Biophysical Chemistry 21 (1985) 57–60
Elsevier

BPC 00897

BINDING OF INSULIN DIMERS TO RECEPTORS

Peter D. JEFFREY

Department of Physical Biochemistry, The John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia

Received 6 August 1984
Accepted 30 August 1984

Key words: Insulin polymerization; Insulin-receptor binding site; Receptor cross-linking

A synthesis of current views of the nature of the self-association of insulin in solution, of the identity of the amino acid residues in the insulin molecule constituting the receptor binding site, and of characteristics of the apparent 'negative cooperativity' of the binding leads to the seemingly contradictory conclusions that only the monomeric form of the protein is available and capable of binding, yet that other species must bind as well. A solution that has been proposed is that an alternative binding site exists, one which is not involved in insulin dimerization. Here, these conclusions are re-examined in the light of the characteristics of a new model for the polymerization of insulin. Firstly, it is found that the idea that dimers and higher polymers of insulin can bind to receptors is plausible, even necessary, secondly, that the postulate of an alternative binding site on insulin molecules is not required, and finally, that the characteristics of the insulin-receptor interaction warrant further investigation specifically in terms of the ability of some insulins to polymerize in solution and their potential to cross-link receptors.

1. Introduction

The association of insulin molecules in solution has been described by several different models, all of which involve the formation first of dimers and then of higher polymers [1–3]. Recently [4], a polymerization scheme was presented which differs from previous ones in allowing for the formation of two different kinds of dimer, a possibility that may be inferred from the two different sets of inter-monomer contacts seen in hexameric zinc insulin [5]. One of these sets, which we may call α-α, usually identified as 'the monomer contact region', contains most of the residues thought to comprise the receptor binding site [6]. The new model has been shown to fit all of the experimentally observed association behaviour of insulin from pH 2–7 to pH 10 and at different ionic strengths and temperatures [7]. One of its features is that an insulin solution is now visualized as containing some molecules of every type of polymer, odd-numbered as well as even (fig. 1). More importantly for the present discussion, representatives of every type of insulin polymer have exposed receptor binding sites: the odd-numbered polymers (and monomer) have one site, the even-numbered polymers have two. Since all previous descriptions of the association of insulin molecules in solution have implied a mechanism of dimerization which buries the receptor site, the scheme outlined above raises some new possibilities in relation to the interaction of insulin with its receptor. The discussion here will be in terms of the potential receptor binding competence of an insulin dimer, since it can serve as a representative of all species larger than monomer and, in any event, is likely to be the only significant insulin species other than monomer that needs to be considered because of the magnitudes of the association constants and the concentration range involved.

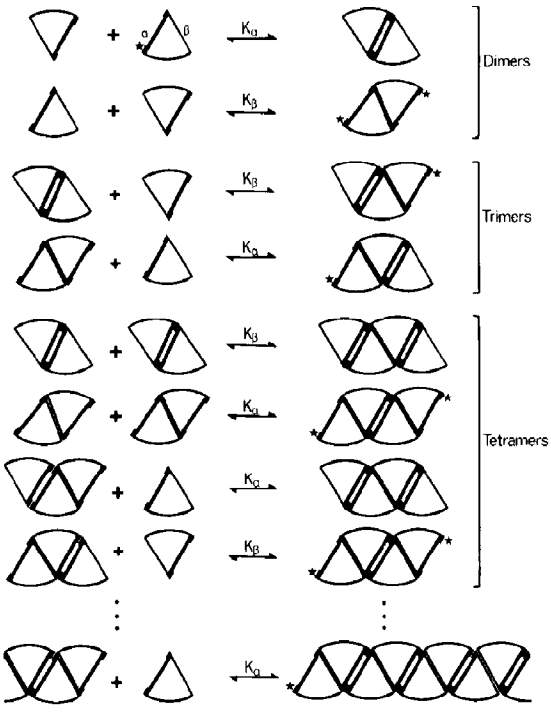

Fig. 1. Diagrammatic representation of the new model [4,7], for the indefinite self-association of insulin in solution discussed in the text. The wedge-shaped monomer has two faces, labelled α and β, available for like-like interaction to form two types of dimer with association constant K_α and K_β, respectively. The face labelled α is that involved in 'monomer-monomer' interactions in the hexamer (about the 2-fold axis OP [5]) and contains the receptor binding site, here marked by a star. Polymeric forms of insulin are assembled by the addition of monomers either through α-α or β-β interactions, resulting in a solution containing all types of polymer in equilibrium with monomer. It can be seen that representatives of each polymeric form have either one or two free receptor binding sites.

2. Results and discussion

2.1. Binding of non-monomeric insulin species

In considering the phenomenon of 'negative cooperativity' in insulin receptor binding, De Meyts [8] presented results (fig. 2) which show the effect reaching a maximum at an insulin concentration near 10^{-7} M and then falling away to zero as the insulin concentration is increased further, up to about 10^{-4} M. On the same curve, the author plotted what he called 'the theoretical % of dimers' and observed that this increased in exact correspondence with the decrease in negative cooperativity. Two points may now be made with respect to the curve obtained by De Meyts [8] and redrawn in fig. 2. Firstly, the curve is calculated on the basis of a dimerization constant of 2×10^5 M^{-1} and the assumption that dimerization only is occurring over the concentration range in question. In fig. 2 I have plotted the actual percentage of all types of dimer and of dimers containing two exposed receptor binding sites for the presently favoured model of insulin association at pH 7 [4]. It can be seen that the fraction of these dimers, while low, is significant over the concentration range of interest but does not follow the decreasing curve of negative cooperativity. In fact, the plot presented by De Meyts [8] represents not dimer but all species other than monomer. Secondly, the very fact of the decrease in the binding

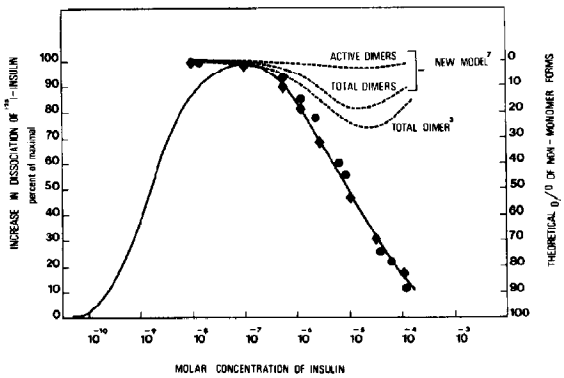

Fig. 2. Change in negative cooperativity with total insulin concentration (———) redrawn from ref. 8. The fraction of insulin existing as dimer when the dimerization constant is 2×10^5 M^{-1} and it is assumed that only dimerization is occurring [8] is also shown (◆). The dashed lines show the fraction of dimeric forms of insulin that can be calculated for the model represented in fig. 1 with $K_\alpha = 5.8 \times 10^4$ M^{-1}, $K_\beta = 0.85 \times 10^4$ M^{-1} at pH 7.0, $I = 0.2$, $T = 25°C$ [7] and for an earlier model for the association of insulin under the same conditions [3]. In the former, 'active dimers' means those with two free receptor binding sites (see fig. 1); in the latter, no dimers have such free sites. The filled circles are calculated on the basis of the model represented in fig. 1 as the fraction of all forms of insulin except monomer as a function of total insulin concentration.

effect referred to as negative cooperativity with the increasing fraction of insulin species other than monomers implies that species other than monomer must also be binding to receptor – total monomer is increasing with insulin concentration so, for the effect to appear, other molecules must be competing with monomer for the receptor sites. Saunders [9] recognized this and because current models for the insulin association allowed only for the existence of dimer and higher polymers with the accepted receptor binding site being inaccessible, he argued for the existence of a different site and suggested a region on the insulin A chain. It is now clear that this is not necessary, since both the proposed model for the association [4] and the result in fig. 2 allow insulin dimers (and higher polymers) to bind to receptors. It might also be noted that the effect identified in fig. 2 is evidence in favour of the proposed new model for the association of insulin in solution, since only this model generates binding species other than monomer.

Fig. 2 also implicates species other than dimer in reducing the apparent negative cooperativity because dimer alone is not present in a large enough fraction to reduce the effect to the required extent. Cuatrecasas and Hollenberg [10] suggested that the dimerization itself might be responsible for generating the binding effect denoted negative cooperativity and under certain conditions binding theory predicts that this is feasible [11]. It seems unlikely that such an effect can be the only one operating since some insulins that apparently do not dimerize in solution do show negative cooperativity. Also, they do not show the effect of reduction in the negative cooperativity at higher concentrations [8]. This is quite logical in terms of their known structures and biological activities. On the one hand, enough of the amino acid residues incorporating the receptor binding region are conserved to allow binding and (demonstrably) negative cooperativity. On the other, enough are altered to lower the ability to associate to undetectably low levels in solution and to confer a concomitant low potency, possibly via reduced flexibility to conformational change and/or lowered ability to cross-link receptors on the membrane.

2.2. Cross-linking of receptors

In the latter connection it may be added that the ability of insulin monomers to associate through two different sets of interactions was invoked in a previous paper [12] as a possible mechanism for cross-linking insulin receptors and promoting the clustering phenomenon which may be a factor in biological activity. The current association model (fig. 1) allows for the possibility of pre-existing insulin dimers and higher polymers binding to receptors, thus providing the additional potential for insulin bridges of various lengths between receptors. Why then does the apparent negative cooperativity fall off with increasing insulin concentration? On the evidence of fig. 2 the explanation does seem to be connected with the formation of insulin polymers. It has been pointed out before [12] that systems like this in which cross-linking can occur have the potential to generate binding responses resembling negative cooperativity and it may be that the observed reduction in the effect at high concentration is related to an inability of some (perhaps all but one) of the binding species of the present self-association model to cross-link receptors. Conformational changes may be involved. The possible requirement for a conformational change in insulin, either before or after its interaction with receptor, has been commented upon [13]. It is clear that the interconnections between the biological potency of insulins and their potential to self-associate, to change conformation, and to induce negative cooperativity and receptor clustering are complex. The development of binding theory specifically related to our present picture of the association pattern of insulin in solution and of the properties of receptors should provide a powerful method for elucidating which of these factors is significant in producing insulin's observed effects.

Acknowledgements

I would like to thank Professor L.W. Nichol and Mr. A.E. Mark for helpful discussions.

References

1 P.D. Jeffrey and J.H. Coates, Biochemistry 5 (1966) 489.
2 A.H. Pekar and B.H. Frank, Biochemistry 11 (1972) 4013.
3 P.D. Jeffrey, B.K. Milthorpe and L.W. Nichol, Biochemistry 15 (1976) 4660.
4 L.W. Nichol, M.J. Sculley, P.D. Jeffrey and D.J. Winzor J. Theor. Biol. 109 (1984) 285.
5 T.L. Blundell, G.G. Dodson, D.C. Hodgkin and D.A. Mercola, Adv. Protein Chem. 26 (1972) 279.
6 R.A. Pullen, D.G. Lindsay, I.J. Tickle, T.L. Blundell, A. Wollmer, G. Krail, D. Brandenburg, H. Zahn, J. Glieman and S. Gammeltoft, Nature 259 (1976) 369.
7 A.E. Mark, P.D. Jeffrey and L.W. Nichol, Proc. Aust. Biochem. Soc. 16 (1984) 17 (Abstr.).
8 P. de Meyts, in: Hormones and cell regulation, eds., J. Dumont and J. Nunez vol. 4. (Elsevier/North-Holland, Amsterdam, 1980) p. 107.
9 D.J. Saunders, Diabetologia 23 (1982) 386.
10 P. Cuatrecasas and M.D. Hollenberg, Biochem. Biophys. Res. Commun. 62 (1975) 31.
11 L.W. Nichol and D.J. Winzor, in: Protein-protein interactions, eds. C. Frieden and L.W. Nichol (John Wiley and Sons, New York, 1981) p. 337.
12 P.D. Jeffrey, Diabetologia 23 (1982) 381.
13 E.J. Dodson, G.G. Dodson, R.E. Hubbard and C.D. Reynolds, Biopolymers 22 (1983) 281.