THEORETICAL CONFORMATIONAL ANALYSIS OF GLUCAGON MOLECULE

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The conformational properties of the peptide hormone glucagon were investigated by the theoretical conformational analysis method. Two families of lowest energy conformations were found corresponding to: a) conformations having α - helix within the C - terminal residues 6-26 and b) conformations including two α -helices (residues 6-11 and 16-26) connected by a β -turn. As previously investigated for related peptide hormone secretin, a similar mechanism of the packing of the structural elements was found for glucagon by these energy calculations.

INTRODUCTION

Glucagon is polypeptide with 29 residues. The effect of glucagon is to release glucose from liver cells to boost blood glucose concentrations. Its action is antagonistic to that of another pancreatic hormone, insulin, which is responsible for lowering unduly high circulating glucose levels. Glucagon plays a central role in glucose homoeostasis and an understanding of this complicated process at the molecular level is not possible without a detailed knowledge of the glucagon conformation. Its conformation has been extensively investigated experimentally and theoretically. In previous studies [1], trimeric glucagon in crystals has been shown to have a high content of α-helix. In dilute aqueous solution, glucagon exist as a monomer. Glucagon is flexible in dilute aqueous solution, but forms a well-defined largely helical conformer in a hydrophobic environment such as organic solvents, lipid bilayers and probably the receptor. α-Helical structure has also been suggested for glucagon in chlorethanol and in the presence of detergents or micelles [2]. Circular dichroism and optical rotatory dispersion indicate that glucagon conformers of 35 % α-helical content are induced in concentrated solutions [3]. The sequence-predictive method of Chou and Fasman does support the presence both α-helix and β-sheet formation for glucagon, and suggest that the conformation is delicately balanced between these two conformations [4].

Pancreatic glucagon is a member of a large family of sequentially related polypeptidea, which also includes secretin. Glucagon exhibits 52 % amino acid sequence homology to secretin. The conformations of these hormones have been investigated by nuclear magnetic resonance spectroscopy under conditions of reduced water activity [5,6]. Both these hormones were found to have a similar secondary structure. In the case of these molecules the application of results of NMR studies is much more difficult because precise information on each dihedral angle is not available.

In earlier study we have investigated the conformational properties of secretin by the theoretical conformational analysis method [7,8]. The spatial structure of secretin can be described by two families of low-energy conformations, possessing relatively conformational valid (residues 7-22) and variable (residues 1-6 and 23-27) fragments. One of these families is comprized by five twists of the α-helix, while the second isoenergetic family possesses two short segments of the α-helix, divided by a β-turn.

Calculations produced the values of all dihedral angles of the backbones and side chains of these structures as well as intra- and inter-residue interaction energies. Therefore, in this paper our purpose is to search for an energetically probable conformation of the glucagon, satisfying experimental data relating to physiological conditions.

I.GLUCAGON H-HIS1-SER2-GLN3-GLY4-THR5-
II.SECRETIN H-HIS1-SER2-ASP3-GLY4-THR5-

I.- PHE6-THR7-SER8-ASP9-TYR10-SER11-LYS12-
II.-PHE6-THR7-SER8-GLU9-LEU10-SER11-ARG12-

I.- TYR13-LEU14-ASP15-SER16-ARG17-ARRG18-
II.-LEU13-ARG14-ASP15-SER16-ALA17-ARG18-

I. -ALA19-GLN20-ASP21-PHE22-VAL23-GLN24-
II.-LEU19-GLN20-ARG21-LEU22-LEU23-LEU24-

I. -TRP25-LEU26-MET27-ASN28-THR29-NH_2
II.-GLY25-LEU26-VAL27-NH_2

Fig. Comparison of the amino acid sequence of glucagon with that of secretin.

CALCULATIONS

This investigation were carried out using theoretical conformational analysis method as described in Refs.[7-9]. Computations were carried out on the IBM586 using programs written by Godjayev et al [9] in FORTRAN. This program calculates the conformational energy of a peptide as a sum of nonbonded (E_{NB}), hydrogen-bonded (E_{HB}) and electrostatic energies (E_{EL}) for pairwise atomic interactions and torsional potential energies (E_{TOR}) for rotation about bonds. Bond lengths and bond angles are fixed at standard values [10], and only dihedral angles are allowed to vary.

For a stable conformation, the φ,ψ dihedral angles of backbone chain are located in a low energy regions:

$R(\varphi,\psi = -180°\text{-}0°)$, $B(\varphi = -180°\text{-}0°,\ \psi = 0°\text{-}180°)$,
$L(\varphi,\psi = 0°\text{-}180°)$ and $P(\varphi = 0°\text{-}180°,\ \psi = -180°\text{-}0°)$.

The conformational state of each amino acid residue is conveniently described by backbone φ,ψ and side chain $\chi_1\chi_2\chi_3$... dihedral angles.

RESULTS AND DISCUSSION

Study of the data on receptor binding and activity of glucagon suggest that almost the entire molecule is required for full biological potency. It is therefore of interest to examine the conformational behavior of the complete molecule of glucagon. The determination of the stable conformations of

glucagon molecule, represented here, is based on the detailed analysis of secondary structure elements of the separate its fragments. This analysis is need for detailed studies of the geometrical and energetical features of the packing of these structural elements.

Calculations of the glucagon molecule can be divided into three stages. The three glucagon large regions: residues 1-12, 9-18 and 18-29 were investigated separately by minimizing the energies of all possible combinations of low-energy structures of the component fragments and then reducing the total number of states at each stage by cutting off the higher-energy ones. By using combinations of appropriate single-residue conformations it was possible to search the low-energy structures of the di-, tri- and tetrapeptide segments adequately. In each situations, the large fragments are built up from smaller ones: di-, tri- and tetrapeptides.

The lowest structures of the dodecapeptide His1-Lys12 are produced by various combinations of almost all favorable states of the two partially overlapping fragments His1-Thr7 and Phe6-Lys12. A comparatively large number of low-energy conformations of His1-Lys12 make be attributed to the fact that the five residues of the N-terminal region forms relatively flexible structure, but the second part Phe6-Lys12 exhibits an α-helical segment. This is a conformational rigid nucleation, which is observed in all low-energy conformations of His1-Lys12 with no changes in its structure.

Conformational properties of the middle fragment Asp9-Arg18 have been investigated basing on the low-energy structures of the three components tetrapeptide fragments Asp9-Lys12, Lys12-Asp15 and Asp15-Arg18. This fragment of glucagon has a few polar amino acid residues, five of which are charged (Fig.). A main goal of this research is to find the position of the possible reverse turn structure. This calculation was used also to test the important role of the various ionic pairs formation.

Calculation indicated only two most favorable conformations for this central decapeptide of glucagon. First of them structure $R_2R_3R_3R_{1222}\ R_{12}\ R_{3222}\ R_2\ R_1\ R_{2222}\ R_{1222}$ (E_{REL} = 0 kcal/mol) forms α-helical conformation, but the second low-energy structure $R_2\ R_3\ R_3\ B_{1222}\ R_{21}\ B_{2122}\ B_2\ R_1\ R_{2222}\ R_{1222}$ (E_{REL}=2.5 kcal/mol) includes two α-helical coils, connected by a β-turn. At this conformation the distance between the C^{α} atoms of Lys12 and Asp15 is found to be approximately 6.8 Å, which indicates the presence of a β-turn. A β-turn leads to the hydrogen bonds between the ε-amino group of Lys12 and the carboxyl group of Asp15 and between the carboxyl group of Asp9 and the guanidine function of Arg18. It is shown that the interactions between negatively charged aspartyl residues and positively charged residues stabilize α-helical segments also.

The next stage in the analyses of glucagon comprises calculation of stable conformations of the C-terminal dodecapeptide Arg18-Thr29 NH_2. Combining all low-energy conformations of the separate overlapping fragments were calculated sequentially increasing larger C-terminal segments: Phe22-Met27, Gln20-Met27, Arg18-Met27 and, at last, Arg18-Thr29NH_2 (Fig.). The results showed that among

Table. The geometrical (in degree) and energetical parameters of lowest energy conformations of glucagon. (χ^3,χ^4,χ^5=180^0)

Aminoacid	First family structure					Second family structure				
N	φ	ψ	ω	χ^1	χ^2	φ	ψ	ω	χ^1	χ^2
HIS1	62	-63	177	172	-94	62	-63	177	172	-94
SER2	-89	-62	173	55	175	-89	-62	173	55	175
GLN3	-61	-33	183	76	-69	-61	-33	183	76	-69
GLY4	-87	71	176	-	-	83	-71	176	-	-
THR5	-132	162	173	60	179	-132	162	173	60	179
PHE6	-74	-29	173	68	82	-74	-29	173	68	83
THR7	-60	-41	-172	56	182	-60	-45	-175	56	182
SER8	-57	-36	180	178	181	-56	-44	179	178	180
ASP9	-49	-43	184	180	89	-53	-47	183	180	89
TYR10	-86	-34	-175	61	85	-75	-25	180	-60	-90
SER11	-68	-33	-180	-60	180	-69	-47	178	-60	180
LYS12	-75	-42	184	75	170	-81	157	-172	72	185
TYR13	-77	-27	-171	87	83	-97	-70	184	178	60
LEU14	-69	-39	-177	-65	170	-120	112	174	180	60
ASP15	-69	-36	180	180	87	-115	154	-173	182	88
SER16	-79	-32	178	64	180	-97	-48	-173	61	180
ARG17	-67	-44	182	180	179	-57	-47	-171	180	180
ARG18	-76	-36	-173	73	179	-69	-37	178	70	183
ALA19	-70	-30	172	180	-	-67	-40	-174	180	-
GLN20	-63	-53	-172	181	62	-51	-53	-172	180	62
ASP21	-78	-38	-172	61	90	-78	-38	188	61	90
PHE22	-57	-44	185	180	91	-57	-44	185	180	91
VAL23	-83	-34	180	-51	63	-83	-34	180	-51	63
GLN24	-68	-62	-163	176	63	-68	-62	-163	176	63
TRP25	-97	-48	-165	-60	105	-97	-48	-165	-60	105
LEU26	-80	-47	-175	-60	180	-80	-47	-175	-60	105
MET27	-92	154	-170	182	165	-92	154	-170	182	165
ASN28	-68	-51	177	181	91	-68	-51	177	181	91
THR29	-72	-48	178	57	182	-72	-48	178	57	182
Energy (kcal/mol)	E_{NB}	E_{EL}	E_{TOR}	E_{REL}		E_{NB}	E_{EL}	E_{TOR}	E_{REL}	
	-191.3	16.9	28.7	1.5		-188.9	15.3	26.1	0.0	

plausible spatial forms of C-terminal dodecapeptide a α-helix is energetically the most stable structure. The global conformation of this fragment R_{1222} R_2 R_{212} R_1 R_2 R_{322} R_{211} R_{32} R_{3222} R_{2222} R_2R_1 (E_{REL}= 0 kcal/mol) contains of α-helical structure, stabilized by networks of regular hydrogen bonds. All of other computed low-energy conformations have partially helical structures with relatively variable C-terminal tripeptide. This calculation strongly supports a model in which the C-terminal region of the glucagon is helical. The empirical method of Chou and Fasman has also been used to predict several structures for glucagon, one of which has a helical segment at aminoacid residues 19-27.

At the final stage of the investigation, all combinations of possible conformations of the separately overlapping fragments His1-Lys12, Asp9-Arg18 and Arg18-Thr29NH_2 had to be considered and then used as starting conformations for computing the structure of the whole molecule. A number of conformational states with the same backbone form and different orientations of side chains were also calculated for a particular shape whenever reasonable.

The results of conformational calculations of glucagon showed two families of lowest energy conformations with the variable N-terminal (residues 1-5) and C-terminal (residues 27-29) regions. One of these families is comprised a great α-helical segment at the 6-26 residues, but the second isoenergetic family contains two short α-helix segments (residues 6-11 and 16-26) connected by a β-turn at the Lys12-Asp15 level. The second family has an energy more than 1.5 kcal/mol lower than that of the first family. The lowest energy conformation of the second family is stabilized by two hydrogen bonds between oppositely charged side chains of the Lys12 and Asp15 and Asp9 and Arg18. The energies of these medium- and long-rang interactions between Lys12 and Asp15 and between Asp9 and Arg18 are equal -11.9 and -11.3 kcal/mol respectively. The conformations of the first family with a high content of α-helix are stabilized by networks of regular backbone hydrogen bonds and hydrogen bonds involving the charged group of side chains. Numerical values of dihedral angles of rotation about the backbone (φ,ψ) and side (χ_1,χ_2) bonds in the two lowest energy conformations of glucagon are listed in Table. There are represented its energetical parameters in this Table too. The resulting conformations of glucagon were compared with the known experimental results. It was shown that conformations of the first family are consistent with the X-ray crystal structure [1], but the conformations of the second families are similar to the proposed structure in NMR investigations [6]. These studies lead to the conclusion that glucagon can form one of these calculating conformations depending on the conditions.

The data in this paper show that glucagon molecule have a similar conformational properties with secretin. As previously observed for secretin [7,8], a similar mechanism of the packing of the structural elements was found for glucagon by these energy calculations. This is not too surprising for families of peptides with similar or identical functions and very extensive amino acid homologies, such as the glucagon and secretin. A more detailed analysis of these results and further structure-function relationships investigation of glucagon will be presented elsewhere.

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QLÜKAQON MOLEKULUNUN NƏZƏRİ KONFORMASİYA ANALİZİ

Peptid hormonu qlükaqonun konformasiya xüsusiyyətləri nəzəri konformasiya analizi üsulu ilə tədqiq olunmuşdur. Qlükaqon molekulu üçün iki tipli aşağı enerjili konformasiyalar müəyyən olunmuşdur: a) C-tərəf 6-26 fraqmentində α-spiral quruluşu əmələ gətirən konformasiyalar və b) β-dönmə ilə birləşən iki α-spiral quruluşları (6-11 və 16-26 fraqmentləri) əmələ gətirən konformasiyalar. Enerji hesablamaları nəticəsində qlükaqon molekulu üçün ona oxşar peptid hormonu sekretin üçün tədqiq olunmuş quruluş mexanizmi müəyyən olunmuşdur.

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ТЕОРЕТИЧЕСКИЙ КОНФОРМАЦИОННЫЙ АНАЛИЗ МОЛЕКУЛЫ ГЛЮКАГОНА

Методом теоретического конформационного анализа исследованы конформационные возможности молекулы глюкагона. Были определены два семейства низкоэнергетических конформаций глюкагона: а) конформации, формирующие α-спиральную структуру на C-концевом 6-26 фрагменте молекулы и б) конформации, содержащие два α-спиральных участка (6-11 и 16-26), соединенных β-изгибом цепи. На основании энергетических расчетов для глюкагона был выявлен похожий механизм укладки структурных элементов как ранее найденный для родственной ему молекулы секретина.

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