy) during the chemical reaction of translation at the ribosomes that produces preproglucagon from amino acids. Table V shows changes in thermodynamic properties for the entire process, while Figure 3 shows changes in thermodynamic properties per peptide bond formed (there are 179 peptide bonds in preproglucagon).

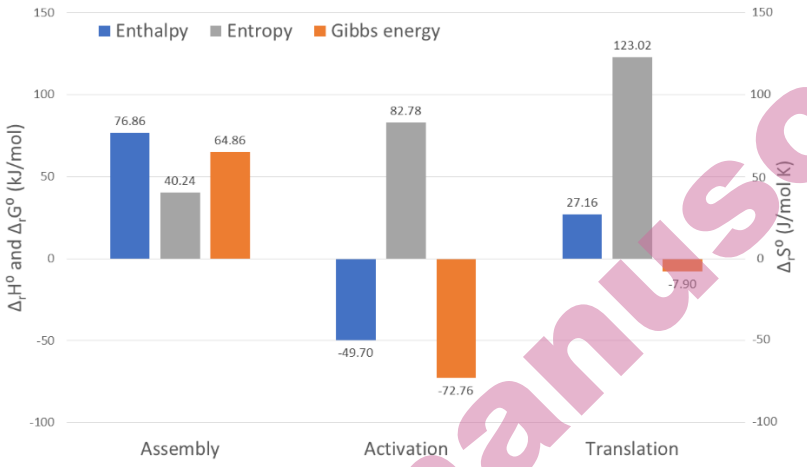

Figure 3: Thermodynamic analysis of translation at the ribosomes. This graph shows standard reaction thermodynamic properties at 25ºC, for polymerization of amino acids into a polypeptide (assembly), hydrolysis of ATP into AMP and Pi (activation), and total translation reaction. The blue columns represent enthalpy change, gray columns entropy change and orange columns Gibbs energy change. Changes in all thermodynamic properties are presented per peptide bond formed.

The translation process can be divided into subprocesses: assembly of amino acids into a polypeptide and activation of amino acids by hydrolysis of ATP. The assembly part represents the changes in thermodynamic properties during polymerization of amino acids into a polypeptide without ATP. The activation part represents hydrolysis of ATP into AMP and Pi to provide energy. The sum of the subprocesses represents the entire reaction of polymerization where amino acids activated by ATP polymerize to produce a polypeptide.

The reaction of assembly of amino acids into a polypeptide without ATP hydrolysis has a positive enthalpy change. This means that the enthalpy change is unfavorable. The assembly process also has a positive entropy change. The positive entropy change originates from the release of a water molecule during the reaction in which the peptide bond is formed: R_1-COOH + H_2N-R_2 → R_1-CO-NH-R_2 + H_2O, where R_1-COOH and H_2N-R_2 are the carboxyl and amino ends of the amino acids that react and R_1-CO-NH-R_2 is the newly formed peptide. Release of the water molecule makes the entropy of the reaction positive. The positive entropy change contributes favorably to the feasibility of the process. However, the enthalpy change is dominant, which makes Gibbs energy change positive for the assembly part. Therefore, assembly of amino acids into the polypeptide (preproglucagon) without activation of amino acids by ATP hydrolysis is not favorable and will not occur spontaneously.

The activation subprocess with ATP hydrolysis has a negative enthalpy change, which is favorable. Moreover, it has a highly positive entropy change. The

positive entropy change originates from hydrolysis of pyrophosphate. During ATP hydrolysis to AMP, ATP reacts with water to produce AMP and pyrophosphate PPi, in the reaction ATP + H_2O → AMP + PPi. The pyrophosphate then spontaneously hydrolyzes into phosphates Pi in the reaction PPi + H_2O → 2 Pi. This makes the entire reaction ATP + 2 H_2O → AMP + 2Pi have a highly positive entropy change. The positive entropy change contributes favorably to the feasibility of the reaction. Due to the negative enthalpy and positive entropy changes, the Gibbs energy change of the ATP hydrolysis reaction is highly negative. This means that the ATP hydrolysis reaction has a large driving force and is highly favorable.

Table III: Thermodynamic properties of translation. This table shows changes in thermodynamic properties (enthalpy, entropy and Gibbs energy) during the translation process, in which amino acids form preproglucagon. Thermodynamic properties are shown for assembly of amino acids into preproglucagon without ATP, hydrolysis of ATP that activates the amino acids and entire translation process in which preproglucagon is formed from amino acids activated by ATP hydrolysis. Symbols: standard reaction enthalpy at 25°C, $\Delta_r H^o$, standard reaction entropy at 25°C, $\Delta_r S^o$, standard reaction Gibbs energy at 25°C, $\Delta_r G^o$.

Name	Reaction	$\Delta_r H^o$ (kJ/mol)	$\Delta_r S^o$ (J/mol K)	$\Delta_r G^o$ (kJ/mol)
Assembly (of amino acids into preproglucagon)	13 Ala + 16 Arg + 8 Asn + 16 Asp + 13 Glu + 10 Gln + 9 Gly + 4 His + 8 Ile + 12 Leu + 10 Lys + 5 Met + 11 Phe + 3 Pro + 17 Ser + 9 Thr + 4 Trp + 4 Tyr + 8 Val → (Preproglucagon) + 179 H_2O	13757.55	7202.25	11609.96
Activation (by energy from ATP hydrolysis)	179 ATP + 358 H_2O → 179 AMP + 358 $H_2PO_4^-$	-8896.3	14817.62	-13024.04
Total translation with ATP	13 Ala + 16 Arg + 8 Asn + 16 Asp + 13 Glu + 10 Gln + 9 Gly + 4 His + 8 Ile + 12 Leu + 10 Lys + 5 Met + 11 Phe + 3 Pro + 17 Ser + 9 Thr + 4 Trp + 4 Tyr + 8 Val + 179 ATP + 179 H_2O → (Preproglucagon) + 179 AMP + 358 $H_2PO_4^-$	4861.25	22019.87	-1414.08

The translation process is the sum of assembly of amino acids into the polypeptide and their activation by ATP hydrolysis. The enthalpy change of the translation process is positive, which is unfavorable. However, the entropy change is highly positive. The reason is that entropy changes of the subprocesses (amino acid assembly and ATP hydrolysis) are positive. The positive entropy contributes favorably towards feasibility of the translation reaction. The favorable entropy

dominates over the unfavorable enthalpy and makes the Gibbs energy change negative and the translation process feasible.

The favorable positive entropy change and unfavorable positive enthalpy change imply that the translation process is overall entropy driven. The positive entropy originates from release of a water molecule during polymerization of amino acids and hydrolysis of the pyrophosphate released during ATP hydrolysis. Therefore, even though many small amino acids polymerize into a large polypeptide molecule, the release of the small H_2O and Pi molecules allows the entropy change of the process to be positive. The positive entropy change makes the Gibbs energy change favorable, which provides the driving force for the translation process.

Biothermodynamic analysis of post-translational processing

Table IV presents thermodynamic properties of the reactions of post-translational processing. Figure 4 shows Gibbs energy changes of the reactions in the metabolic pathway for production of glucagon. Reaction A is translation at the ribosomes, where amino acids activated by ATP hydrolysis polymerize to produce preproglucagon (blue column in Figure 4a). Reactions B-G represent post-translational processing of preproglucagon into glucagon (green columns in Figure 4a). Gibbs energy change of the translation reaction is highly negative. The reason for the highly negative Gibbs energy of the translation reaction is hydrolysis of ATP that activates amino acids that are loaded to tRNAs before they react at the ribosomes to produce the polypeptide. The highly negative Gibbs energy means that the translation reaction has a large driving force and is highly favorable. On the other hand, the reactions of post-translational processing (B-G) have slightly positive Gibbs energy changes. This means that these reactions alone are not favorable and will not occur unless they are coupled with reactions with negative Gibbs energy.

The initial reactant of the post-translational processing reactions is preproglucagon, while their final product is glucagon (Figure 4b). Preproglucagon is the product of the translation reaction, which has a highly negative Gibbs energy change. This means that the translation reaction is highly favorable and will continuously produce preproglucagon, which is the reactant of the post-translational processing reactions. Therefore, the reactant of the post-translational processing reactions is continuously produced by the translation reaction with its great driving force. Moreover, the final product of the post-translational processing reactions is glucagon, which is excreted from the cell into the blood. This means the product of the post-translational processing reactions is continuously removed from the system (cell). The continuous input of reactant (preproglucagon) and removal of product (glucagon) shifts the post-translational processing reactions towards production of more products, in accordance with the Le Chatelier's principle. Therefore, the Le Chatelier's principle allows the coupling of the

translation reaction that has a highly negative Gibbs energy change with the post-translational processing reactions that alone are not favorable, which makes the entire metabolic pathway for production of glucagon feasible. Indeed, the total Gibbs energy change of the metabolic pathway is highly negative (grey column in Figure 4a).

Table IV: Stoichiometry and thermodynamic properties of the reactions in the metabolic pathway for production of glucagon from amino acids at the physiological temperature of 37°C. Symbols: standard reaction enthalpy at 37°C, $\Delta_r H'$, standard reaction entropy at 37°C, $\Delta_r S'$, and standard reaction Gibbs energy at 37°C, $\Delta_r G'$.

Name	Reaction	Δ_rH' (kJ/mol)	Δ_rS' (J/mol K)	Δ_rG' (kJ/mol)
A	13 Ala + 16 Arg + 8 Asn + 16 Asp + 13 Glu + 10 Gln + 9 Gly + 4 His + 8 Ile + 12 Leu + 10 Lys + 5 Met + 11 Phe + 3 Pro + 17 Ser + 9 Thr + 4 Trp + 4 Tyr + 8 Val + 179 ATP + 179 H_2O → (Preproglucagon) + 179 AMP + 358 $H_2PO_4^-$			-1666.65
B	(Preproglucagon) + H_2O → (Proglucagon) + (Signal peptide)	-0.56	-28.14	8.17
C	(Proglucagon) + H_2O → (Glicentin-KR) + MPGF	-0.56	-28.14	8.17
D	(Glicentin-KR) + H_2O → (Oxyntomodulin-KR) + GRPP-KR	-0.56	-28.14	8.17
E	(Oxyntomodulin-KR) + H_2O → Oxyntomodulin + KR	-0.56	-28.14	8.17
F	(Oxyntomodulin) + H_2O → (Glucagon-KR) + (Hexapeptide)	-0.56	-28.14	8.17
G	(Glucagon-KR) + H_2O → (Glucagon) + KR	-0.56	-28.14	8.17

In summary, the translation reaction A that produces preproglucagon from amino acids at the ribosomes has a highly negative Gibbs energy change, due to hydrolysis of ATP during activation of amino acids, which means that it has a large driving force and is highly favorable. The post-translational processing reactions B-G have slightly positive Gibbs energy changes and are not feasible unless coupled with a reaction with a negative Gibbs energy change. The initial reactant of the post-translational processing reactions is preproglucagon, which is continuously produced by the highly favorable translation reaction. Moreover, the final product of the post-translational processing reactions is glucagon, which is continuously removed from the cell (system) by excretion into the blood. The continuous input of reactant (preproglucagon) and removal of product (glucagon) makes the post-translational processing reactions proceed towards formation of more product, due to the Le Chatelier's principle.

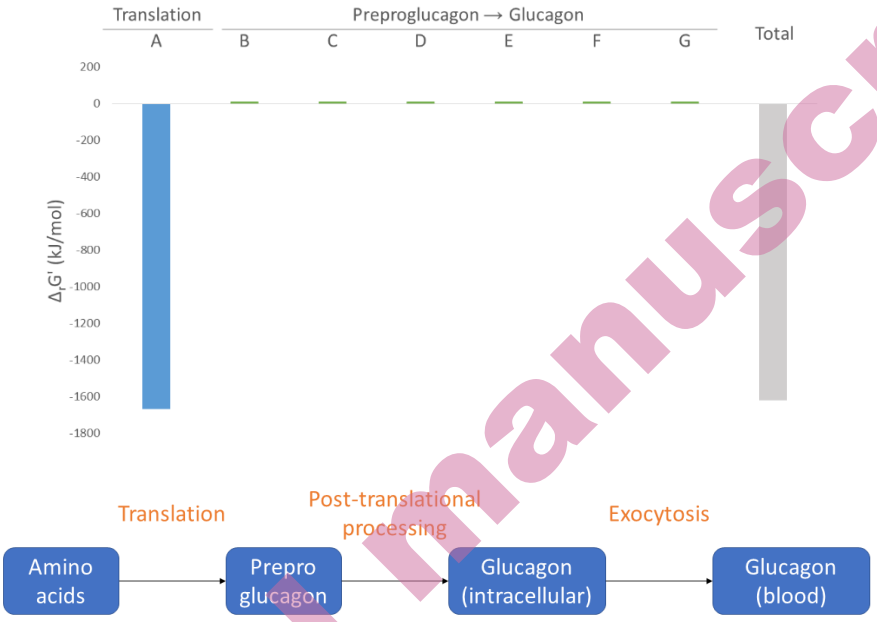

Figure 4: Driving force of glucagon synthesis. This figure shows standard reaction Gibbs energy at physiological temperature, $\Delta_r G'$, of the reactions in the metabolic pathway for synthesis of glucagon. Reaction A (blue column) is translation at the ribosomes, where preproglucagon is synthetized from amino acids activated by ATP. Reactions B-G (green columns) represent post-translational processing of preproglucagon to produce glucagon. The last (grey) column gives the total Gibbs energy change for the entire metabolic pathway.

In this research, the methodology of biothermodynamics was used to calculate changes in Gibbs energy during the reactions of the metabolic pathway for production of glucagon, based on protein sequences. Gibbs energy change represents the driving force of chemical reactions and allows to determine their feasibility. Based on the determined Gibbs energies, the feasibility of the metabolic pathway for production of glucagon was analyzed. This approach could be useful in omics research, where a lot of data on biomolecules is collected and based on it many potential metabolic pathways can be proposed. The biothermodynamic methodology described in this research allows to determine which potential metabolic pathways are feasible and can occur in the cell. This makes the biothermodynamic approach presented in this paper useful for omics research.

CONCLUSIONS

Chemical and thermodynamic characterization was performed of glucagon, proglucagon, preproglucagon and related peptides. The determined properties include molecular formulas, empirical formulas, molar masses, standard enthalpies of formation, $\Delta_f H^o$, standard molar entropies, $S_m{}^o$, standard Gibbs energies of

formation, $\Delta_f G^o$, standard molar heat capacities at constant pressure, $C^o_{p,m}$, standard entropies of formation, $\Delta_f S^o$, and standard enthalpies of combustion, $\Delta_C H^o$.

Biothermodynamic analysis was made of the translation process where amino acids activated with ATP polymerize at the ribosomes to produce a polypeptide (preproglucagon). The translation process is made feasible by ATP hydrolysis during activation of amino acids. The translation process has a positive enthalpy and positive entropy change, which means that the process is entropy driven.

The metabolic pathway for the production of glucagon was analyzed with the methodology of biothermodynamics. The driving forces (Gibbs energy changes) of the reactions in the metabolic pathway were determined. The reaction of translation has a highly negative Gibbs energy and is favorable. The reactions of post-translational processing have slightly positive Gibbs energy changes and are not feasible unless they are coupled with a process with a negative Gibbs energy. The initial reactant of the post-translational processing reactions is preproglucagon, while their final product is glucagon. Preproglucagon is continuously produced by translation at the ribosomes, which has a large driving force - highly negative Gibbs energy. Moreover, glucagon is continuously removed by excretion into the blood. The continuous introduction of reactant and removal of product allows the post-translational processing reactions to proceed in accordance with the Le Chatelier's principle. Therefore, the Le Chatelier's principle makes the entire metabolic pathway feasible, because it provides coupling between the highly favorable translation process and slightly unfavorable post-translational processing reactions.

The biothermodynamic methodology used in this study can be applied in omics research for metabolic pathway analysis. Based on protein sequences, driving forces of metabolic pathways were calculated. The driving forces were used to find the feasibility of the metabolic pathway. Therefore, the methodology presented in this paper can be used in omics research to find feasibility of metabolic pathways.

NOMENCLATURE

GRPP glicentin-related pancreatic peptide, GRPP-KR glicentin-related pancreatic peptide with a Lys-Arg extension at the C-terminus, Glicentin-KR glicentin with a Lys-Arg extension at the C-terminus, Oxyntomodulin-KR oxyntomodulin with a Lys-Arg extension at the C-terminus, Glucagon-KR glucagon with a Lys-Arg extension at the C-terminus, MPGF major proglucagon fragment, GLP-1 glucagon-like peptide 1, t-GLP-1 truncated glucagon-like peptide 1, GLP-2 glucagon-like peptide 2, KR Lys-Arg dipeptide.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13558, or from the corresponding author on request.

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И З В О Д

ЛЕ ШАТЕЉЕОВ ПРИНЦИП И МЕТАБОЛИЗАМ: БИОТЕРМОДИНАМИЧКА АНАЛИЗА МЕТАБОЛИЧКОГ ПУТА ЗА СИНТЕЗУ ГЛУКАГОНА

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Глукагон је основни катаболички хормон у људском организму. Глукагон је добро проучен са аспекта биолошких и медицинских наука. Међутим, у литератури се не може наћи анализа глукагона заснована на хемијској термодинамици. Приступ биотермодинамике би омогућио да се одреди *driving force* производње глукагона, као и разумевање процеса са аспекта фундаменталних закона природе. У овом истраживању извршена је анализа глукагона помоћу методологије биотермодинамике. На основу протеинских секвенци, извршена је хемијска и термодинамичка карактеризација глукагона, проглукагона, препроглукагона и повезаних пептида, помоћу *atom counting* метода и *Patel-Erickson-Battley* модела. Формулисане су реакције транслације на рибозомима као и посттранслационе обраде и израчуната је њихова *driving force* (промена Гибсове енергије). Процес транслације на рибозомима који производи препроглукагон изучен је са аспекта хемијске термодинамике. На основу Гибсове енергије, извршена је анализа метаболичког пута за производњу глукагона. Изучена је улога Ле Шатељеовог принципа у спрезању реакција у метаболичком путу. На крају, разматрана је примена биотермодинамичке методологије у омици за одређивање изводљивости метаболичких путева.

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