

Insulin-like growth factor: A model for tertiary structure accounting for immunoreactivity and receptor binding

(nonsuppressible insulin-like activity/molecular model building/evolution of insulin/amino acid sequence homology/quaternary structure)

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Communicated by Donald F. Steiner, October 25, 1977

ABSTRACT A model for the three-dimensional structure of insulin-like growth factor (IGF) is proposed based on the close sequence homology of IGF with insulin, the tertiary structure of which is known. The IGF molecule is postulated to have an insulin-like main chain conformation for residues equivalent to B6-B27 and A1-A21 and a hydrophobic core nearly identical to that of insulin. A short connecting peptide of twelve residues and an extension at the COOH-terminus are easily accommodated on the molecular surface. The surface involved in dimer formation in insulin is largely conserved, but the zinc-binding histidine and many residues involving hexamerization are very different from those of insulin and it is unlikely that IGF forms zinc hexamers. The model provides a ready explanation for the inability of IGF to bind antibodies to insulin and for its ability to bind insulin receptors with low affinity.

Nearly 15 years ago, Froesch and coworkers (1) showed that most insulin-like activity in human serum is immunologically different from insulin; only 5-10% can be suppressed by guinea pig antibodies to insulin. This nonsuppressible insulin-like activity has been purified from a Cohn fraction of human serum (2). Two polypeptides with molecular weights of 7,500 could be isolated (3), and they were shown to have insulin-like effects *in vitro* and *in vivo* (4). Moreover, these polypeptides show growth-promoting effects *in vitro*, such as stimulation of DNA, RNA, protein, and proteoglycan synthesis (3-7), ornithine decarboxylase (8), and cell proliferation (5, 8). The two polypeptides with nonsuppressible insulin-like growth activity (NSILA) have therefore been named insulin-like growth factors (IGF I and II).[‡] IGF binds to two sets of specific receptors, each of which also binds insulin (9). The "insulin receptor" has a greater affinity for insulin than for IGF, while the reverse is true for the "IGF receptor." More recently we (E. Rinderknecht and R. E. Humbel) have determined the complete sequence of IGF-I,[§] which shows clearly homology with proinsulin. In this paper we present a model for the tertiary structure of IGF-I based on the known three-dimensional structure of porcine insulin (10, 11) and show how this model may account for its immunoreactivity and its affinity for insulin and IGF receptors.

MATERIALS AND METHODS

The model was built in several stages. The proposed structure was first constructed using Lapquip model parts at a scale of 1 cm = 1 Å. This was examined for unfavorable intramolecular contracts and readjusted before the coordinates of each atom were read off using a mechanical device. The approximate coordinates were then regularized using the "modelfit" com-

puter program of Isaacs *et al* (12), and bond lengths and angles and other intramolecular distances were calculated using an IBM 360/65 computer. The model was then displayed on a computer graphics system (Digital Equipment Corp. graphics with a PDP11 computer) using programs written by D. Richardson, P. Pauling, and C. Chothia, and adjustments were made using the interactive facilities of the graphics system to optimize the intramolecular distances. Finally, the model was regeometricized using "modelfit" and stereo pairs of the model were generated in hard copy.

RESULTS AND DISCUSSION

Three-dimensional model for IGF I

Table 1 shows the sequences of human IGF I, IGF II, and porcine insulin aligned so that the maximum homology is obtained. The numbering for the insulin A and B chains is indicated, as is the numbering for the IGF I polypeptide. The sequence of the IGF connecting peptide of 12 residues is also included, but because this shows no homology with the proinsulin connecting peptide, the latter is omitted. IGF I has an extension of eight residues at the COOH terminus of the A chain. Table 2 gives the differences in numbers of amino acids between the 51 amino acids of porcine insulin and the equivalent residues of other insulins, the protein hormone relaxin (13-15), which also appears to be homologous with insulin, and IGF.

The sequence comparison shows a close homology for residues 5-25 of IGF (B6-B26 of insulin) and 42-61 (A1-A20). The arrangement of cystines is identical in IGF and insulin, and glycines 7 (B8), 19 (B20), and 22 (B23), which have dihedral angles that are disallowed for residues with side chains, are conserved, so that the polypeptide backbone can assume the same three-dimensional structure as insulin. We began by building the sequence 5-26 and 42-61 into an insulin-like structure. This conformation is shown schematically in Fig. 1. Residues 8-18 (B9-B19) and 43-48 (A2-A7) are right-handed α -helices and residues 54-60 (A13-A19) formed a less organized right-handed helix. Cys 47 and 52 (A6 and A11) and Cys 61 and 18 (A20 and B19) have their disulfides placed in the core, while the Cys 48 and 6 (A7 and B7) disulfide is on the surface. We then added side chains to residues Leu 5 (B6), Leu 10 (B11), Val 11 (B12), Ala 13 (B14), Leu 14 (B15), Val 17 (B18), Phe 23 (B24), Ile 43 (A2), Val 44 (A3), Leu 57 (A16), and Tyr 60 (A19), which are entirely or partially buried in the hydrophobic core of insulin and are identical in IGF and porcine insulin. The

Abbreviation: IGF, insulin-like growth factor.

[‡] E. Rinderknecht and R. E. Humbel, unpublished.

[§] Unpublished data. A preliminary communication has been presented at the 11th Acta Endocrinologica Congress, Lausanne, 1977.

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Table 1. Sequences of porcine and human IGF I and II[‡]

B chain number	-1	0	1	2	3	4	5	⑥	⑦	⑧	9	10	⑪	⑫	13	14	⑮	16	17	18	⑲	20	21	22	⑳	㉑	25	26	27	28	29	30	
Porcine insulin	NH ₂ -Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala-COOH																																
B chain	HO O O H O IH O O O Z I IDr DH IH I Or H I I H O Or OR DR ODR Dr O O O O																																
Human IGF I	NH ₂ -Gly-Pro-Glu-Thr-Leu-Cys-Gly-Ala-Glu-Leu-Val-Asp-Ala-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Tyr-Phe-Asn-Lys-Pro-Thr																																
Human IGF II	NH ₂ -Ala-Tyr-Arg-Pro-Ser-Glu-Thr-Leu-Cys-Gly-Gly-Glu-Leu-Val-Asp-Thr-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Tyr-Phe-Ser-Arg-Pro																																
IGF number	1	2	3	4	⑤	⑥	⑦	8	9	⑩	⑪	12	⑬	⑭	15	16	⑰	⑱	⑲	20	㉑	㉒	㉓	㉔	㉕	㉖	㉗	㉘	㉙	㉚	㉛	㉜	㉝
Human IGF connecting peptide	Gly-Tyr-Gly-Ser-Ser-Ser-Arg-Arg-Ala-Pro-Gln-Thr																																
IGF number	30	31	32	33	34	35	36	37	38	39	40	41																					
A chain number	①	②	3	4	⑤	⑥	⑦	8	9	10	⑪	12	13	14	15	⑮	17	18	⑲	⑳	㉑												
Porcine insulin	NH ₂ -Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn-COOH																																
Human IGF	Gly-Ile-Val-Asp-Glu-Cys-Cys-Phe-Arg-Ser-Cys-Asp-Leu-Arg-Arg-Leu-Glu-Met-Tyr-Cys-Ala-Pro-Leu-Lys-Pro-Ala-Lys-Ser-Ala-COOH																																
IGF number	④②	④③	④④	45	46	④⑦	④⑧	49	50	51	⑤②	53	⑤④	55	56	⑤⑦	⑤⑧	59	⑥①	⑥②	⑥③	⑥④	⑥⑤	⑥⑥	⑥⑦	⑥⑧	⑥⑨	70					

The numbering for insulin A and B chains is given above the sequences; residues conserved amongst insulins are circled. The numbering system for IGF I is given beneath the sequences; residues identical between IGF I and porcine insulin are circled. The letters below the insulin sequence indicate the position of the residue side chain in the insulin hexamer: I, inside, contributes to hydrophobic core of monomer; D, dimer contact; H, hexamer contact; Z, binds zinc; O, outside of hexamer; R, in center of putative receptor binding region; r, on periphery of receptor-binding region. Some residues play more than one role. For instance, Phe 25 is involved in contacts between monomers in the dimer (D) but is still partly exposed in the hexamer (O) and is probably involved in receptor binding (R). Hence the notation ODR for this residue.

atoms in the main chain of residues 5–26 and 42–61 and those in the side chains of the residues built on to the model at this stage have precisely the same relative positions as those equivalent identical residues in insulin determined by x-ray analysis. We also added Phe 25 in the place of Tyr B26 of insulin. In this way the whole of the hydrophobic core was built up. This clearly demonstrates the three-dimensional structural homology of insulin and IGF.

The remaining side chains of the residues 5–25 (B6–B26) and 42–61 (A1–A20) are almost entirely on the surface of the molecule and are very easily accommodated in an insulin-like structure. Most, but not all, are hydrophilic and differ between insulins and IGF. Residues 1–4 of IGF (B2–B5) differ but also lie on the outside of the molecule and can be added to the model without disruption of the tertiary structure. We have placed them in a conformation similar to that of insulin in rhombohedral crystals. However, for insulin it is clear that residues B1–B3 play little role in the stabilization of the tertiary structure and the same is almost certainly true for IGF residues 1 and 2. Residues 26–29 of IGF can also be accommodated on the surface of the tertiary structure in positions approximately equivalent to residues B27–B30 of insulin; however, the rather surprising reversal of the Pro,Lys sequence in IGF leads the chain away from the main core of the molecule and makes the structure “looser” at this point. These features can be observed in the two stereo views of the molecule shown in Fig. 1 and 2.

It is difficult to be precise about conformations of surface

polar side chains. For example, although the side chain of Lys 27 can be folded against the side chain of Phe 25, we have placed it pointing directly into solution. This decision was influenced by the finding in the x-ray refinement of insulin that Lys B29, the equivalent group in insulin, is less important structurally than was originally thought, and does not form an intramolecular ion pair with Glu A4 (M. Vijayan, personal communication).

The residues 30–41 comprising the connecting peptide sequence of IGF I easily span the positions of the B30 and A1 of insulin. Indeed, it has often been remarked (11, 16) that the connecting peptide of insulin (about 30 amino acid residues in length) need only be three residues in length to achieve the simple object of spanning the two chains and allowing the polypeptide to achieve the correct three-dimensional structure. In fact, a synthetic bridge of about 10 Å can mimic the role of the connecting peptide in guiding chain folding and ensuring correct pairing of sulfurs in the cystines (17). The connecting peptides of proinsulins differ much more widely than the corresponding A and B chains, both in length and in amino acid composition. It appears that either changes are selectively neutral in evolution (18) or, alternatively, the functional requirements for the connecting peptides differ widely (1, 6, 19).

In the absence of an x-ray analysis of proinsulin and of any homology of the connecting (C) peptides of insulin and IGF, we have considered possible conformations from first principles. We have tried to keep polar residues exposed to the solvent or forming ion pairs or hydrogen bonds. Conversely, we have placed non-polar groups against similar groups where possible. Of the predictive methods, that of Chou and Fasman (19) is quite successful in predicting the insulin structure. The helices found in insulin by x-ray analysis were A2–A8, A13–A19, and B9–B19, while those predicted were A2–A7, A13–A18, and B10–B19. The extended chain β sheet found in insulin by x-ray analysis includes B2–B7 and B24–B28, and these regions are predicted by Chou and Fasman (19) to have sheet structure. We were thus encouraged to use the method as an aid to the prediction of secondary structure of the C peptide of IGF. Residues 28–34 are all residues found in β turns. By using the method of

Table 2. Numbers of amino acid differences

	Guinea				IGF	Relaxin
	Porcine insulin	Cod insulin	pig insulin	Hagfish insulin		
Porcine insulin	0	15	18	19	27	40
Cod insulin		0	19	19	22	40
Guinea pig insulin			0	25	31	41
Hagfish insulin				0	30	39
IGF					0	40
Relaxin						0

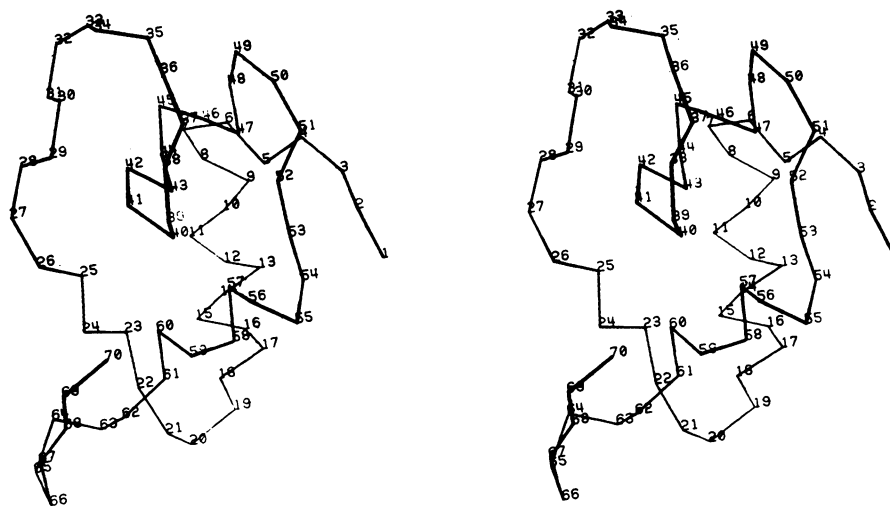


FIG. 1. Stereo view of the proposed three-dimensional structure of IGF I, showing α -carbon positions.

Chou and Fasman (19), at least two β turns are predicted for these residues. The sequence contains very few helix or β -sheet-inducing residues, but on the contrary includes the sequence Ser-Ser-Ser-Arg-Arg (33–37), whose residues are rarely found in helical or sheet structures (19). The connecting peptide as a whole is very hydrophilic and the sequence Ser-Ser-Ser-Arg-Arg must almost certainly be loose on the surface of the molecule.

For those reasons we have built chain with two β turns in the residues 28–34, so that the polypeptide of residues 33–37 lies away from the surface with all the side chains available to the solvent. In this way Tyr 31 has its side chain towards the top of the hydrophobic surface comprising 11 (B12), 23 (B24), 24 (B25), and 25 (B26) that is involved in dimer formation in insulin. Also Arg 36 and Arg 37 have their side chains in the vicinity of the acid groups of Asp 45 (A4) and Glu 46 (A5). The remaining residues of the connecting peptide (38–41) are then easily folded to join Gly 42 (A1). We attempted to build these residues as an extension of the helix 43–48, but this makes it impossible to fold the preceding chain residues to give the ion pairs described above and at the same time keep the chain packed against the protein surface. A β turn is more easily accommodated at this point.

Residues 63–70 have fewer constraints on their conformation. The presence of Pro 63 and Pro 66 and the hydrophilic groups such as Lys 65, Lys 68, and Ser 69 make helix unlikely (19). However, the Leu 64 would probably pack against the more hydrophobic surface residues such as Met 59 (A18), Tyr 60 (A19), and Tyr 24 (B25) in this region. As can be seen in Figs.

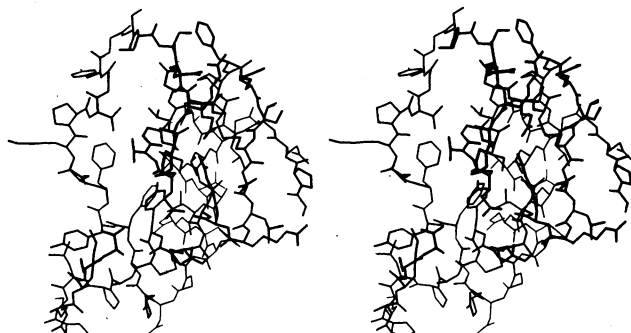


FIG. 2. Stereo view equivalent to that of Fig. 1 of the proposed three-dimensional structure of IGF I. All atomic positions are shown.

1 and 2, we have chosen to fold the residues back on themselves; this allows the hydrophilic side chains to be in contact with solvent and brings the COOH-terminus into the region of the guanidinium group of Arg 21 (B22).

The tertiary structure described here leads to a reasonably compact globular molecule. The many charged groups are distributed over the surface of the molecule, giving rise to a complicated series of ionic interactions. For instance, Arg 21 (B22) is close to Glu 58 (A17) and Ala 70 α -carboxylate, and an extensive arrangement of charged groups is formed, as illustrated in Fig. 3.

Similar model-building studies have been used to predict the structure of proinsulin (11, 20, 21, ¶). Also the protein hormone relaxin, from the corpus luteum, has an insulin-like primary structure and we have recently shown by model building that the conformation may be very similar to that of insulin (22).

The conformation of insulin determined by x-ray analysis and proposed conformations for proinsulin and relaxin (22, ¶) are compared to that proposed here for IGF in the schematic representation of Fig. 4.

Quaternary structure?

In summary, the structure of IGF I proposed here may be described as a "mini" proinsulin. The connecting peptide, residues 31–41, and the extension at the COOH-terminus, residues 63–70, occupy positions on the more hydrophilic surface of the molecule that in insulin forms the surface of the 2-Zn insulin hexamer and over which the C peptide of proinsulin probably lies (11, 16, 22, ¶). Much of the remaining surfaces of insulin and IGF I differ less. These surfaces are involved in the formation of dimers and hexamers of both insulin and proinsulin. We may then ask: is it possible that IGF I can self-associate to give an insulin-like quaternary structure?

Let us first consider dimerization. In insulin this involves the association of two equivalent predominantly hydrophobic surfaces (B12 Val, B16 Tyr, B24 Phe, B25 Phe, and B26 Tyr). These are symmetry related by an approximate 2-fold axis that allows residues B24–B26 from the two molecules to form an antiparallel pleated sheet. In IGF the equivalent residues probably have a similar conformation; Val 11 (B12) and Phe 23 (B24) are conserved and Tyr 24 (B25) and Phe 25 (B26) are

¶ S. P. Wood and T. L. Blundell, unpublished results.

¶ G. Dodson, N. Isaacs, A. C. T. North, and A. Evans, personal communication.

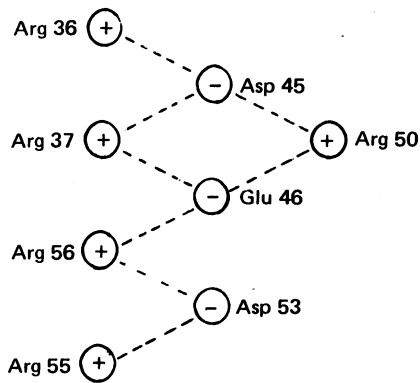


FIG. 3. The arrangement of surface charged groups that occurs in the proposed model of the IGF I molecule.

reversed. Of greater consequence is the existence of Gln 15 in place of B16 Tyr, but even this is not a very significant change. Residues such as Ala 8 (compare B9 Ser) and Asp 12 (compare B13 Glu) which are close to the region of dimerization are also rather similar. It therefore seems quite possible that IGF I could dimerize, although the reversal of Pro and Lys at 27 and 28 might tend to weaken the association by disturbing conformation of the adjacent residues.

The formation of zinc insulin hexamers from dimers proceeds by coordination of zinc ions at B10 His and association through hydrophobic surfaces involving B6 Leu, B14 Ala, B17 Leu, B20 Gly, and A13 Leu. Residues B1–B4 and A14 are also involved in hexamer formation, but variations in the sequence in these positions occur for insulins such as porcine, cod, and turkey, which all form zinc insulin hexamers (16). The insulin of certain hystricomorph rodents (guinea pig, coypu) have diverged from those of other mammals and cannot form zinc insulin hexamers (13). B10 His has been replaced by Asn, and residues such as B4 (Gln → Arg), B14 (Ala → Ser), B17 (Leu → Ser), B20 (Gly → Gln), and A13 (Leu → Arg) in the region involved in dimer-dimer contacts have become more hydrophilic or larger or both (16). On the other hand, the most primitive insulin sequenced, that of the hagfish (23) is also unable to form hexamers, although dimers are formed (24), but this insulin is more similar to porcine insulin than that of the hystricomorphs. B10 His is replaced by Asp, and B17 Leu and A14 Leu are replaced by Ile, but residues B14 and B20 are Ala and Gly as in porcine insulin.

In IGF I and IGF II, Glu 9 occupies the position of the zinc-binding B10 His of insulin. Although Leu 5 (B6), Ala 13 (B14), Gly 19 (B20), and Leu 54 (A13) are identical in IGF I and insulin, Thr 13 (B14) in IGF II and Phe 16 (B17) are residues that would weaken hexamer formation. The rather similar degree of change in IGF and hagfish insulin suggests that IGF probably does not form hexamers with zinc. It is, however, of interest that in both IGF and hagfish insulin, as in porcine insulin, the *surface* equivalent to that involved in hexamer formation in porcine insulin is mainly hydrophobic, unlike that of guinea pig insulin, which is more hydrophilic.

It therefore appears that IGF, like the most primitive insulin (hagfish), may be able to form dimers but almost certainly cannot form zinc insulin hexamers. These suggestions await the availability of further IGF for experimental verification. The rather hydrophobic surface involved in association of dimers to hexamers in porcine insulin appears to have evolved before hexamer formation. On the other hand, it is possible that the ability to dimerize appeared before insulin and IGF diverged.

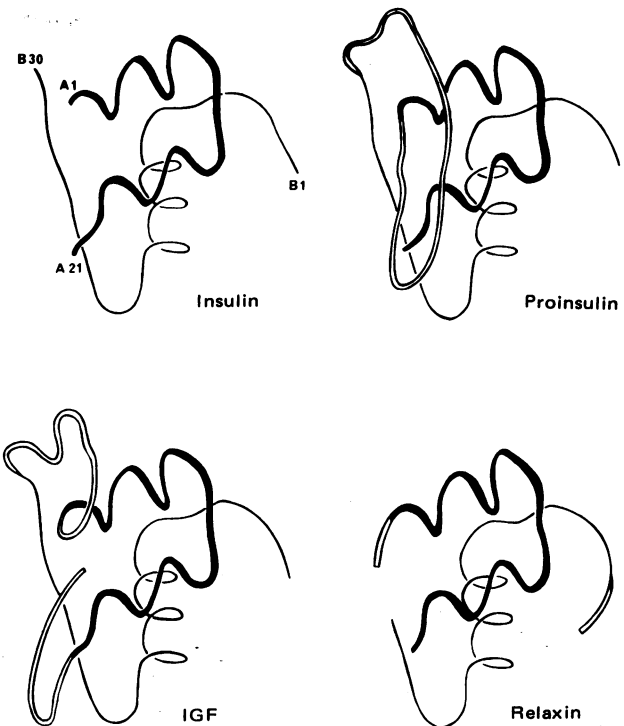


FIG. 4. Schematic representations of the three-dimensional structure of insulin based on the x-ray analysis of rhombohedral porcine 2-Zn insulin crystals (10, 11) and proposed conformations based on model building for proinsulin (20, 21, ¹¹), porcine relaxin (22, ¹¹), and IGF I showing the close structural homology.

Immunoreactivity and receptor binding of IGF

IGF was recognized first by virtue of its inability to bind guinea pig antibodies to bovine insulin. Arquilla and coworkers (25) have shown that strains of guinea pigs raise antibodies to different regions of the insulin tertiary structure. Some recognize a region involving the B1 terminal region and the adjacent region of the A chain (A8–A14). Others recognize a region involving A1, A19, A21, and the B-chain COOH-terminus; this antibody does not recognize proinsulin, and this is explained by the presence of C peptide covering this surface area. Assuming that either or both of these antibodies may be present in laboratory guinea pigs used, the model must explain why IGF is different in both these regions from both bovine and guinea pig insulins.

In the first region the residues equivalent to B1–B5 are quite different; in particular B5 His of the insulins is replaced by Thr in IGF. The adjacent residues A8, A9, A10, A12, A13, A14, are Ala, Ser, Val, Ser, Leu, Tyr in bovine insulin, Thr, Gly, Thr, Thr, Arg, His in guinea pig insulin, and Phe, Arg, Ser, Asp, Leu, Arg in IGF. An antibody recognizing the region of one would not be expected to bind the other. We have proposed that the connecting peptide (residues 30–41) and the extension of the COOH terminus (residues 63–70) cover the second binding region, which would inhibit antibody binding. Thus, the fact that IGF does not react with antibodies against insulin is fully accounted for by the model.

It has been demonstrated that both insulin and IGF have wide but remarkably similar spectra of biological activities (4, 26), although insulin is more potent in stimulating acute metabolic effects (3–5). These different effects probably arise through the interaction of the polypeptides with two separate membrane receptors, one of which has a higher affinity for IGF and the other a higher affinity for insulin (9).

The insulin molecule appears to bind its receptor through noncovalent interactions involving a surface region possibly including A1 Gly, A19 Tyr, A21 Asn, B23 Gly, B24 Phe, B25 Phe, B12 Val, B13 Glu, and B16 Tyr (11, 16, 27). The exact dependence of the receptor-hormone interaction on each of these residues has not been established unequivocally owing to the difficulty of their specific chemical modification and the complications due to conformational changes consequent upon modification. However, it appears that their three-dimensional arrangement is critically important because the insulin native tertiary structure must be conserved for full biological activity. The receptor interaction resembles that of dimerization and may involve similar, but more extensive, hydrophobic and other interactions including a β -sheet formation involving residues B24-B26.

From these considerations it is apparent that some part of the insulin receptor binding region is conserved in IGF but the remainder is radically changed. Thus Val 11 (B12), Gly 22 (B23), Phe 23 (B24), and Tyr 60 (A19) are identical and Asp 12 (B13) and Phe 24 (B25) are conservatively varied. This area (with the exception of Tyr 60) may be responsible for the reactivity of insulin and IGF for the other's receptors. However, A1 and A21 are extended in IGF by the connecting peptide and extension at the COOH terminus, which we have suggested covers this surface region. Thus IGF, like proinsulin, would be expected to bind weakly to the insulin receptor, as observed. Parts of the connecting peptide and COOH-terminal region of IGF may enhance IGF binding to IGF receptors. Alternatively, this specificity may be a consequence of the replacement of B16 Tyr of insulin by Gln 15 in IGF.

We thank Dr. J. Finney and Mr. J. D. Nicholas for the use of their mechanical device for measuring coordinates, and Dr. P. Pauling, Dr. C. Chothia, and Mr. D. Richardson for generously making available their computer graphics system at University College, London. We are grateful to Dr. S. Wood, Mr. J. Jenkins, Mr. J. Pitts, and Mr. T. Sewell for assistance with computing and for useful discussions. T.L.B. and S.B. thank the Science Research Council (United Kingdom) for financial support.

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