INTERACTION OF INSULIN WITH METAL(II) IONS

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1. Introduction

The importance of protein–metal ion interactions for biological activity and physiology of proteins is apparent in the examle of insulin, which is stored in the pancreatic β-cells in microcrystals with high content of Zn^{2+} [1,2]. It is also known that Ca^{2+} and Mg^{2+} have an important role in the mechanism of secretion and action of insulin [2,3]. In vitro studies have shown that presence of Zn^{2+} and other metals (e.g., Cd^{2+}, Co^{2+}) facilitates the formation of insulin crystals [4,5] and aggregates [6,7]. The hypothesis about coordination of Zn^{2+} to insulin through histidine residues has been confirmed by crystallographic studies [8].

The influence of Zn^{2+} on the electronic spectra of insulin has been studied [9] in acid media. This question has been touched recently in [10,11]. This paper deals with the interaction of divalent metal cations with insulin in neutral and mildly alkaline solutions, i.e., under pH values near to the physiological.

Interaction of the insulin with Zn^{2+}, Cd^{2+}, Co^{2+}, Ca^{2+} and Mg^{2+} has been studied by ultraviolet difference spectroscopy. In the neutral media, the presence of Zn^{2+}, Cd^{2+} and Co^{2+} causes the red shift of tyrosyl bands in the insulin spectrum. Interaction of insulin with Ca^{2+} and Mg^{2+} at pH 7–8 as well as with all metals in acid medium did not lead to spectral changes. The plausible interpretation consists in the conformational changes of insulin aggregates probably near the tyrosines of insulin A chain.

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2. Materials and methods

Insulin (beef) was supplied by Sigma, London (lot no. 24 C 3130, 24.1 units/mg). Zn^{2+} content (0.3%) was determined by atomic absorption spectroscopy (Varian 1200, Melbourne); water content was checked to be 10.5%.

Preparation of stock solution of Zn^{2+}-free insulin: a solution of insulin (4 g/l) in 0.01 M HCl was dialysed 36 h against 1.0 mM HCl to remove Zn^{2+} [12], then shortly against 0.01 M NaOH to obtain alkaline pH. Ionic strength was adjusted by dialysis against 0.1 M NaCl overnight. For each measurement, stock solution was diluted by 0.1 M NaCl to 1.6×10^{-4} M and finally pH was adjusted.

Difference spectra were made in a two-cell arrangement: one cell contained insulin solution with perturbant (metal chloride); the second cell contained insulin solution alone. To assure the same insulin concentration in both cuvettes, a solution of metal chloride was placed into the cell, dried, then insulin solution added. Spectra were recorded on the Specord UV VIS (Carl Zeiss, Jena) spectrophotometer with the EZ 2 (Labora, Prague) external recorder, 1 cm-quartz cuvettes were used in a thermostated holder (25°C). Insulin concentration was checked spectrophotometrically ($\epsilon_{275} = 6100$) [13].

3. Results and discussion

Interaction of insulin with Zn^{2+} at pH 6.8–8.0 resulted in the difference spectrum (fig.1) which corresponds to the red shift of the tyrosine absorption bands. In the spectra, no significant perturba-

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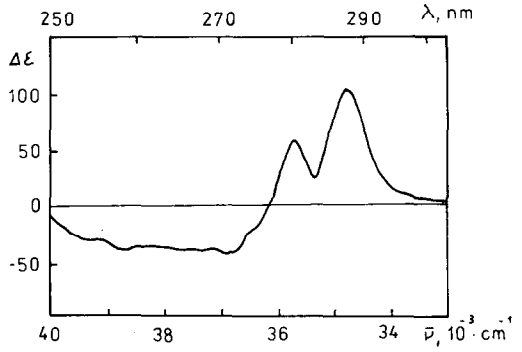

Fig.1. Insulin difference spectrum. Zn^{2+}, 1.6×10^{-4} M; Zn^{2+}-free insulin, 1.6×10^{-4} M; pH 7.45.

tion of phenylalanine chromophores (250–270 nm) was present. Figure 2A shows the dependence of the perturbation magnitude (expressed as $\Delta\epsilon' = \Delta\epsilon_{288} - \Delta\epsilon_{273}$) on the insulin : Zn^{2+} molar ratio, fig.2B dependence on the pH at 6.8–8.0. In the acid medium (pH 2–3) no difference spectra were observed. It can be easily explained by the coordination of Zn^{2+} through histidine residues, which are protonized in acid media. The absence of the insulin spectrum perturbation [9] is thus consistent with this interpretation.

The nature of the interaction of both transition metals (Cd^{2+}, Co^{2+}) with insulin is similar. Observed difference spectra are nearly the same in shape but greater in magnitude (fig.2A). On the other hand, both Ca^{2+} and Mg^{2+} do not perturb the insulin spectrum either in neutral or in acid medium. This fact

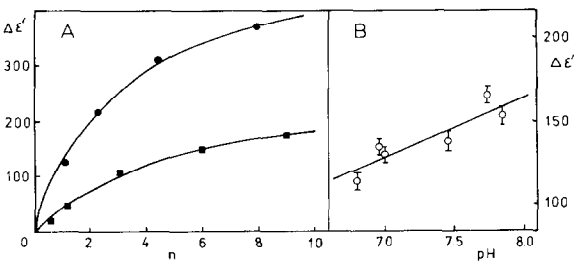

Fig.2. (A) Dependence of the perturbation magnitude $\Delta\epsilon'$ (defined as $\Delta\epsilon_{288} - \Delta\epsilon_{273}$) on the no. metal atoms/insulin hexamer n. Full circles, Cd^{2+}; full squares, Zn^{2+}; pH 7.45. (B) Dependence of $\Delta\epsilon'$ on the pH (Zn^{2+}; 2.4×10^{-4} M). Insulin, 1.6×10^{-4} M.

may be ascribed to the different ability of transition and alkaline earth metals to complex with nitrogen bases [14]. In the light of absence of the insulin conformational changes due to Ca^{2+}/Mg^{2+}–insulin interaction, it seems that the regulatory mechanisms [2,3] involving Ca^{2+} and/or Mg^{2+} cannot include the direct metal–insulin interaction.

The appearence of the difference spectra in the case of neutral and mildly alkaline media can be ascribed to:

(1) Direct interaction of metal cations with tyrosine residues of insulin (parallel to this with histidine);
(2) Conformational changes due to insulin aggregation conditioned by presence of metal;
(3) Conformational changes of existing aggregates caused by interaction with metal, and combination of all these possibilities.

To verify (1), a separate experiment with tyrosine and Zn^{2+} has been carried out. No perturbation of tyrosine chromophore spectra caused by interaction with Zn^{2+} has been observed. To discuss the involvement of insulin aggregation, one must consider that aggregations of monomers to dimers as well as of dimers to hexamers must involve conformational changes in the neighborhood of phenylalanine, namely the B 24 and B 25 for dimer and B 1 for hexamer formation [8,9]. However, such conformational changes have not been detected (see fig.1). Moreover, the perturbation of tyrosyls continues after the metal : insulin molar ratio when only hexamers are present [7]. Thus, the aggregation cannot be the principal cause of observed spectral changes. In the light of the reasons presented, the plausible interpretation is conformational changes of the insulin aggregates presented in solution in the neighborhood of tyrosine residues*. Among 4 tyrosine residues (A 14, A 19, B 16, B 26) in the insulin molecule the tyrosines of A chain are still (after formation of hexamer) exposed [8,9,11]. Because the observed red shift of tyrosine bands corresponds to the lower accessibility of chromophore [15], changes of tyrosine exposure occur most probably in tyrosines of insulin A chain.

* A similar conclusion about the interaction of Zn^{2+} with insulin was reached independently by CD study of Arquilla et al. (1978) Biochem. J. 175, 289–297; whose work was brought to our attention after this manuscript had been prepared

Although it is likely that the active insulin species is the insulin monomer [8], the importance of metal–insulin interaction follows from the fact that these monomers originate from the pancreatic microcrystals with Zn^{2+} high content. Thus, during the transport of secreted insulin from portal circulation to the target place a dissociation of zinc–insulin complex probably occurs which is accompanied with a conformational change. The differences in the insulin conformation in the presence and absence of zinc have been shown [16] to be reflected in the changed ability of insulin to react with antibodies and, moreover, in vitro and in vivo experiments have shown that the presence of Zn^{2+} strongly enhances the binding of insulin to liver plasma membranes.

It can be concluded that the observed conformational changes, which most probably involve the A-chain tyrosines at the surface of the insulin molecule, are a key event in the proposed [16] mechanism for fine modulation and control of insulin.

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