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Association of Zn-free Insulin

KJELD MARCKER

Universitetets Fysisk-Kemiske Institut, Copenhagen, Denmark

In this investigation the association of Zn-free insulin in dependence on concentration and pH has been investigated by means of osmometry. The minimum molecular weight at acid pH has been found to 12 000, and at basic pH to 6 000. The association monomer to dimer seems to proceed by a reaction which is different from the one leading to association of dimers to higher degrees of association.

Molecular weights for insulin determined by several methods have already been published.[1–6] In these investigations it has been observed that the molecular weight of insulin is dependent upon pH, temperature, ionic strength and protein concentration of the solution.

The insulin used in these experiments was crystalline Zn-insulin and therefore it seemed worth while to investigate the association of Zn-free insulin in aqueous solution. Fredericq [5] has found indications of association of insulin molecules by means of Zn-ions. On account of this finding it was therefore expected that a difference between crystalline Zn-insulin and Zn-free insulin would exist, and this was found to be the case.

The insulin used was Zn-free pig insulin prepared from crystalline Zn-insulin by precipitation at pH 2 with NaCl. The colloid osmotic pressures were measured by means of the inverted micro-osmometer described by Christiansen and Jensen [7]. The semipermeable membranes were made according to Adair [8]. The membranes prepared by this method have proved to be impermeable to molecules with molecular weights as low as five to six thousands. The collodion solution used was made in the following way: 4 g of completely dry pyroxolin (Parlodion Mallinckrodt) were dissolved in a mixture of 50 ml of anhydrous alcohol and 50 ml of anhydrous ether; to this mixture were added 6 ml of anhydrous ethylene glycol. The preparation of the semipermeable membranes turned out to be a difficult problem. In the drying process the membranes shrink, thus giving rise to tensions in the membranes. Besides the membranes lose their elasticity which makes it very difficult to place them on the capillary. In order to avoid these difficulties smaller modifications in the membrane making procedure have been tried, but unfortunately without success. On account of the above mentioned irregularities about 50 % of the membranes had to be discarded. The tension in the membranes could easily be detected, because in the case of tension it was impossible to obtain a straight line in the interpolation diagram.

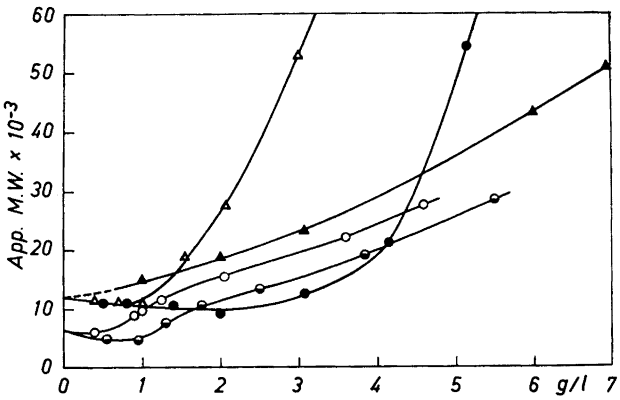

Fig. 1. Association of Zn-free insulin: Ordinate: Apparent molecular weight; Abscissa: Concentration in g/l. △ pH = 2.10; ● pH = 2.98; ▲ pH = 7.70; ○ pH = 8.31; ◒ pH = 9.10. Temp. 20°C.

Fredericq [5] and Gutfreund [3] have observed a specific anion effect upon the association of crystalline Zn-insulin. In this investigation no attempt has been made to evaluate a possible anion effect upon the association of Zn-free insulin.

The buffers used were: HCl-KCl, citrate-HCl and veronal-HCl. All buffers were adjusted to the ionic strength 0.2 with KCl. According to our experience the influence of the Donnan-effect is then negligible. The results are shown in Fig. 1.

It should be mentioned that the points in the diagram refer to apparent molecular weights and not to the true molecular weights. On account of the association it is impossible to extrapolate to infinite dilution in order to obtain the true molecular weight. The difference, however, between the true and the apparent molecular weight is probably not great, because of the relatively small electrical charge on the insulin molecule.

The surprisingly high degree of association in the acid region might be explained as a "salting out" effect. At pH 2 and 3 insulin is easily precipitated by KCl. At acid pH Fredericq [5] has obtained evidence for the existence of the insulin monomer in very dilute solution. In the present investigation there is no indication for the existence of the monomer at acid pH. On the contrary the results strongly support the generally accepted view that the minimum molecular weight in acid solution is 12 000 which corresponds to the dimer. In nearly neutral solution crystalline zinc insulin is reported to have a molecular weight of 36 000. The results of this investigation show that this is not the case with zinc-free insulin. The results reported here indicate a molecular weight of 12 000 at a pH about 7.7. Schlichtkrull [9] has shown that the minimum amount of zinc in crystalline zinc insulin is two atoms pr 36 000. It therefore seems reasonable to assume that three molecules having a molecular weight of 12 000 are associated by means of two zinc ions. Until recently it was generally accepted that the monomer insulin molecule did not exist in aqueous solution. By sedimentation experiments at pH 9 and 10 Fredericq [4],

however, demonstrated the presence of the monomer in dilute aqueous solution. The results reported here are in agreement with the findings of Fredericq as they clearly show that in dilute basic solution insulin molecules exist as monomers. By closer examination of the curves representing the results obtained at pH 8.31 and pH 9.10, a difference in the course of the curves in the concentration range 0 to 1.5 g/l compared to that from 1.5 g/l to higher values is apparent. This might indicate that the reaction of monomers to dimers is different from the reaction of dimers to higher association compounds.

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REFERENCES

1. Gutfreund, H. *Biochem. J.* **40** (1946) 432.
2. Gutfreund, H. *Ibid.* **42** (1948) 544.
3. Gutfreund, H. *Ibid.* **50** (1952) 564.
4. Fredericq, E. *Nature* **171** (1953) 570.
5. Fredericq, E. *Arch. Biochem. Biophys.* **65** (1956) 218.
6. Tietze, F. and Neurath, H. *J. Biol. Chem.* **194** (1952) 1.
7. Christiansen, J. A. and Jensen, C. E. *Acta Chem. Scand.* **7** (1953) 1247.
8. Adair, G. S. *Biochem. J.* **62** (1955) XXVI.
9. Schlichtkrull, J. *Acta Chem. Scand.* **10** (1956) 1455.

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