Acta Cryst. (1965). **19**, 686

Insulin: preliminary X-ray studies of chloride crystals and a crystalline derivative. By BARBARA W. LOW and CELIA C. H. CHEN, *Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York* 10032, *U.S.A.*

(*Received* 19 *January* 1965)

A new orthorhombic crystalline form of zinc-free porcine insulin has been described by Sundby (1962). The crystals grow when a 1 % solution of porcine insulin in 0·5*N* hydrochloric acid is stored for some days at 4°C. The preparations described here were prepared by a modified procedure (Sundby, 1963), in which the 1 % solutions in 0·5*N* hydrochloric acid were stored for one or a few days at 4°C, and then allowed to stand at room temperature. Under these conditions, insulin is slowly deamidated. The method gives large (2 mm × 0·5 mm × 0·4 mm) well-developed crystals elongated along *a* and bounded by {*h*0*l*} and {*hk*0}. A prismatic habit is sometimes observed, {010}, {001} terminating in {*h*0*l*}, {*hk*0}. The crystals show low birefringence with $\alpha \| c$, $\beta \| a$, $\gamma \| b$.

X-ray studies were carried out at room temperature using the Buerger precession camera and Cu $K\alpha$ radiation. The wet crystals were mounted in the usual manner in thin-walled glass capillaries. Xylene-dried crystals were studied in air. The densities of the wet crystals were measured in water-saturated bromobenzene–*m*-xylene solution, and of the xylene-dried crystals in dried bromobenzene–*m*-xylene solution by the modified gradient tube procedure (Low & Richards, 1952; Richards & Thompson, 1952).

Insulin chloride crystals were also studied after immersion in a solution of hydrochloric acid and *N* potassium chloride at *p*H 3·0, and in such solutions containing three different heavy-atom salts. One of the latter preparations with 0·0005*M* K_2HgI_4 gave a heavy-atom derivative which is being studied in detail in this laboratory. A search for other heavy-atom derivatives is in progress.

All the crystals examined have the space group $P2_12_12_1$. There are four molecules (of molecular weight 5777) in the asymmetric unit. The minimum observed spacing for the normal wet crystals is 1·9 Å, and for the xylene-dried crystal 10 Å.

In calculating the weight fraction of protein in the crystal given in Table 1, we assumed four molecules of protein per asymmetric unit. However, in determining the compatible value of the partial specific volume of the liquid of crystallization, employing the equation

$$1/D_m = X_p v_p + (1 - X_p) v_{l.c.}$$

where

D_m = density of wet crystal
X_p = weight fraction of the non-aqueous component
$1 - X_p$ = weight fraction of the aqueous component
v_p = partial specific volume of the protein
$v_{l.c.}$ = partial specific volume of the liquid of crystallization

we assumed a non-aqueous component of protein and 24 chloride ions (Cl^-) per asymmetric unit. This corresponds to chloride binding appropriate to the net charge on the protein below *p*H 1·5.

We are grateful to Dr F. Sundby, Novo Research Institute, Copenhagen, for a generous gift of insulin chloride crystals and of pork insulin for our studies. This investigation was supported in part by Public Health Research Grants AM 01320-07 and AM 01320-08 from the National Institute of Arthritis and Metabolic Diseases; in part by a Research Grant GB 157 from the National Science Foundation; in part (BWL) by a Public Health Service research career program award 5-K3-GM-15, 246-05 (06) from the National Institute of General Medical Sciences; and in part by a Public Health Service Fellowship (CCHC) 1-Fl-GM-22, 756-01 from the National Institute of General Medical Sciences.

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Table 1. *Data for insulin chloride preparations*

Insulin chloride	Cell dimensions (Å) *a*	*b*	*c*	Volume (Å^3)	D_m	Weight fraction of protein	Density of liquid of crystallization* (1)	(2)
Wet	103 ± 0·4	50·2 ± 0·2	39·7 ± 0·2	2·05 × 10^5	1·23 ± 0·01	0·612 ± 0·012	1·004	1·022
Xylene-dried	100 ± 2·0	44·2 ± 0·5	30·4 ± 0·6	1·34 × 10^5	1·32 ± 0·01			
Soaked in:								
N KCl, *p*H 3·0	104 ± 0·5	49·9 ± 0·2	37·6 ± 0·3					
N KCl, *p*H 3·0 + 0·0005 *M* K_2HgI_4	104 ± 0·8	49·9 ± 0·6	38·1 ± 0·3					

* These values are calculated on the assumption that the partial specific volume of the protein in dilute solution is appropriate for the wet crystal study (see Low & Richards, 1954) and using values: (1) $v_p = 0{\cdot}71$ and (2) $v_p = 0{\cdot}72$ (Oncley *et. al.*, 1952).