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A proposed interaction model of the insulin molecule with its receptor *

Dong-cai Liang [1], Wen-rui Chang, Zhu-li Wan

National Laboratory of Biomacromolecules, Institute of Biophysics, Academia Sinica, Beijing 100101, People's Republic of China

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Abstract

Based on the extensive structural comparisons among the determined structures of the different species and crystal forms of insulin and its derivatives in our laboratory, it was suggested that the binding interaction with the receptor molecule should take place mainly on an amphipathic surface of the insulin molecule. In the middle of this amphipathis surface, there was a hydrophobic surface with an area of about 150 $Å^2$, while the polar and charged groups distributing around the hydrophobic surface constructed a hydrophilic zone. The hydrophobic surface was usually covered by the extended B-chain C-terminal peptides with great mobility. The angle between the proposed binding interaction surface and the surface of dimerization was about 20°. The results from studies on structures of A1–(L-Trp) insulin and A1–(D-Trp) insulin confirmed the interaction mechanism model we proposed.

Key words: Structure and function of insulin; Binding interaction model of insulin with its receptor

The insulin structure analysis has a long history. The initiative work was done by Professor Dorothy Hodgkin in Oxford. More than 20 years ago the 2Zn insulin structure was successfully achieved by the Oxford research group [1,2], and not much later the insulin structure was solved independently by the Peking Insulin Structure Research Group as well [3,4].

As a hormone, insulin has various important biological functions and very complicated processes for exerting its biological activities. However, the binding interaction of the insulin molecule to its receptor is the first and important step for explaining its activity. The crystal structure analysis of the complex of insulin and its receptor may be the best way to understand the structure-function relationship of insulin. Unfortunately, so far none of us has succeeded in crystallization of the complex or the insulin receptor itself. Therefore, X-ray diffraction studies on insulin derivatives of varying biological activity and their structural comparisons are perhaps the most powerful approach to elucidation of the insulin structure-function relationship, in particular as the first step to the understanding of the molecular mechanism of the binding interaction

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of insulin to its receptor. Since 1980 a series of activity-sensitive analogues of insulin modified at the C-terminus and N-terminus of B-chain and the N-terminus of A-chain has been investigated. The crystals of some insulin analogues were successfully achieved and studied by X-ray crystallography in our laboratory as shown in Table 1. Fourteen structures from these crystals have been determined. Among them, the structures of 2Zn insulin and despentapeptide (B26–B30) insulin (so-called DPI) were refined at 1.2 Å and 1.5 Å resolution with *R*-factors of 12.8% and 14.4% respectively [5,6], and they have given us very important and very precise structural information for understanding the three-dimensional structure and function of insulin.

Fig. 1 shows the electron density of the hydrogen atoms in the main chains of A-chain and B-chain, hydrogen atoms in hydrogen bonds, hydrogen atoms in the methyl groups and so on. Actually, about 80% of hydrogen atoms in the insulin molecule were found with the electron densities which were two or three times higher than the noise in the difference map. Fig. 2 shows the anisotropic shape of the electron density of two sulphur atoms in the disulphur bridge. The top part in Fig. 2 was before anisotropic refinement while the last part was after several cycles of anisotropic refinement. It means that this very precise structure of 2Zn insulin at very high resolution provided us the very important and very valuable information about the thermodynamic motion of the insulin molecule.

The structural comparisons of 2Zn insulin with DPI and many other insulin derivatives were performed by the least-squares superimposition tech-

Table 1
X-ray crystallographic analysis of insulin and its derivatives in our laboratory

Insulin	Space group	Parameters of unit cell						Structure determination at resolution (Å) [a]
		a (Å)	*b* (Å)	*c* (Å)	α (deg)	β (deg)	γ(deg)	
porcine 2Zn	R3	82.5	82.5	34.0	90	90	120	1.2
bovine 4Zn	R3	80.8	80.8	37.4	90	90	120	1.9
DPI	C2	58.7	27.9	24.0	90	100.6	90	1.5
DPI	C2	72.7	57.1	62.4	90	93.4	90	
DPI	$P2_12_12_1$	70.8	62.4	57.6	90	90	90	
DPI	$P4_12_12_1$	92.6	92.6	98.9	90	90	90	
des(B1-2)DPI	$P2_1$	27.1	24.6	27.8	90	104	90	
DHI	C2	42.6	37.8	27.2	90	125.4	90	2.0
DHPI	$P2_12_12_1$	59.2	54.8	23.0	90	90	90	3.0
DHPI	$P2_12_12_1$	57.3	56.0	23.0	90	90	90	
B0 (L-Met)	$P2_13$	73.5	73.5	73.5	90	90	90	3.0
B0 (L-Arg)	R3	81.8	81.8	35.0	90	90	120	2.0
B0 (D-Ala)	R3	82.5	82.5	34.0	90	90	120	1.9
B0 (L-Leu)	R3	82.3	82.3	34.0	90	90	120	2.2
Des (B1–B2)	$P4_13$	97.4	97.4	97.4	90	90	90	
B31–Arg	R3	80.7	80.7	37.6	90	90	120	3.0
A1–(L-Trp)	R3	80.3	80.3	37.4	90	90	120	2.1
A1–(D-Trp)	R3	78.6	78.6	50.0	90	90	120	2.2
A1–(L-Ala)	$P2_1$	61.5	62.2	48.3	90	110.9	90	2.5
A1–(L-Ala)	R3	80.9	80.9	37.6	90	90	120	1.9
A1–(D-Ala)	$I2_13$ I23	78.9	78.9	78.9	90	90	90	
duck	$P4_1$	47.5	47.5	138.1	90	90	90	
snake	$P4_132$	67.3	67.3	67.3	90	90	90	
snake (phenol) insulin(I)	$P4_122$	131	131	124	90	90	90	
(phenol) insulin(II)	$P2_1$	49.2	60.9	48.2	90	95.8	90	
	$P2_1$	61.4	61.7	48.0	90	110.8	90	

[a] Crystal structure was determined in our laboratory.

nique and the graphic technique. Structural comparisons indicated that all insulin derivatives with certain biological activity of insulin have the specific three-dimensional structure of insulin molecule in 2Zn hexamer. In other words, the essential features of insulin molecule structure found in the 2Zn crystal form are the essentially structural requirements for exerting the biological functions of insulin. Any destruction of the specific three-dimensional structure should bring about a serious loss of the biological activity of insulin.

The evidence from the structural comparisons implied that the three pieces of α-helixes and a β-bend may be assembled into the insulin monomer as rigid bodies and they may move to each other in limited degrees around certain peptide-like hinges. We suggested that the peptides of A10, B4, B8, B24, and two other glycines of B20 and B23 might be as the hinge peptides in the insulin monomer to mediate the limited relative movements between the helical segments of A1–A9 and A12–A21, between the α-helix B9–B19 and the β-bend B20–B23, as well as the large-scale mobility of the extended peptides segments in N- and C-terminal regions of B-chain as shown in Fig. 3.

A dimer in a 2Zn insulin hexamer with a non-crystallographic 2-fold axis p–p was shown in Fig. 4. Here the surface m in the middle of the

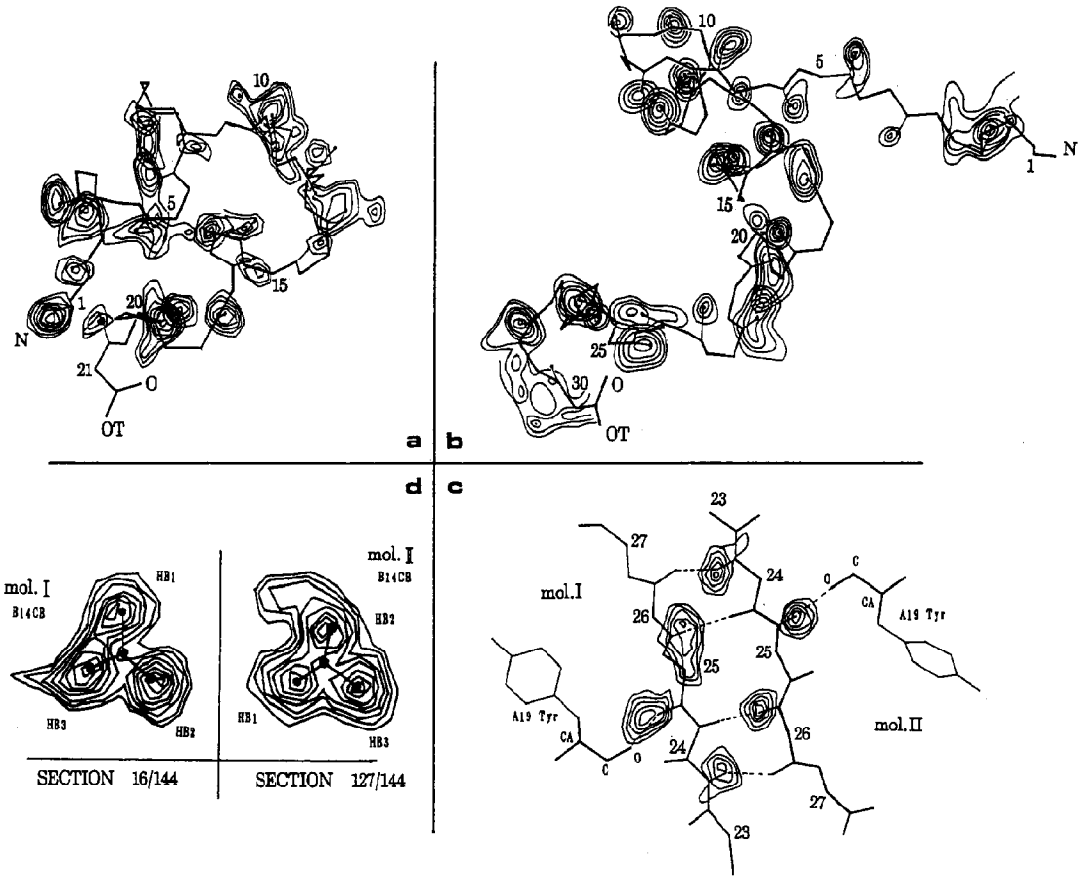

Fig. 1. The electron density of hydrogen atoms on the difference maps in (a) amide-N groups of the main chain of A-chain, (b) amide-N groups of the main chain of B-chain, (c) the hydrogen bonds of anti-parallel β-sheet (B24–B26) between molecule I and II of dimer, (d) methyl groups of B14 of molecule I and molecule II.

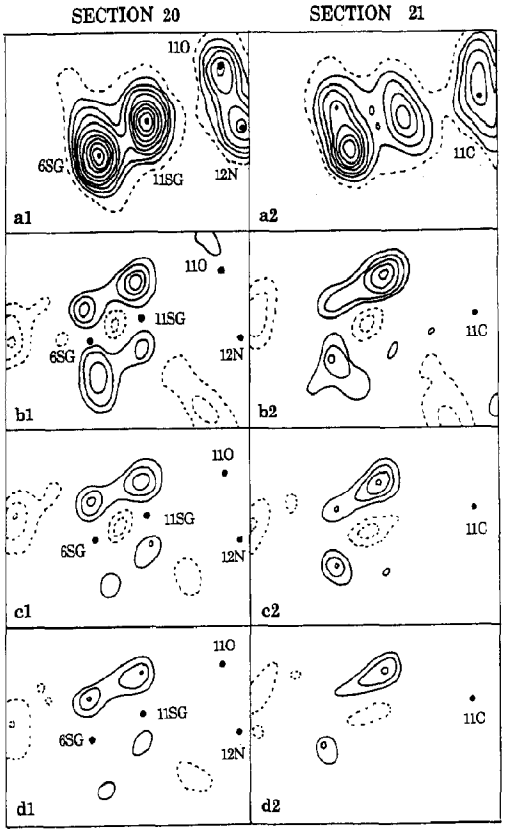

Fig. 2. The anisotropic distribution of electron density of sulphur atoms (molecule I, A6–A11). (a) 3Fo–Fc map; (b) Fo–Fc map; (c) Fo–Fc map (after two cycles of anisotropic refinement); (d) Fo–Fc map (final).

dimer is the molecular aggregation surface of dimer formation. As we know, the C-terminal of B-chain, which forms an anti-parallel β-sheet between two molecules in a dimer is the important part of the dimerization surface. A suggestion about the possible binding district of the insulin molecule with its receptor was first made by the Insulin Research Group of China [7,8] in 1974 and later by the Peking Insulin Structure Research Group [4]. They considered that the molecular aggregation surface of dimer formation would be the possible binding surface of the insulin molecule with its receptor and this dimerization surface was shown in Fig. 4 with a mark of "m". Since then, a series of discussions about the binding interaction mechanism of insulin with its receptor were made on the basis of studies by X-ray analysis, thermodynamics, CD spectrum and some other methods [9–13]. However, the binding interaction model presented so far has not had much change. All the proposed models of binding interaction have not been lifted out of the surface of dimer formation in 2Zn insulin structure [14].

Despentapeptide (B26–B30) porcine insulin (DPI) is one of the important derivatives of insulin. It exists in monomeric form and still with comparatively high biological activity. After the monomeric structure of DPI was solved [15] and refined at high resolution in our laboratory [6], we found that the molecular conformation of monomeric DPI produced an amphipathic surface due to the removal of five amino acides from the C-terminus of B-chain which were very flexible in 2Zn insulin molecules as shown in Fig. 5. Naturally in 2Zn insulin structure this surface was located behind the B-chain C-terminal peptides. Early in 1985 we proposed that this amphipathic surface may be the important interaction surface with insulin receptor [16].

Based on the detailed investigation of the structures of DPI, 2Zn pig insulin, 4Zn bovine insulin and other insulin derivatives, we suggested that the binding interaction of insulin receptor should occur on a surface of insulin molecule, and this interaction surface of insulin molecule consists of two parts. In the middle of the interaction surface there is a large and flat hydrophobic surface with an area of about 150 $Å^2$, as marked with the dotted line in Fig. 6, and it consists mainly of A2–Ile, A3–Val, A19–Tyr, B11–Leu, B12–Val, B15–Leu, B16–Tyr, B24–Phe, B25–Phe and perhaps B26–Tyr. Around this hydrophobic surface there are polar and charged groups which form a hydrophilic part. It consists of the α-aminyl group of A1–Gly, the side-chain groups of A4–Glu, A5–Gln, A18–Asn, A21–Asn, B9–Ser, B10–His B13–Glu, B21–Glu, B22–Arg and the carbonyl group at the C-terminus of B-chain.

What is the important difference between our proposed binding interaction surface and the molecular aggregation surface in a dimer? First of all, the surface we suggested here was located behind the C-terminal extending peptides of B-

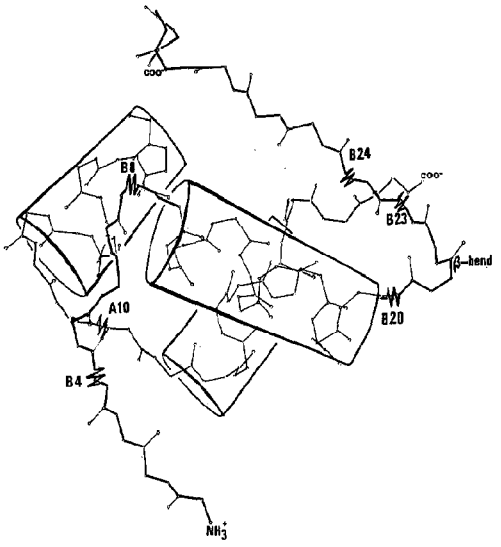

Fig. 3. The illustration of the main chain motion characteristics and the hinge peptides of insulin molecule.

chain in 2Zn insulin molecules, but it was not the dimerization surface involving right the C-terminal extending peptides of B-chain. The angle between these two surfaces was about 20° as shown in Fig. 4. Secondly, our suggested binding surface was obviously different from the dimerization surface in the construction and properties of residues. And furthermore, the construction of our proposed binding surface involved varied residues as well as the invariant and conservative residues as shown in Fig. 6.

The biological evidence pointed out that the hydrophobic interaction is original. We proposed that the monomeric insulin molecule comes close to its receptor, the extended peptides at C-terminus of B-chain (including B30–Ala, B29–Lys, B28–Pro, B27–Thr and perhaps B26–Tyr, which all are very flexible in conformation) will move away from the amphipathic surface of the insulin molecule and therefore the hydrophobic surface of the insulin molecule bares fully to the hydrophobic region of its receptor. The recognizing and binding interaction therefore may occur between two molecules, and following the hydrophobic interaction the hydrophilic interaction may take place. It could be understood from Fig. 6 that most of the polar and charged groups dispersing around the hydrophobic surface are the long side-chains with much flexibilities, such as the side-chains of B22–Arg, B13–Glu, B21–Glu, A4–Glu, A5–Gln and some others. They may move up and down, something like the suckers of an octopus, and may get in touch with the corresponding polar and charged groups of the receptor molecule. With any impediment to the hydrophobic interaction or hydrophilic interaction and therefore to the recognizing and binding interaction of the insulin molecule with its receptor, the biological activity of the insulin molecule should reduce seriously. Studies on the structures of insulin derivatives modified at Al–Gly with L-configurational and D-configurational amino acids provided us evidence strongly supporting the model of binding interaction we proposed above.

As we know, the α-aminyl group of Al–Gly with positive charge located rightly at the edge of the hydrophobic surface (Fig. 6) is a very important charged group for the interaction of the insulin molecule with its receptor. The biological activity of insulin derivatives modified at Al–Gly with different L-configurational and D-configurational amino acids which were prepared by Geiger

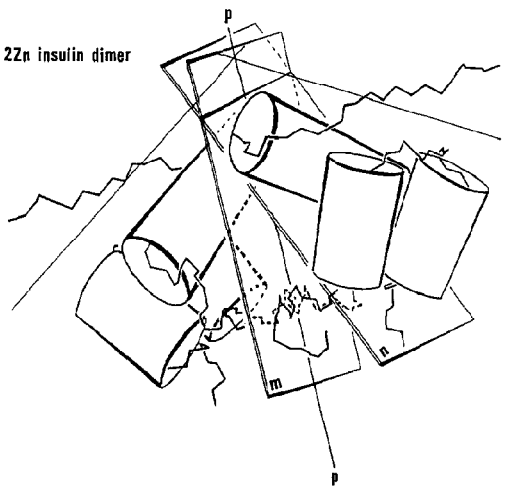

Fig. 4. The illustration of the aggregation surface (m) of molecule I and molecule II of a dimer in rhombohedral 2Zn insulin and our proposed binding interaction surface (n) of molecule I with its receptor. The angle between the surfaces m and n is ≈ 20°.

Table 2
Biological activity of insulin-analogs at A1 position [a]

A1-amino acid	Biological activity	
	L-configuration	D-configuration
Gly (Insulin)	100	100
Trp	14	82
Ala	12	74
Leu	16	112
Lys	4	9
Glu	9	79

[a] Refs. [17–19].

[17–19] was shown in Table 2. The molecule with a substitution of glycine by a L-configurational tryptophan at A1 maintained only 14% of natural activity, while A1–(D–Trp) insulin kept a high activity of 82%. The substitution of glycine by other amino acids with L-configuration and D-configuration gave a similar result [17–19]. The crystal structure of A1–(L-Trp) insulin and A1–(D-Trp) insulin were determined and refined at 2.1 Å and 2.2 Å resolution respectively in our laboratory [20,21].

The structural comparison showed that both A1–(L-Trp) insulin and A1–(D-Trp) insulin kept the specific three-dimensional structure of natural insulin molecule and the conformation of their molecule I was very similar, except for some side-chains. The results from structural comparison told us that A1–(L-Trp) insulin and A1–(D-

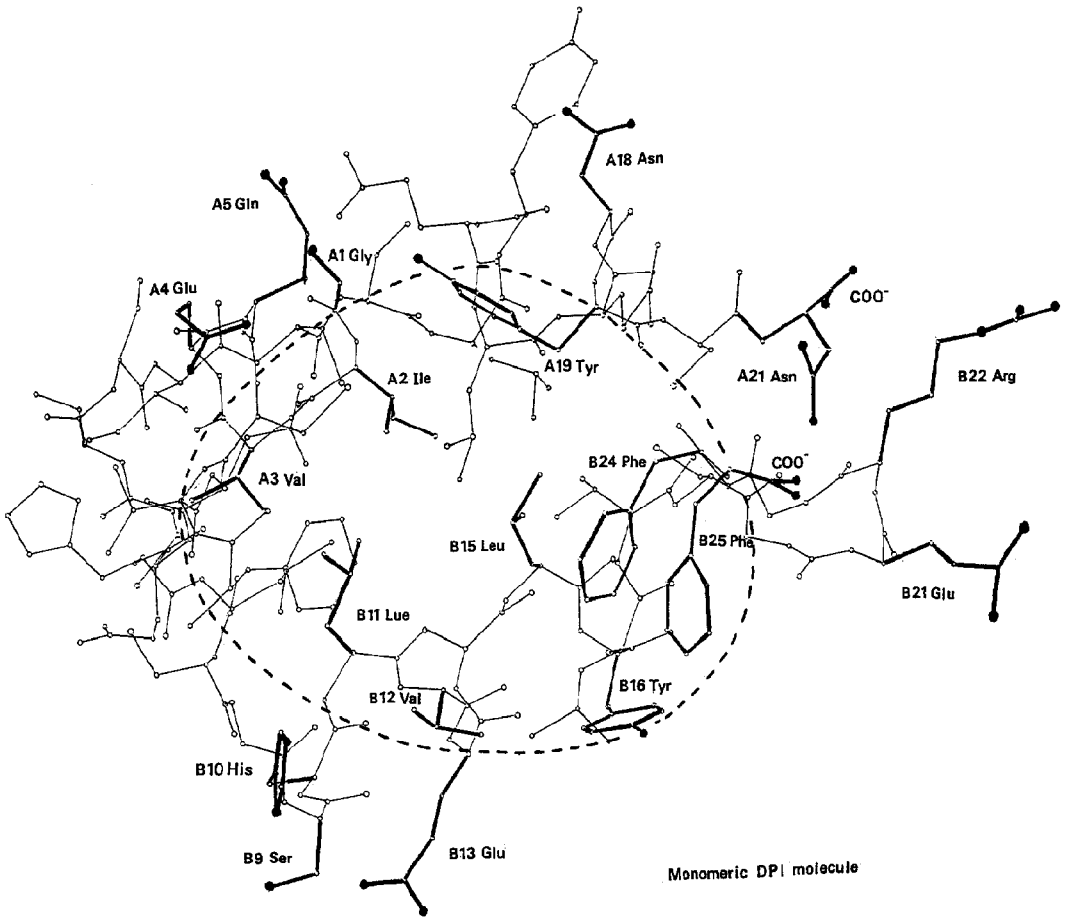

Fig. 5. The amphipathic surface on the DPI molecule. The hydrophobic surface (bounded by dotted line) consisting of hydrophobic residues is in the middle and the hydrophilic zone consisting of polar and charged groups is around it.

Trp) insulin were very similar, however big conformational differences were found in the side-chain of Al–Trp residue.

In the hexamer of rhombohedral Al–(L-Trp) insulin structure it was found that the Al–Trp residue of molecule I possessed two distinct positions of 1 and 2 with the occupancies of 0.7 and 0.3 respectively, while molecule II had only position 1 with a full occupancy. The side-chain, indole ring with L-configuration at Al in both positions 1 and 2 stretched upwards from the binding surface and obstructed the insulin receptor from full contact with the hydrophobic surface of insulin molecule as shown in Fig. 7. Furthermore, the indole ring with L-configuration at Al screened the charged α-aminyl group of the N-terminus from the interaction with the corresponding charged group of receptor molecule. And therefore, Al–(L-Trp) insulin remained a very low biological activity.

The structural comparison indicated that the orientation of the indole ring at Al residue in Al–(L-Trp) insulin and Al–(D-Trp) insulin was absolutely different, as shown in Fig. 8. Here the dotted line indicates the projection of the binding interaction surface of insulin molecule with its receptor. It was clear that since the side-chain, indole ring with D-configuration at Al in Al–(D-Trp) insulin goes down and out of the binding interaction surface of insulin molecule, it does not seriously interfere with the binding interaction of the insulin molecule with its receptor.

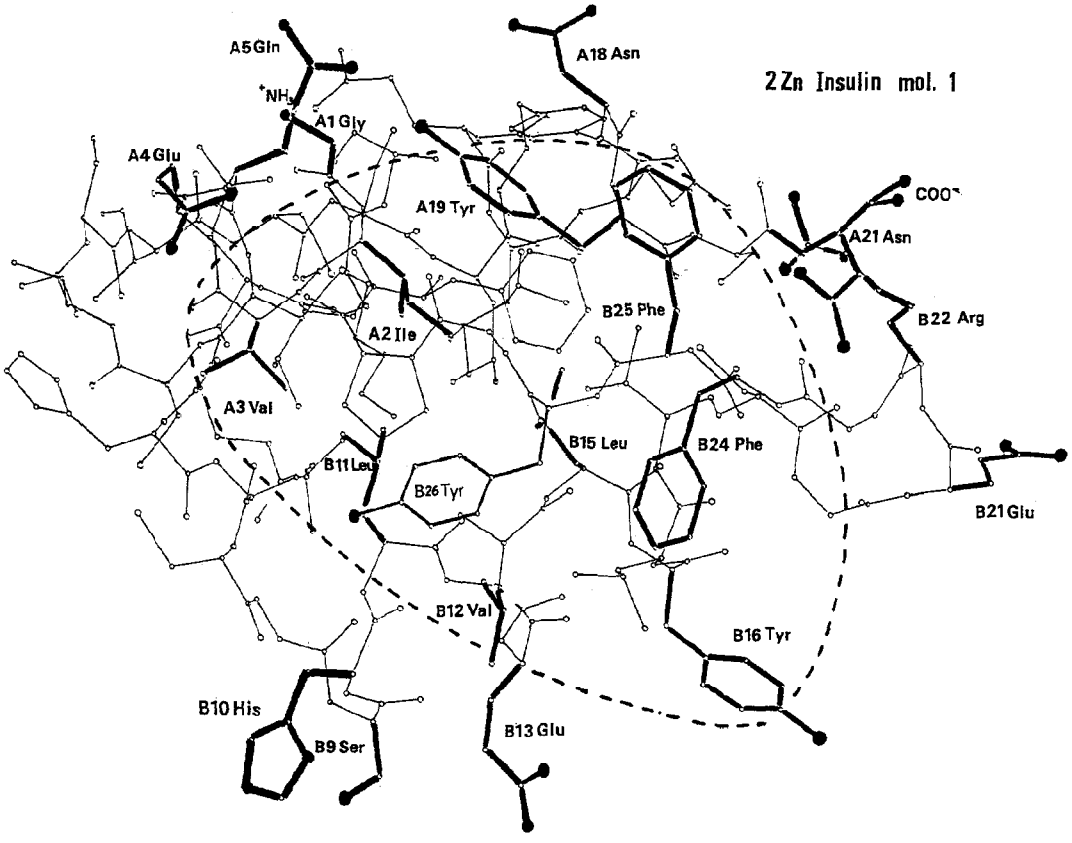

Fig. 6. The proposed binding interaction surface of insulin molecule with its receptor.

This is the reason why Al–(D-Trp) insulin keeps 82% of the biological activity. The results of this investigation not only gave an elucidation for the big difference of biological activity between Al–(L-Trp) insulin and Al–(D-Trp) insulin, but also provided us very important evidence for confirming our proposed binding interaction model of insulin molecule with its receptor.

Insulin is a small protein with a molecular weight of only 6700, while its receptor is a huge membrane protein with a molecular weight of about 300 000. We consider that, no doubt, the insulin molecule should bring with itself the critical and important information, but not all the information for asserting its biological functions. The relationship between the insulin molecule and its receptor is something like the key and its lock. After the key is inserted into the lock and opens it, the task of the key is fulfilled. The information for a series of the following biological events which will take place after the insulin molecule's recognition of, binding to and interaction with its receptor should not come directly from the insulin molecule itself anymore, but from the receptor bound by the insulin molecule. As for the insulin molecule itself, its recognition of, binding to and interaction with its receptor molecule are the extremely important steps for

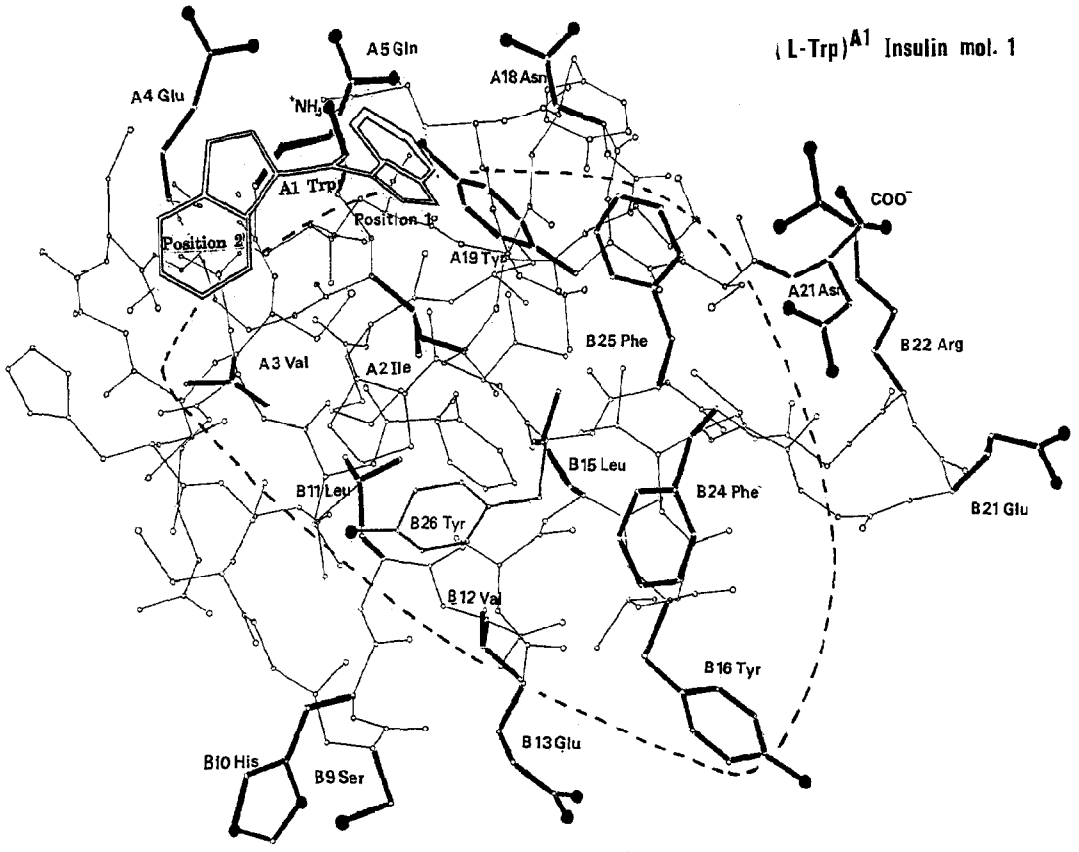

Fig. 7. Two positions of indole ring with occupancies of 0.7 and 0.3 respectively on the proposed binding surface of Al–(L-Trp) insulin molecule I.

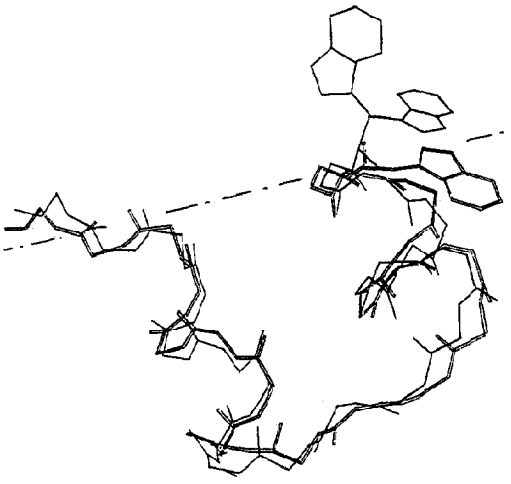

Fig. 8. The A-chain main chain superimposition of Al-(L-Trp) insulin molecule I (thin line connections) and Al-(D-Trp) insulin molecule I (double line connections), showing the different orientation of the Al-Trp side-chain groups (indole rings) of the two molecules. The dotted line indicates the projection of the binding interaction surface of the insulin molecule with its receptor.

performing its biological functions. From our point of view, the binding interaction is the first step and may be the last step.

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