# The crystal forms and molecular weight of α-chymotrypsinogen
## An X-ray study

An examination has been made of all available crystal forms of chymotrypsinogen, where possible using X-ray methods; in addition the molecular weight of the protein has been deduced from the X-ray data for one form (Type D) which is salt-free. The crystal forms which we have investigated are the following.

*Type A*. These crystals are precipitated from an aqueous solution containing 25% saturated ammonium sulphate by addition of sodium hydroxide. They were first described by NORTHROP AND KUNITZ[1]. They are very thin needles, whose length can without too much difficulty be made as great as 0.7 cm; even crystals as long as this, however, are too thin to give interpretable X-ray photographs.

*Type B*. This type of crystal was described by KUNITZ[2], and is prepared from alcoholic solution by raising the pH from 4 to 5. Morphologically type B crystals are tetragonal prisms with prism faces in the form {110}, and which terminate in pyramidal faces {111}.

*Type C*. In the course of preparing type B crystals according to KUNITZ's methods we found that by varying the protein concentration it was possible to obtain larger or smaller proportions of another crystal form, superficially similar to type B but in fact possessing different crystal symmetry. These are orthorhombic prisms bounded by prism faces {101} and terminated by dome faces {011}. Fig. 1 of the above-mentioned paper by KUNITZ[2] appears to show a mixture of the two types.

*Type D*. This is a new form of salt-free (iso-ionic) crystal prepared by Dr. P. E. WILCOX, who was kind enough to send us very large specimens for examination. He prepared them by passing a solution of chymotrypsinogen through an Amberlite exchange resin column, the upper part of which was mixed-bed IR 120-$H^+$ and IR 400-$OH^-$, and the lower part a layer of IR 120-$H^+$. The ion-free solution crystallized spontaneously on emergence from the column. The crystals generally form four-sided prisms flattened on {001} and terminated by pinacoidal faces {010}.

*X-ray examination*

We were able to study the diffraction patterns of crystals of types B, C, and D. We used the rotating-anode X-ray tube designed by Mr. D. A. G. BROAD and a Buerger precession camera, with which we obtained 9° or 17° pictures using Cu Kα radiation. Type B crystals were found to be tetragonal with space group $P4_12_12$, while types C and D were orthorhombic with space group $P2_12_12_1$. The cell dimensions are given in Table I. Some X-ray work has also been done on type B crystals by CARLISLE[3].

TABLE I

UNIT CELL DIMENSIONS AND SPACE GROUPS OF CRYSTAL FORMS OF α-CHYMOTRYPSINOGEN

| *Type* | *Space group* | *Cell dimensions* | | | *Mols/cell* |
|---|---|---|---|---|---|
| | | *a* | *b* | *c* | |
| B | $P4_12_12$ | 112.1 A | 112.1 A | 54.6 A | 8 |
| C | $P2_12_12_1$ | 105.1 | 60.4 | 77.0 | 8 |
| D | $P2_12_12_1$ | 42.6 | 54.6 | 91.9 | 4 |

Assuming that the molecular weight of the protein lies between 20,000 and 30,000 (see below) it may be concluded with some confidence that crystals of types B and C contain 8 molecules per unit cell (*i.e.* one per asymmetric unit in type B, two per asymmetric unit in type C), while those of type D contain 4 per unit cell (*i.e.* one per asymmetric unit).

We calculated Patterson projections for type D crystals, but these are not reproduced in the present paper because they contain no striking or readily-interpretable features.

*Determination of molecular weight*

Salt-free protein crystals, such as type D crystals of chymotrypsinogen, contain only protein and water, so it is possible to arrive simply at a value of the molecular weight of the protein by measuring (a) the volume of the unit cell, (b) the density of the crystal, and (c) its water content. The expression to be evaluated is

$$M = \frac{V w N \varrho}{n}$$

Where $V$ = volume of unit cell (ml)
$w$ = proportion by weight of protein in the crystal
$\varrho$ = density of crystal, g/ml
$n$ = number of molecules per unit cell

The determination of these quantities will be considered in turn.
(a) *Volume of the unit cell.* The value derived from the measured cell dimensions is 213,800 $A^3$; the standard deviation is estimated to be 1.2%.
(b) *Dry weight.* The difficulties inherent in the measurement of this quantity have been discussed by LOW AND RICHARDS[4]. Following their methods our practice was to dry crystals *in vacuo* over phosphorus pentoxide for 48 hours, by which time it was found by experiment that the crystals had settled down to a constant weight. We found $w$ = 0.642, and estimate the standard error of the measurements to be 2%.
(c) *Crystal density.* This was measured in a gradient tube by the methods developed by LOW AND RICHARDS[5], with the modification, demonstrated to us by Dr. RICHARDS, that the gradient tube was calibrated by weighing a glass plunger of known volume and density suspended at the same level as the crystal in its equilibrium position. The density of the crystals was found to be 1.215 with a standard deviation of 1%.

Inserting these values in the equation it is found that the molecular weight is 25,000 with a standard deviation of $\pm$ 800.

This value is in good agreement with more recent determinations of the molecular weight by other techniques all of which have yielded results somewhat higher than those current in the earlier literature*. Among these recent determinations may be mentioned the following: TIETZE AND NEURATH[6] obtained 25,000 by light scattering; NEURATH[7] interpreted the amino-acid data of LEWIS, SNELL, HIRSCHMANN AND FRAENKEL-CONRAT[8] in terms of a molecular weight of 25,000; GUTFREUND[9] obtained a value of 24,000 $\pm$ 500 by osmotic pressure measurement. Our value is also in good agreement with the most recent molecular weight determinations based on light-scattering measurements, amino-acid analysis and sedimentation and diffusion measurement, all of which converged[10] towards a value of about 25,000.

*Acknowledgements.* The authors wish to record their indebtedness to Dr. P. E. WILCOX of the Department of Biochemistry, School of Medicine, University of Washington, Seattle, U.S.A., for sending samples of salt-free chymotrypsinogen crystals; and to Dr. F. M. RICHARDS, lately of Harvard University, for demonstrating his technique of density determination by means of the gradient column.

M. M. BLUHM
J. C. KENDREW

*Medical Research Council Unit for Research on the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge (England)*

1 M. KUNITZ AND J. H. NORTHROP, *J. Gen. Physiol.*, 18 (1935) 433.
2 M. KUNITZ, *J. Gen. Physiol.*, 32 (1948) 265.
3 C. H. CARLISLE, personal communication (1953).
4 B. W. LOW AND F. M. RICHARDS, *J. Am. Chem. Soc.*, 76 (1954) 2511.
5 B. W. LOW AND F. M. RICHARDS, *J. Am. Chem. Soc.*, 74 (1952) 1660.
6 F. TIETZE AND H. NEURATH, *J. Biol. Chem.*, 194 (1952) 1.
7 H. NEURATH, in E. S. G. BARRON, *Modern Trends in Physiology and Biochemistry*, Academic Press, New York, 1952.
8 J. C. LEWIS, N. S. SNELL, D. J. HIRSCHMANN AND N. FRAENKEL-CONRAT, *J. Biol. Chem.*, 186 (1950) 23.
9 H. GUTFREUND, *Trans. Faraday Soc.*, 50 (1954) 624.
10 J. KRAUT, P. E. WILCOX, H. NEURATH AND R. D. WADE, to be published in *Biochim. Biophys. Acta.*

Received February 3rd, 1956

* Summarized by N. M. GREEN AND H. NEURATH in Chap. 25, Vol. II B of *The Proteins* (Eds. H. NEURATH AND K. BAILEY), Academic Press, New York, 1954.