



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

MARKO E. POPOVIĆ^{1*}, DONG PEI² AND Marija Mihailović¹

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

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: MKSIYFVAGLFVMLVQGSWQSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSSRAQDFVQWLMNTKRNRRNNIAKRHDEFER 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 HSQGTFTSDYSKYLDSSRAQDFVQWLMNT [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 $\text{CH}_{n\text{H}}\text{O}_{n\text{O}}\text{N}_{n\text{N}}\text{S}_{n\text{S}}$, where $n\text{H}$, $n\text{O}$, $n\text{N}$ and $n\text{S}$ 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, $\Delta_f H^\circ$, standard molar entropy, S_m° , standard Gibbs energy of formation, $\Delta_f G^\circ$, standard molar heat capacity at constant pressure, $C_{p,m}^\circ$, standard entropy of formation, $\Delta_f S^\circ$, and standard enthalpy of combustion, $\Delta_c H^\circ$. 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, $\Delta_r H^\circ$, standard reaction entropy at 25°C, $\Delta_r S^\circ$, standard reaction Gibbs energy at 25°C, $\Delta_r G^\circ$, standard reaction heat capacity at constant pressure at 25°C, $\Delta_r C_p^\circ$. 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 [UniProt, 2025; PubChem, 2025; NCBI, 2025; Bataille, 1996; Thomsen et al., 1972].

Name	Start	End	Sequence	Length
Preproglucagon	1	180	MKSIYFVAGLFVMLVQGSWQ RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKRHDEFERHAE GTFTSDVSSYLEGQA AKEFI AWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILDNL AARDFINWLIQTKITDRK	180
Proglucagon	21	180	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKRHDEFERHAE GTFTSDVSSYLEGQA AKEFI AWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILDNL AARDFINWLIQTKITDRK	160
GRPP	21	50	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKR	30
GRPP-KR	21	52	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKR	32
Glicentin-KR	21	91	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKR	71
Glicentin	21	89	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIA	69
Oxyntomodulin-KR	53	91	HSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIAKR	39
Oxyntomodulin	53	89	HSQGTFTSDYSKYLDSRR AQDFVQWLMNTKRNRRNNIA	37
Glucagon-KR	53	83	HSQGTFTSDYSKYLDSRR AQDFVQWLMNTKR	31
Glucagon	53	81	HSQGTFTSDYSKYLDSRR AQDFVQWLMNT	29
Mini-glucagon	71	81	AQDFVQWLMNT	11
Hexapeptide	84	89	NRNNIA	6
GLI9000	21	81	RSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQGTFTSDYSKYLDSRR AQDFVQWLMNT	61
MPGF	92	180	HDEFERHAE GTFTSDVSSYLEGQA AKEFI AWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILDNL AARDFINWLIQTKITDRK	89
GLP-1	92	128	HDEFERHAE GTFTSDVSSYLEGQA AKEFI AWLVKGRG	37
t-GLP-1	98	128	HAEGTFTSDVSSYLEGQA AKEFI AWLVKGRG	31
GLP-2	145	178	RHADGSFSDEMNTILDNL AARDFINWLIQTKITD	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 $\text{CH}_{n_H}\text{O}_{n_O}\text{N}_{n_N}\text{S}_{n_S}$, 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, M_r in grams per mole of carbon (C-mole).

Name	n_H	n_O	n_N	n_S	M_r (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, $\Delta_f H^\circ$, standard molar entropy, S_m° , standard Gibbs energy of formation, $\Delta_f G^\circ$, standard molar heat capacity at constant pressure, $C_{p,m}^\circ$, standard entropy of formation, $\Delta_f S^\circ$, standard enthalpy of combustion, $\Delta_c H^\circ$. The properties in this table are for the empirical formulas and are given per mole of carbon (C-mol).

Name	$\Delta_f H^\circ$ (kJ/C-mol)	S_m° (J/C-mol K)	$\Delta_f G^\circ$ (kJ/C-mol)	$C_{p,m}^\circ$ (J/C-mol K)	$\Delta_f S^\circ$ (kJ/C-mol)	$\Delta_c H^\circ$ (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, $\Delta_r H^\circ$, standard reaction entropy at 25°C, $\Delta_r S^\circ$, standard reaction Gibbs energy at 25°C, $\Delta_r G^\circ$, standard reaction heat capacity at constant pressure at 25°C, $\Delta_r C_p^\circ$.

Na me	Reaction	$\Delta_r H^\circ$ (kJ/mol)	$\Delta_r S^\circ$ (J/mol K)	$\Delta_r G^\circ$ (kJ/mol)	$\Delta_r C_p^\circ$ (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 ₂ O → (Preproglucagon) + 179 AMP + 358 H ₂ PO ₄ ⁻	4861.25	21047.55	-1414.08	
B	(Preproglucagon) + H ₂ O → (Proglucagon) + (Signal peptide)	0.00	-26.29	7.84	-46.75
C	(Proglucagon) + H ₂ O → (Glicentin-KR) + MPGF	0.00	-26.29	7.84	-46.75
D	(Glicentin-KR) + H ₂ O → (Oxyntomodulin-KR) + GRPP-KR	0.00	-26.29	7.84	-46.75
E	(Oxyntomodulin-KR) + H ₂ O → Oxyntomodulin + KR	0.00	-26.29	7.84	-46.75
F	(Oxyntomodulin) + H ₂ O → (Glucagon-KR) + (Hexapeptide)	0.00	-26.29	7.84	-46.75
G	(Glucagon-KR) + H ₂ O → (Glucagon) + KR	0.00	-26.29	7.84	-46.75

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