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The pH-dependence of the deuterium exchange of insulin

In continuation of previous experiments[1,2,4] the rate of the reaction of deuterium-loaded pork insulin with H_2O at 0° was studied at different hydrogen ion concentrations. The method applied is outlined in reference[1] and described in detail in references[3,4,5]. The results are seen in Fig. 1 wehre n_i is the number of deuterium atoms exchanged per insulin monomer (Mw 5777). In the experiments at pH 2.66 and 3.50 the protein was loaded with deuterium at these pH values while in that at pH 7.1 the protein was deuterated at pH 3, lyophilized, dried (see [1,3,4]), and dissolved in H_2O + NaHO to bring pH to 7.1. The process of dissolution took about 2 minutes. For the sake of comparison the values experimentally found for the number of deuterium atoms exchanged (n) were corrected by means of the formula

$$n_i = n - i$$

where i is the net charge — reckoned with sign — of the monomer during the loading with deuterium. The justification of this correction is found in the fact that the hydrogen atoms involved in the ionization process must be instantaneously exchangeable[6]. It will immediately be clear that, independent of pH, the values of n_i will all approach 85 valid for isoelectric insulin, the value 89 previously given for insulin with net charge + 4 being used as a basis (see [1,4]). As appears from Fig. 1 the rate of the exchange reaction is strongly dependent upon the hydrogen ion concentration and falls with decreasing pH. At pH 7.1 the exchange is complete after 3 hours. The curves are generally of the same type as those found for ribonuclease[6] and confirm the observations made by LENORMANT AND BLOUT[7] in their study of other proteins (bovine serum albumin and ovalbumin). Since preliminary experiments on β-lactoglobulin have shown the same pH dependence of the exchange of this protein it would appear that the phenomenon is fairly general. Our experiments cannot at present serve as a basis for quantitative calculations of the relationship between pH and rate of exchange. Refined technique and additional experimental material are required to solve this problem. They show, however that the exchange mechanism is more complicated than tentatively assumed in a recent article[2].

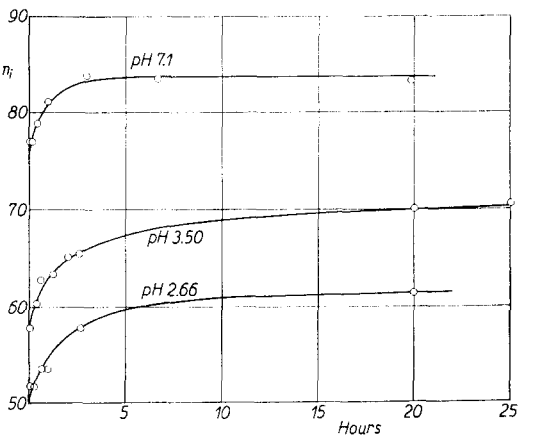

Fig. 1.

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