Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 91 (3) S107–S113 (2026) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

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

MARKO E. POPOVIĆ[1]*, DONG PEI[2] and MARIJA MIHAILOVIĆ[1]

[1]*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia and* [2]*Research Center for Natural Medicine and Chemical Metrology, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China*

J. Serb. Chem. Soc. 91 (3) (2026) 227–244

Table S-I presents the amino acid sequences of glucagon, proglucagon, preproglucagon and related peptides. The sequences were taken from [UniProt, 2025; PubChem, 2025; NCBI, 2025; Bataille, 1996; Thomsen et al., 1972]. The amino acid sequence of preproglucagon can be found under the UniProt access number P01275 (section *Sequence*) [UniProt, 2025] and NCBI access number NP_002045.1 [NCBI, 2025]. The amino acid sequence of preproglucagon is: MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFER HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRGRRDFPEEVAIVEELGRRH ADGSFSDEMNTILDNLAARDFINWLIQTKITDRK [UniProt, 2025; NCBI, 2025]. The amino acid sequence of glucagon can be found under the UniProt access number P01275 (section *PTM/Processing*) [UniProt, 2025], PubChem CID 16132283 [PubChem, 2025] and in references [Bataille, 1996; Thomsen et al., 1972]. The amino acid sequence of glucagon is HSQGTFTSDYSKYLDSRRAQDFVQWLMNT [UniProt, 2025; PubChem, 2025; Bataille, 1996; Thomsen et al., 1972]. The amino acid sequences of proglucagon and related peptides can be found under the UniProt access number P01275 (section *PTM/Processing*) [UniProt, 2025] and in reference [Bataille, 1996].

Table S-II shows the empirical formulas of glucagon, proglucagon, preproglucagon and related peptides. Empirical formulas express the numbers of atoms present in a molecule per carbon atom. The empirical formulas have the general form $CH_{nH}O_{nO}N_{nN}S_{nS}$, where n_H, n_O, n_N and n_S represent the numbers of hydrogen, oxygen, nitrogen and sulfur atoms in the empirical formula,

* Corresponding author. E-mail: marko.popovic@ihtm.bg.ac.rs

respectively. They were determined with the atom counting method, based on protein sequences, as described in [Popovic et al., 2024a; Popovic, 2022].

Table S-II gives thermodynamic properties of empirical formulas of glucagon, proglucagon, preproglucagon and related peptides. The determined thermodynamic properties of entire molecules include 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, $\Delta_f S^o$, and standard enthalpy of combustion, $\Delta_C H^o$. Thermodynamic properties of empirical formulas are expressed per mole of carbon (C-mol). They were determined with the Patel-Erickson-Battley and Hurst-Harrison models, based on empirical formulas, as described in [Popovic et al., 2024b; Ozilgen and Sorguven Oner, 2016].

Table S-IV shows the stoichiometry and changes in thermodynamic properties of the chemical reactions in the metabolic pathway for synthesis of glucagon at room temperature (25°C). The determined 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 determined based on the molecular formulas and thermodynamic properties of the analyzed molecules, with the methodology of thermochemistry, as described in [Popovic et al., 2024a; Atkins and de Paula, 2011].

Table S-I: Amino acid sequences of glucagon, proglucagon, preproglucagon and related peptides. All peptides in this table are derived by post-translational processing of preproglucagon. For every sequence, the start and end amino acid within preproglucagon is given, as well as its length. Abbreviations: 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. Data taken from taken from [UniProt, 2025; PubChem, 2025; NCBI, 2025; Bataille, 1996; Thomsen et al., 1972].

Name	Start	End	Sequence	Length
Preproglucagon	1	180	MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADP LSDPDQMNEDKRHSQGTFTSDYSKYLDSRRAQDFVQWLM NTKRNRNNIAKRHDEFERHAEGTFTSDVSSYLEGQAAKEFI AWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILD NLAARDFINWLIQTKITDRK	180
Proglucagon	21	180	RSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSD YSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAE GTFTSDVSSYLEGQAAKEFIAWLVKGRGRRDFPEEVAIVEE LGRRHADGSFSDEMNTILDNLAARDFINWLIQTKITDRK	160
GRPP	21	50	RSLQDTEEKSRSFSASQADPLSDPDQMNED	30
GRPP-KR	21	52	RSLQDTEEKSRSFSASQADPLSDPDQMNEDKR	32
Glicentin-KR	21	91	RSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSD YSKYLDSRRAQDFVQWLMNTKRNRNNIAKR	71
Glicentin	21	89	RSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSD YSKYLDSRRAQDFVQWLMNTKRNRNNIA	69
Oxyntomodulin-KR	53	91	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKR	39
Oxyntomodulin	53	89	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA	37
Glucagon-KR	53	83	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKR	31
Glucagon	53	81	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT	29
Mini-glucagon	71	81	AQDFVQWLMNT	11
Hexapeptide	84	89	NRNNIA	6
GLI9000	21	81	RSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSD YSKYLDSRRAQDFVQWLMNT	61
MPGF	92	180	HDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGRGRRD FPEEVAIVEELGRRHADGSFSDEMNTILDNLAARDFINWLI QTKITDRK	89
GLP-1	92	128	HDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG	37
t-GLP-1	98	128	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG	31
GLP-2	145	178	RHADGSFSDEMNTILDNLAARDFINWLIQTKITD	34
Signal peptide	1	20	MKSIYFVAGLFVMLVQGSWQ	20
KR			KR	2

Table S-II: Empirical formulas of glucagon, proglucagon, preproglucagon and related peptides. The empirical formulas have the general form $CH_{nH}O_{nO}N_{nN}S_{nS}$, where n_H, n_O, n_N and n_S represent the numbers of hydrogen, oxygen, nitrogen and sulfur atoms in the empirical formula, respectively. The table also gives the molar masses of empirical formulas, *Mr* in grams per mole of carbon (C-mole).

Name	n_H	n_O	n_N	n_S	Mr (g/C-mol)
Preproglucagon	1.5470	0.3140	0.2932	0.0055	22.88
Proglucagon	1.5547	0.3259	0.3035	0.0037	23.16
GRPP	1.5809	0.4265	0.3015	0.0074	24.89
GRPP-KR	1.6149	0.4054	0.3176	0.0068	24.79
Glicentin-KR	1.5795	0.3438	0.3239	0.0057	23.82
Glicentin	1.5647	0.3500	0.3176	0.0059	23.83
Oxyntomodulin-KR	1.5637	0.3039	0.3284	0.0049	23.21
Oxyntomodulin	1.5365	0.3125	0.3177	0.0052	23.18
Glucagon-KR	1.5091	0.3091	0.2970	0.0061	22.83
Glucagon	1.4706	0.3203	0.2810	0.0065	22.76
Mini-glucagon	1.4590	0.2951	0.2459	0.0164	22.17
Hexapeptide	1.7778	0.3704	0.4444	0.0000	25.95
GLI9000	1.5349	0.3588	0.2990	0.0066	23.70
MPGF	1.5398	0.3142	0.2876	0.0022	22.69
GLP-1	1.4785	0.3172	0.2742	0.0000	22.42
t-GLP-1	1.5099	0.3113	0.2649	0.0000	22.22
GLP-2	1.5556	0.3275	0.2807	0.0058	22.94
Signal peptide	1.5091	0.2364	0.2182	0.0182	20.95
KR	2.1667	0.2500	0.5000	0.0000	25.20

Table S-III: Thermodynamic properties of empirical formulas 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 empirical formulas and are given per mole of carbon (C-mol).

Name	$\Delta_f H^o$ (kJ/C-mol)	$S^o{}_m$ (J/C-mol K)	$\Delta_f G^o$ (kJ/C-mol)	$C^o{}_{p,m}$ (J/C-mol K)	$\Delta_f S^o$ (kJ/C-mol)	$\Delta_C H^o$ (kJ/C-mol)
Preproglucagon	-66.42	31.24	-25.92	32.36	-135.83	-550.35
Proglucagon	-69.77	31.74	-28.64	32.75	-137.98	-547.41
GRPP	-91.98	33.97	-47.95	34.31	-147.70	-530.37
GRPP-KR	-88.54	34.27	-44.12	34.57	-148.98	-538.43
Glicentin-KR	-74.01	32.76	-31.54	33.58	-142.43	-547.49
Glicentin	-74.87	32.59	-32.63	33.44	-141.68	-544.59
Oxyntomodulin-KR	-64.86	31.88	-23.54	33.01	-138.60	-554.07
Oxyntomodulin	-65.82	31.52	-24.96	32.72	-137.04	-549.33
Glucagon-KR	-63.96	30.75	-24.10	32.09	-133.71	-547.62
Glucagon	-65.09	30.22	-25.93	31.65	-131.37	-541.17
Mini-glucagon	-56.46	29.02	-18.84	30.69	-126.17	-552.06
Hexapeptide	-87.76	37.82	-38.74	37.63	-164.42	-559.82
GLI9000	-75.67	32.06	-34.11	32.99	-139.40	-539.82
MPGF	-67.11	31.04	-26.88	32.16	-134.94	-547.34
GLP-1	-66.44	30.09	-27.43	31.46	-130.83	-538.37
t-GLP-1	-66.11	30.20	-26.97	31.45	-131.28	-543.19
GLP-2	-69.59	31.38	-28.91	32.38	-136.44	-548.55
Signal peptide	-44.51	28.02	-8.19	29.78	-121.82	-571.87
KR	-73.37	41.26	-19.89	40.00	-179.37	-629.79

Table S-IV: Stoichiometry and thermodynamic properties of the reactions in the metabolic pathway for production of glucagon from amino acids at 25°C. 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, $\Delta_r G^o$, standard reaction heat capacity at constant pressure at 25°C, $\Delta_r C_p{}^o$.

Name	Reaction	$\Delta_r H^o$ (kJ/mol)	$\Delta_r S^o$ (J/mol K)	$\Delta_r G^o$ (kJ/mol)	$\Delta_r C_p{}^o$ (J/mol K)
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 $H_2PO_4^-$	4861.25	21047.55	-1414.08	
B	(Preproglucagon) + H_2O → (Proglucagon) + (Signal peptide)	0.00	-26.29	7.84	-46.75
C	(Proglucagon) + H_2O → (Glicentin-KR) + MPGF	0.00	-26.29	7.84	-46.75
D	(Glicentin-KR) + H_2O → (Oxyntomodulin-KR) + GRPP-KR	0.00	-26.29	7.84	-46.75
E	(Oxyntomodulin-KR) + H_2O → Oxyntomodulin + KR	0.00	-26.29	7.84	-46.75
F	(Oxyntomodulin) + H_2O → (Glucagon-KR) + (Hexapeptide)	0.00	-26.29	7.84	-46.75
G	(Glucagon-KR) + H_2O → (Glucagon) + KR	0.00	-26.29	7.84	-46.75

REFERENCES

1. Atkins, P. W., & de Paula, J. (2011). *Physical Chemistry for the Life Sciences (2nd edition)*, W. H. Freeman and Company. ISBN-13: 978-1429231145
2. Bataille, D. (1996). Preproglucagon and Its Processing. In: Lefèbvre, P.J. (eds) Glucagon III. Handbook of Experimental Pharmacology, vol 123. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-61150-6_3
3. NCBI (2025). Pro-glucagon preproprotein (Homo sapiens) [Online] National Center for Biotechnology Information. Available at: https://www.ncbi.nlm.nih.gov/protein/NP_002045.1?report=genbank&log$=protalign&blast_rank=6&RID=7J5YEGKW014 (Accessed on August 9, 2025)
4. Ozilgen, M., & Sorguven Oner, E. (2016). Biothermodynamics: Principles and Applications (1st ed.). CRC Press. https://doi.org/10.1201/9781315374147
5. Popovic, M., Tadić, V., & Mihailović, M. (2024a). From genotype to phenotype with biothermodynamics: empirical formulas, biosynthesis reactions and thermodynamic properties of preproinsulin, proinsulin and insulin molecules. *Journal of biomolecular structure & dynamics*, *42*(19), 10388–10400. https://doi.org/10.1080/07391102.2023.2256880
6. Popović, M. E., Stevanović, M., & Pantović Pavlović, M. (2024b). Biothermodynamics of Hemoglobin and Red Blood Cells: Analysis of Structure and Evolution of Hemoglobin and Red Blood Cells, Based on Molecular and Empirical Formulas, Biosynthesis Reactions, and Thermodynamic Properties of Formation and Biosynthesis. *Journal of molecular evolution*, *92*(6), 776–798. https://doi.org/10.1007/s00239-024-10205-9
7. Popovic M. (2022). Atom counting method for determining elemental composition of viruses and its applications in biothermodynamics and environmental science. *Computational biology and chemistry*, *96*, 107621. https://doi.org/10.1016/j.compbiolchem.2022.107621
8. PubChem (2025). Compound summary: Glucagon [Online] Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Glucagon (Accessed on August 9, 2025)
9. Thomsen, J., Kristiansen, K., Brunfeldt, K., & Sundby, F. (1972). The amino acid sequence of human glucagon. *FEBS letters*, *21*(3), 315–319. https://doi.org/10.1016/0014-5793(72)80192-3
10. UniProt (2025). P01275 GLUC_HUMAN [Online] Available at: https://www.uniprot.org/uniprotkb/P01275/entry (Accessed on August 9, 2025).