ACTA CHEMICA SCANDINAVICA 13 (1959) 2036–2038

Preparation of a New Zinc-rich Insulin Compound

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It is shown, that crystalline insulin, when treated with alcoholic solutions of zinc chloride in concentrations up to 10 % w/w in the limit combines with two moles of Zn and five moles of $ZnCl_2$. Thus the stoichiometric composition of the compound is insulin,2Zn,5$ZnCl_2$. The compounds with $MgCl_2$, $CaCl_2$, $NiCl_2$, $MnCl_2$, $CdCl_2$, and $CoCl_2$ have been prepared in a similar way. Their compositions are approximately: (Insulin, 1/2 Zn, 1/2 Mg); (Insulin, 1/2 Zn, 1 Ca); (Insulin, 1/2 Zn, 1 1/2 Ni) $NiCl_2$; (Insulin, 1/3 Zn, 1 2/3 Mn)6 $MnCl_2$; (Insulin, 1/3 Zn, 1 2/3 Cd)3 $CdCl_2$; (Insulin, 2 Co)5$CoCl_2$.

For a long time it has been known that zinc combines with insulin forming a zinc-insulin complex. Hallas-Møller *et al.*[1] have shown that the zinc content in crystalline insulin under certain conditions rises to a figure of about two moles per Sanger unit or even higher. It is the purpose of this paper to describe the preparation of a new zinc-rich crystalline insulin compound which in chemical composition differs from those previously described.

The following technique is used in preparing the zinc-rich insulin: 0.5 g of crystalline insulin (0.8 % Zn) is suspended in solutions containing various amounts of dry $ZnCl_2$ in 50 ml of absolute alcohol. The suspension is shaken overnight and filtered the following day on a Büchner funnel. The insulin is washed twice with 20 ml of absolute alcohol and once with 30 ml of dry ether. It is then dried in air, and finally the zinc-content is determined by titration with EDTA and Eriochromblack T as indicator. The results are shown in Fig. 1. By gradually increasing the concentration of $ZnCl_2$ in the suspension it is found that the zinc-content in insulin at concentrations higher than 10 % remained constant at a value of about 7.7—7.8 %. Assuming a molecular weight of 6 000 (a Sanger unit = 5 778 + 6 % which is the water content of dry insulin) this figure corresponds fairly closely with seven zinc atoms per molecule of insulin. The zinc-rich insulin contains chloride and the content of chloride is then determined according to Volhard. The results of these analyses are that five atoms of the total zinc-content in insulin are present

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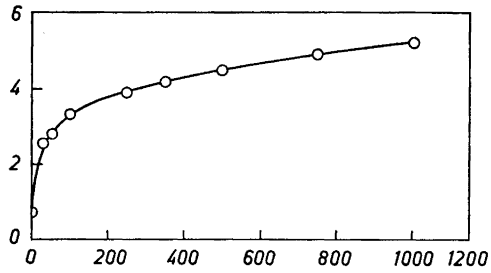

Fig. 1. The reaction between insulin and $ZnCl_2$. Ordinate: moles of Zn per unit of 6 000. Abscissa: mg $ZnCl_2$ in 50 ml of absolute alcohol.

as the chloride. The stoichiometry of the zinc-rich insulin compound is thus as follows: Insulin,2Zn,5$ZnCl_2$.

In order to evaluate the influence of the crystal size of insulin on the zinc uptake from the solution, two different insulin preparations with a crystal diameter of about 10 μ and 20 μ, respectively, were prepared and subjected to the procedure described above. The results were:

Crystal diameter appr. 10 μ	Crystal diameter appr. 20 μ
% Zn in insulin 7.79 =	% Zn in insulin 7.42 =
7.15 moles per unit of 6 000	6.81 moles per unit of 6 000

Although the zinc contents of the two preparations are not identical it is evident that the crystal size is relatively unimportant for the zinc uptake.

A few other zinc salts have been tried. These are $Zn(OOCCH_3)_2$, $ZnBr_2$ and ZnI_2. The results expressed in stoichiometric formulas are: (Insulin, 2Zn) $4ZnBr_2$; (Insulin, 2Zn)$3ZnI_2$ and for $Zn(OOCCH_3)_2$, (Insulin, 2Zn). These results might be explained by assumption of steric hindrances on the sites of binding between the insulin and the metal salt.

By suspending the zinc-rich insulin in absolute alcohol a small amount of the $ZnCl_2$ diffuses out of the crystal until an equilibrium has been attained. By suspending the zinc-rich insulin in water, however, all the $ZnCl_2$ rapidly

Table 1. The reaction between insulin and metal salts. The zinc content in untreated insulin is 0.8 % = 0.73 atoms per unit of 6 000.

Metal chloride	% metal in insulin	moles of metal per unit of 6 000	moles of Zn per unit of 6 000	Proposed stoichiometric formula
$MgCl_2$	0.23 [a]	0.57	0.62	(insulin,$\frac{1}{2}$Zn,$\frac{1}{2}$ Mg)
$CaCl_2$	0.65 [a]	0.97	0.55	(insulin,$\frac{1}{2}$Zn,1 Ca)
$NiCl_2$	2.60	2.65	0.51	(insulin,$\frac{1}{2}$Zn,1$\frac{1}{2}$Ni)1$NiCl_2$
$MnCl_2$	6.80	7.42	0.34	(insulin,$\frac{1}{2}$Zn,1$\frac{1}{2}$Mn)6$MnCl_2$
$CdCl_2$ [b]	9.24	4.93	0.29	(insulin,$\frac{1}{3}$Zn,1$\frac{1}{3}$Cd)3$CdCl_2$
$CoCl_2$	6.70	6.81	<0.1	(insulin,2Co)5$CoCl_2$

[a] These preparations contain no chloride.

[b] $CdCl_2$ is only sparingly soluble in absolute alcohol. The figure quoted is thus probably not the final value.

diffuses out, and the zinc content of the insulin decreases to a value of about 2 moles per Sanger unit. This effect shows that the binding between $ZnCl_2$ and insulin is unstable in aqueous solution.

The reaction between insulin and other metal chlorides has also been investigated. The metal salts used are $NiCl_2$, $CdCl_2$, $MgCl_2$, $MnCl_2$, and $CoCl_2$. The results are shown in Table 1.

As indicated in Table 1 the incorporation of metal chloride into insulin replaces zinc from the insulin. This phenomenon proved to have a remarkable effect on the crystal structure of insulin. When the preparations of insulin contain more than 1/3 mole Zn per unit of 6 000 it is still crystalline, but containing less than this amount the crystal structure is deteriorated. A more detailed report on the above mentioned effects including an X-ray crystallographic investigation on insulin preparations containing varying amounts of zinc will be published elsewhere.

I am gratefully indebted to Dr. med. H. Christensen, Roskilde Medical Company, for his guidance and valuable suggestions and to Miss Tove Ahrens for excellent technical assistance.

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Received August 13, 1959.