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Insulin-Containing Amino Acids and Oligopeptides/β-Cyclodextrin Supramolecular Systems: Molecular Modeling and Docking Experiments

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Abstract: The paper presents the molecular modeling of insulin-containing amino acids and A and B chains and furthermore the docking experiments of these amino acids and oligopeptide moieties in β-cyclodextrin. Best results were obtained for insulin-containing amino acids tyrosine, phenylalanine, and leucine, as well as for the corresponding residues from A and B chains of human insulin, when the maximum number of cyclodextrin molecules was six (for every insulin-containing chain).

Keywords: insulin, amino acids, supramolecular systems, β-cyclodextrin, molecular modeling, docking

1. Introduction

Insulin is a hormone produced in the pancreas which has implications in the decreasing of the sugar level in the blood [1]. It has vital effects on the metabolic energy, cell permeability, and cellular homeostasis. Insulin is the most important physiological factor which controlling the glucose cell concentration (together with the corresponding antagonist, glucagon) [1].

Insulin is stored in the pancreatic β cells as hexameric complex with zinc and consists of 51 amino acids distributed in two chains (A with 21 amino acids and B with 30 amino acids) linked by two disulfide bonds and an extra disulfide bond in chain A (Figure 1) [1-5]; it is biosynthetized from the proinsulin (a polipeptide which contains 84 amino acids). The primary structures of bovine, porcine, and human insulin are very similar [1,3].

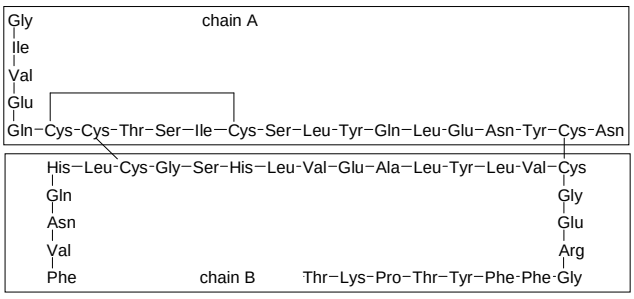


Figure 1. Human insulin structure

Human, bovine, and porcine insulin (or even derivatives) are widely used in the treatment of diabet; these compounds are prepared by enzymatic or genetic engineering methods [1-5].

Some insulin derivatives or insulin-contaning pharmaceutical formulations are developed in order to obtain the controlled release property.

Cyclodextrins (cyclic oligosaccharides formed by 6-8 glucopyranose units for α-, β-, and γ-cyclodextrin) are widely used for molecular encapsulation of bioactive compounds due to the presence of a hydrophobic inner cavity which can accommodate a hydrophobic biomolecule or a hydrophobic rest from a biomolecule [6,7]. The main essential amino acids were studied in order to evaluate the possibility to encapsulate in cyclodextrins, and the influence of the hydrophobicity of amino acid residue on the molecular inclusion process was evaluated [8].

In this paper the possibility of molecular encapsulation of the insulin-containing oligopeptide A and B chains by using the molecular modeling and docking experiments was studied [9-11].

2. Experimental

Molecular modeling of insulin chains, the corresponding insulin-containing amino acids, and β-cyclodextrin. Human insulin chains (builded by using the Amino Acids subprogram from HyperChem package), the main amino acids from the human insulin structure, and β-cyclodextrin were modeled by using the MM+ Molecular Mechanics approach from HyperChem 7.5 molecular modeling package [12], with a 0.01 kcal/mole RMS gradient and the Polak-Ribiere algorithm.

In order to obtain the most stable conformations of amino acids (with minimum internal energy), conformational studies for all structures were conducted by using the Conformational Search program from

HyperChem package. The following steps were run over: (1) for the biocompound structure in a random conformation, but with defined bond length and angles, all flexible bonds and rings were set up and used in the conformational analysis; (2) a random values of these torsion angles were used for every starting conformation; (3) the minimizing of conformation energy was conducted until the RMS gradient was lower than 0.01 kcal/mole; (4) all conformations with energy of maximum 4 kcal/mole above the minimal energy obtained (the most stable conformation) were retained for the docking studies. The conformational search parameters were: variation of the flexible torsion angles of $\pm 60^{o} \div \pm 180^{o}$, acceptance energy criterion 4 kcal/mol above best, duplicate structure if the energy was within the range of 0.05 kcal/mole, skip of the structures which have atoms closer than 0.5 Å and torsion within 15º; the optimization program was MM+, with the Polak-Ribiere algorithm, and RMS gradient of 0.01 kcal/mole; the hydrogen atoms were ignored. The maximum number of iterations and optimizations were set up to 500, and no more than 20 conformations were retained.

β-Cyclodextrin structure, used for the molecular encapsulation of insulin moieties and insulin-containing amino acids, were molecular modeled by using the MM+ molecular mechanics program from HyperChem package. The starting conformations for cyclodextrin were builded by knowing the X-ray structures in crystals [6,7].

3. Results and Discussion

Human insulin contains 51 amino acids distributed in two main chains connected by two *S-S* bonds. The amino acid distribution is: six of L-Cys and L-Leu, four of Gly, L-Val, L-Glu, L-Tyr, three of L-Asn, L-Gln, L-Ser, L-Thr, L-Phe, two of L-Ile and L-His, and one of L-Ala, L-Lys, L-Arg, and L-Pro. The main insulin chains have α-helix configurations and the *R*-amino acid residues are oriented to the exterior (Figs. 2 and 3). Thus, the possibility of molecular encapsulation of *R* residue in β-cyclodextrin exists, especially for hydrophobic moieties, like isobutyl, benzyl, and 4-hydroxybenzyl contained by L-Leu (2 in A chain and 4 in B chain), L-Phe (all in B chain), and L-Tyr (2 in A chain and 2 in B chain), respectively. L-Cys is improbable to form complexes with β-cyclodextrin due to the bonded form of four of these amino acids, but other insulin-containing amino acids can form complexes with cyclodextrin like L-Val, L-Ile, L-Asn, L-Gln, L-Glu, L-Lys, or L-Arg amino acid-containing residues.

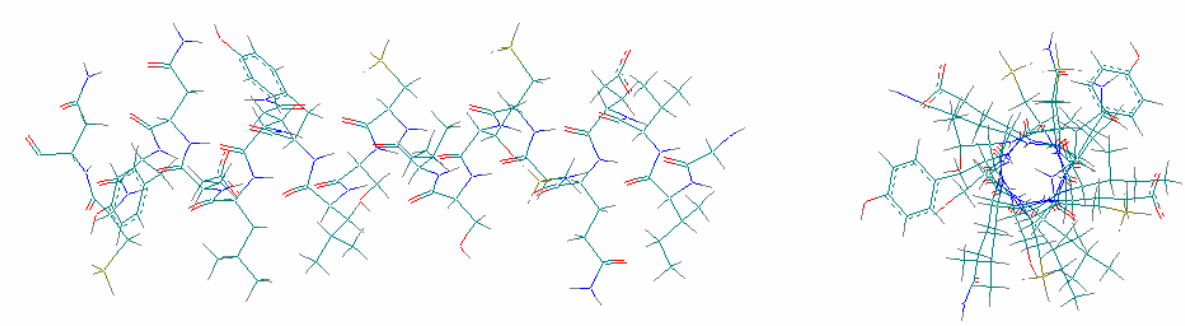


Figure 2. A chain of human insulin (α-helix form)

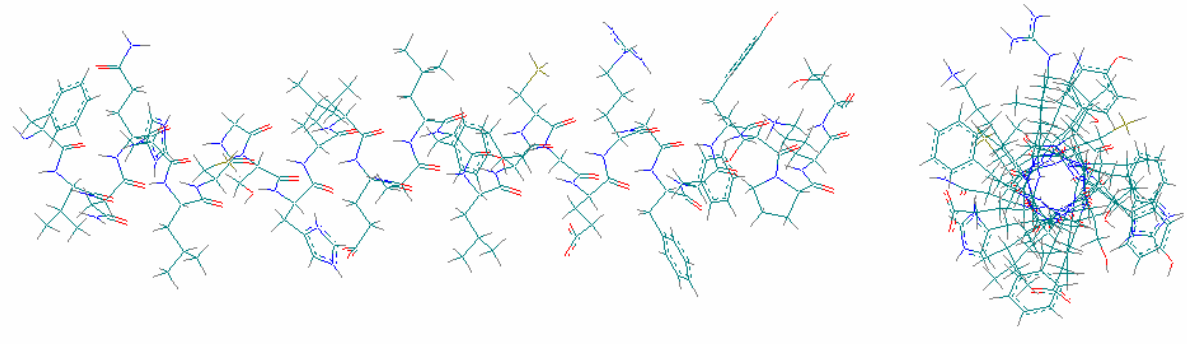


Figure 3. B chain of human insulin (α-helix form)

In order to evaluate the possibility to encapsulate the insulin-containing amino acid moieties, all important amino acids were studied. Thus, all amino acids in minimal energy conformations (obtained by using the MM+ molecular modeling program from the Hyper Chem package) were studied for docking in β-cyclodextrin (in conformation obtained by the same program and by using the RX data for the pure compound), the start positions being with the amino or carboxyl groups of amino acids oriented even to the A or B sides of cyclodextrin, especially with the amino acid gravity centre at ~8Å situed on the *OZ* axix of cyclodextrin.

A higher number of cycles for the amino acid/β-cyclodextrin interaction was observed in the case of

docking of bulky amino acids like L-Cys, L-Tyr, or L-Glu, most probable due to the steric hindrance between the *R* moiety of amino acid and cyclodextrin inner cavity. Furthermore, hydrophyllic groups of amino acids (like amino, carboxyl, hydroxyl, thio) can form hydrogen bonds with the hydroxyl groups from the cyclodextrin structure (this can be observed in the docking experiments). Only in the case of hydrophobic amino acid moiety orinted to the β-cyclodextrin A or B sides can conduct to the complex formation (by van der Waals interactions, Figure 4), which is revealed by the calculated interaction energy (as the difference between the sum of amino acid and cyclodextrin internal energies – calculated in vacuum – and the amino acid/cyclodextrin complex energy). The best interaction energies were obtained in the case of bulky (more hydrophobic) amino acids, like L-Leu, L-Lys, L-Phe, and L-Tyr, which can better interract with the β-cyclodextrin inner cavity (Table 1).

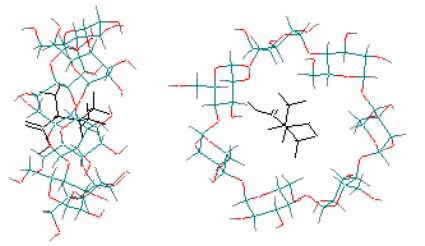


Figure 4. The L-Leu/β-cyclodextrin complex obtained by MM+ docking experiments

TABLE 1. Interaction energies (kcal/mole) in the case of amino acid/β-cyclodexrin complexes

No	Amino acid/ β-cyclodextrin	E(amino acid) (kcal/mole)	E(βCD) (kcal/mole)	E(amino acid)+E(βCD) (kcal/mole)	Ecomplex (kcal/mole)	Einteraction (kcal/mole)
1	L-Asp/βCD	-11.92	82.7	70.78	57.89	12.89
2	L-Cys/βCD	-0.23	82.7	82.47	66.97	15.5
3	L-Glu/βCD	-10.15	82.7	72.55	60.91	11.64
4	L-Ile/βCD	-0.78	82.7	81.92	64.79	15.13
5	L-Leu/βCD	-1.81	82.7	80.89	63.49	17.5
6	L-Lys/βCD	-3.5	82.7	79.2	61.36	17.84
7	L-Phe/βCD	-8.23	82.7	74.47	56.72	17.75
8	L-Tyr/βCD	-8.11	82.7	74.59	56.22	18.37
9	L-Val/βCD	-2.13	82.7	80.57	66.54	14.03

It is possible that the β-cyclodextrin to form a 1:2 complex with the L-Leu, or even with the more bulky aminoacids like L-Tyr (Figure 5).

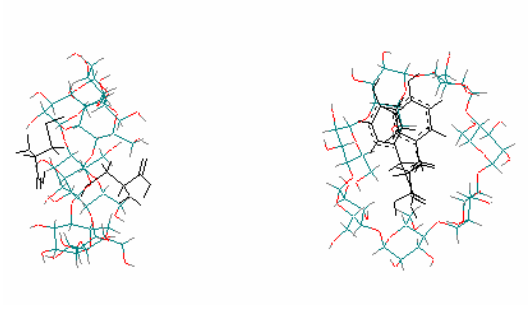


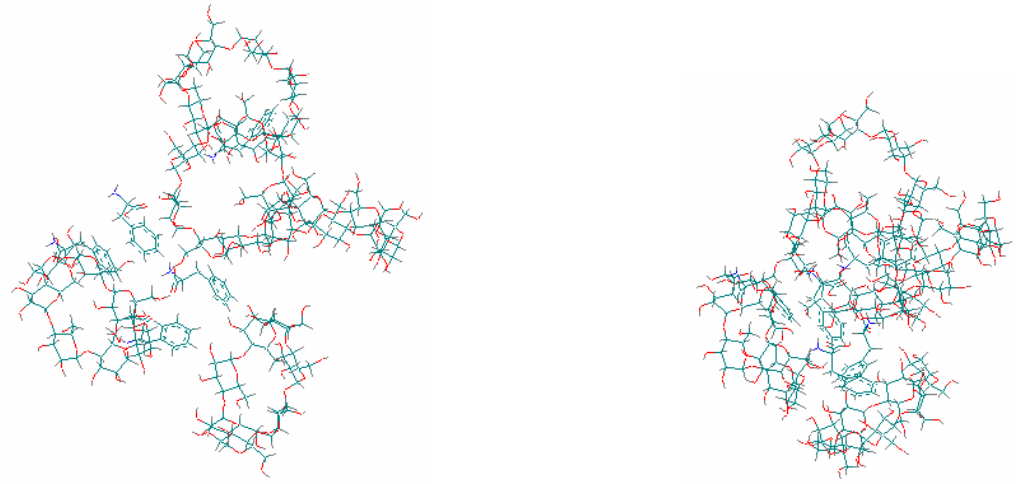
Figure 5. The L-Tyr/β-cyclodextrin complex (in 2:1 ratio) obtained by MM+ docking experiments

The similar docking studies were conducted by using five aminoacids molecules and five cyclodextrin structures randomly oriented one to another, but with the amino acid structure on the B side of every cyclodextrin. The distance between amino acid and cyclodextrin gravity centres were ~8Å. The MM+ docking studies confirm the formation of the complex, especially by the interaction between phenyl (or even benzyl) hydrophobic amino acid moiety and the hydrophobic inner cavity of cyclodextrin. A good interaction energy or internal complex energy was obtained for L-Phe and L-Glu (Figure 6 and Table 2).

TABLE 2. Internal complex energies obtained for five amino acid and five β-cyclodextrin molecules

No	Amino acid/β-cyclodextrin	Ecomplex (kcal/mole)
1	L-Asp/βCD	772
2	L-Cys/βCD	255
3	L-Glu/βCD	213
4	L-Ile/βCD	1364
5	L-Leu/βCD	296
6	L-Lys/βCD	1392
7	L-Phe/βCD	224
8	L-Tyr/βCD	1147
9	L-Val/βCD	757

The same docking experiments were conducted between the amino acid residues from insulin-containing oligopeptides (A and B chains) and one β-cyclodextrin molecule oriented with the B side to the hydrophobic moiety of amino acid at ~8Å. For the A chain, the lower complex energy was obtained especially in the case of l-Tyr, l-Gln, and l-Glu, while in the case of B chain, the best complex energy was obtained in the case of l-Phe, l-Tyr, but also in the case of l-Leu and l-His residues (Table 3 and Fig. 7).



(a) (b)

Figure 6. The cyclodextrin complex obtained from five L-Phe amino acid and five β-cyclodextrin molecules (the start – (a) and stop – (b) positions)

TABLE 3 Internal complex energies obtained for insulin-containing amino acid β-cyclodextrin structures

Chain A

No	Amino acid	Chain position	Ecomplex (kcal/mole)	No of cycles
1	Gly	1	-41.1	2187
2	L-Ile	2	-45	1144
3	L-Val	3	-44.2	823
4	L-Glu	4	-43.6	1820
5	L-Gln	5	-37.7	1049
6	L-Cys	6	-49.9	1533
7	L-Cys	7	-40.9	1096
8	L-Thr	8	-37.8	1187
9	L-Ser	9	-43.9	891
10	L-Ile	10	-46.9	1410
11	L-Cys	11	-36.1	873
12	L-Ser	12	-43.6	1104
13	L-Leu	13	-44.8	1387
14	L-Tyr	14	-52.4	2012
15	L-Gln	15	-54.2	2574
16	L-Leu	16	-46.5	1483
17	L-Glu	17	-51.4	1047
18	L-Asn	18	-49.2	1839
19	L-Tyr	19	-49.7	1726
20	L-Cys	20	-46.3	1503
21	L-Asn	21	-43.6	1513

Chain B

No	Amino acid	Chain position	Ecomplex (kcal/mole)	No of cycles
1	L-Phe	1	-145.1	1351
2	L-Val	2	-140	1084
3	L-Asn	3	-139	799
4	L-Gln	4	-145.6	1293
5	L-His	5	-143.6	937
6	L-Leu	6	-150.8	2039
7	L-Cys	7	-147.3	1249
8	L-Ser	9	-140.8	706
9	L-His	10	-150.6	1690
10	L-Leu	11	-145.3	1649
11	L-Val	12	-145.8	1450
12	L-Glu	13	-144.3	984
13	L-Leu	15	-145	1474
14	L-Tyr	16	-153.2	1022
15	L-Leu	17	-149.1	2044
16	L-Val	18	-148.5	1559
17	L-Cys	19	-146.6	758
18	L-Glu	21	-147	1934
19	L-Arg	22	-148.6	1010
20	L-Phe	24	-149.5	1556
21	L-Phe	25	-151.5	1572
22	L-Tyr	26	-150	817
23	L-Thr	27	-147.9	1270
24	L-Pro	28	-145.3	1555
25	L-Lys	29	-145.1	1644
26	L-Thr	30	-134.4	785

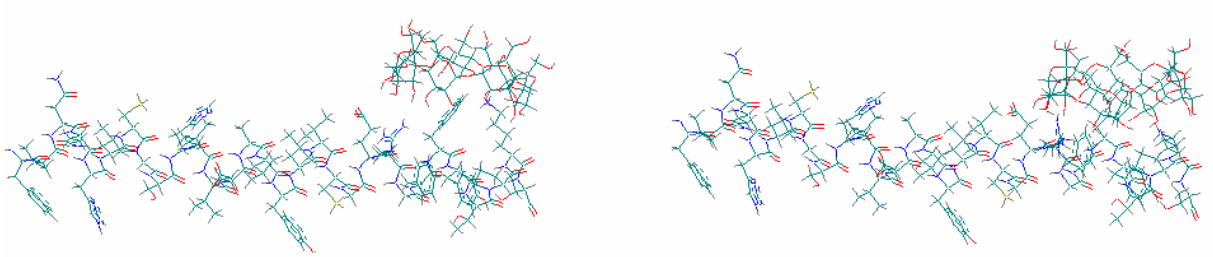


Figure 7. Complex formation in the case of β-cyclodextrin orinted to the L-Phe moiety from the 25-position of B chain of human insulin (the start – left and stop – right positions)

By using these results on the molecular encapsulation of amino acid residues from insulin-containing oligopeptides in β-cyclodextrin, maximum six cyclodextrin molecules can be oriented, from steric considerations, to the oligopeptides, especially to the hydrophobic amino acid moieties (L-Tyr, L-Phe, L-Gln, L-Glu, L-Lys, L-His, L-Leu). The complex formation is revealed by lowering the total internal energy (with the increasing of the overall complex stability, Figs. 8 and 9), with the compacting of the whole complex structure, as can be see in Figures 10 and 11.

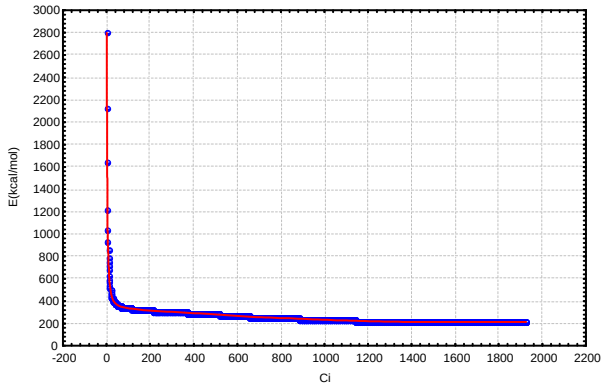


Figure 8. Internal energy (kcal/mole) *vs* number of cycles for the complex formation between A chain of human insulin and six β-cyclodextrin molecules

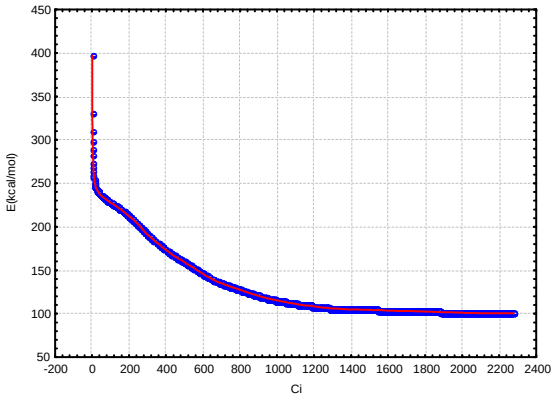


Figure 9. Internal energy (kcal/mole) *vs* number of cycles for the complex formation between B chain of human insulin and six β-cyclodextrin molecules

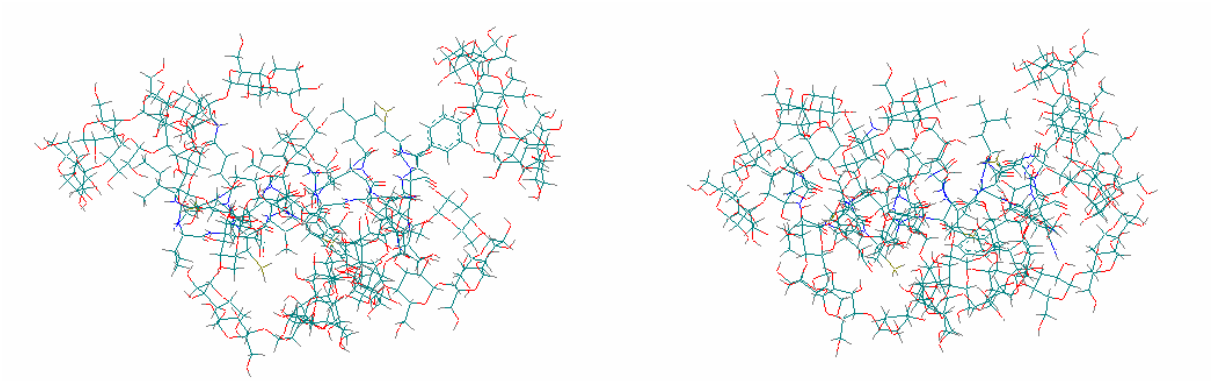


Figure 10. Complex formation in the case of six β-cyclodextrin molecules orinted to the main amino acid moiety from the A chai of human insulin (the start – left and stop – right positions)

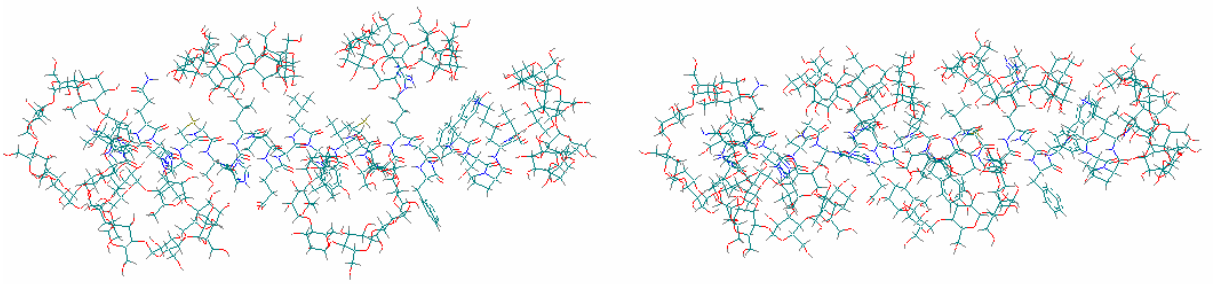


Figure 11. Complex formation in the case of six β-cyclodextrin molecules orinted to the main amino acid moiety from the B chain of human insulin (the start – left and stop – right positions)

4. Conclusion

The following conclusions can be drawn among the molecular modeling and docking experiments on the β-cyclodextrin and insulin-containing amino acids and oligopeptides (especially A and B chains of human insulin): (1) all insulin-containing amino acids can form complexes with β-cyclodextrin, especially from the B side of this cyclic oligosaccharide; (2) the amino acids with more hydrophobic moieties better interract with the inner β-cyclodextrin cavity; the best results were obtained with the L-Tyr, L-Phe, and L-Leu; (3) a good interraction exists even the amino acids are bonded in the human insulin chains; (4) no more than six cyclodextrin molecules can interract with the amino acid residues from the human insulin chains and the possibility of human insulin/β-cyclodextrin complex formation exists.

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