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The three-dimensional structure of crystalline porcine pancreatic elastase

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Now at The Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol

[Plate 55]

1. Introduction

Elastase is a proteolytic enzyme obtained from pig pancreas, which shows a high degree of amino acid sequence homology with other serine proteinases, including bovine trypsin and chymotrypsin (Hartley, this volume, p. 77). It consists of a single polypeptide chain of 240 residues, which corresponds to the single polypeptide chain of trypsin, and the B and C chains of chymotrypsin. Elastase possesses a common catalytic mechanism with these enzymes but differs from them in its substrate specificity, cleaving peptide bonds on the carboxyl terminal side of amino acid residues lacking charged or aromatic side chains (Naughton & Sanger 1961).

Several workers have suggested that homologous enzymes with common catalytic mechanisms have very similar tertiary structures. This prediction was supported by Blow and his co-workers, who found that the two disulphide bridges present in trypsin, but absent in chymotrypsin, could be built into the molecular model of α-chymotrypsin with little or no distortion of the polypeptide chain (Sigler, Blow, Matthews & Henderson 1968), and by Hartley (this volume, p. 77) who has shown that the trypsin and elastase side chains can be substituted for those present in a skeletal molecular model of α-chymotrypsin with no gross distortions of the polypeptide chain.

To test these predictions, and also to discover the detailed structural differences responsible for the varying specificities of the serine proteinases, the three-dimensional structure investigation of elastase was undertaken.

2. The preparation and evaluation of heavy atom derivatives

As previously reported (Shotton, Hartley, Camerman, Hofmann, Nyburg & Rao 1968), elastase crystallizes from dilute sodium sulphate solution in the space group $P2_12_12_1$ with unit cell dimensions $a = 5.15$, $b = 5.80$, $c = 7.55$ nm, corresponding to a unit cell of four molecules, with one molecule per asymmetric unit.

Following the techniques developed for the inhibition of the active centre serine residue of chymotrypsin by sulphonyl fluorides (Sigler, Skinner, Coulter, Kallos, Braxton & Davies 1964; Sigler, Jeffery, Matthews & Blow 1966), we have prepared fully inhibited crystals of *p*-toluenesulphonyl elastase (tosyl elastase) and *p*-chloromercuribenzenesulphonyl elastase (PCMBS elastase) which are isomorphous with crystals of the native enzyme. These form a perfect isomorphous pair of derivatives in which the only difference is the replacement of a methyl group in tosyl elastase by a chloromercuri group in PCMBS elastase. Throughout this work tosyl elastase has been used as the 'parent' structure, rather than using the native enzyme, in order to exploit this high isomorphism in the structure determination, and to provide an active site

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marker to aid in the interpreation of the three-dimensional electron density map. Difference Fourier projections between tosyl elastase and native elastase, obtained by methods similar to those described below for the heavy atom derivatives, are shown in figure 1. The electron density of the additional sulphonyl group of tosyl elastase is clearly visible. It can be seen that the differences in conformation between these two structures are very small and are confined to the local environment of the tosyl group.

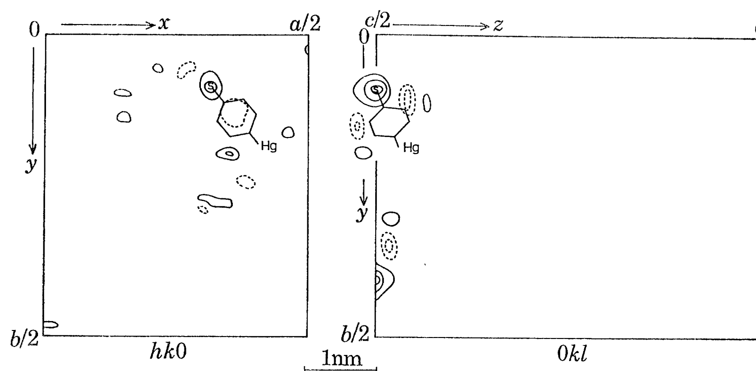

FIGURE 1. $h\,k\,0$ and $0\,k\,l$ difference Fourier projections calculated at a resolution of 0.35 nm between tosyl elastase and native elastase, with phases derived for the three-dimensional structure determination of tosyl elastase (see §3). The orientation of the tosyl group is indicated by the line drawing superimposed on the contours, the position of the sulphur atom being marked by the letter S and that of the mercury atom present in PCMBS elastase (see figure 3*c*) by Hg. Zero contours omitted.

X-ray diffraction photographs of the principal projections of tosyl and PCMBS elastase were taken using a Buerger precession camera, and measured by the computer controlled densitometer developed by Arndt and his co-workers (Arndt, Crowther & Mallet 1968). Easily interpretable difference Patterson projections were calculated from these intensity data, as is shown for example in figure 2*a*, from which the coordinates of the single mercury atom were determined. Further heavy atom derivatives suitable for use with the multiple isomorphous replacement technique (Green, Ingram & Perutz 1954) were prepared by soaking crystals of tosyl and PCMBS elastase in uranyl nitrate solutions. Two-dimensional X-ray diffraction data were collected from these derivatives by the methods described above. The difference Patterson projections of uranyl tosyl elastase (see figure 2*b*) show that a uranyl ion, probably $UO_2(OH)_n^{(n-2)-}$ (Blake, Koenig, Mair, North, Phillips & Sarma 1965), is strongly bound to a single site in these derivatives.

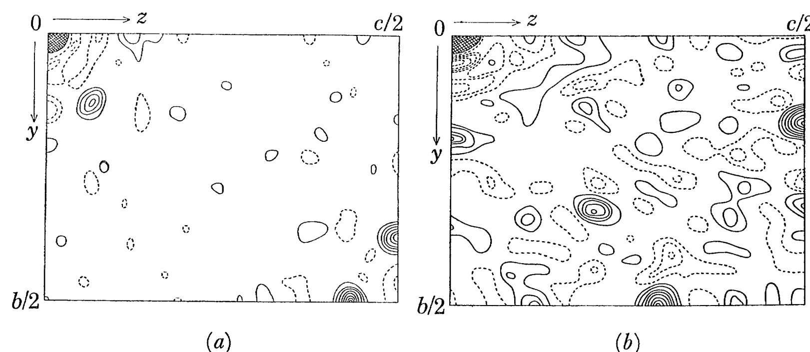

FIGURE 2. $0kl$ difference Patterson projections calculated using tosyl elastase as the parent structure, (*a*) of PCMBS elastase, at 0.28 nm resolution, (*b*) of uranyl tosyl elastase, at 0.35 nm resolution. Zero contours omitted.

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The potential usefulness of these derivatives in providing three-dimensional phase information was checked by calculating difference Fourier projections. Figures 3*a* and *b* show difference Fourier projections of uranyl tosyl elastase and uranyl PCMBS elastase, using phase information derived from PCMBS elastase, and figure 3*c* shows difference Fourier projections of PCMBS elastase calculated with phase information derived from uranyl tosyl elastase. The positions of the metal atoms in these derivatives are clearly visible. From these results it was obvious that these derivatives were suitable for use in determining the three-dimensional structure of tosyl elastase.

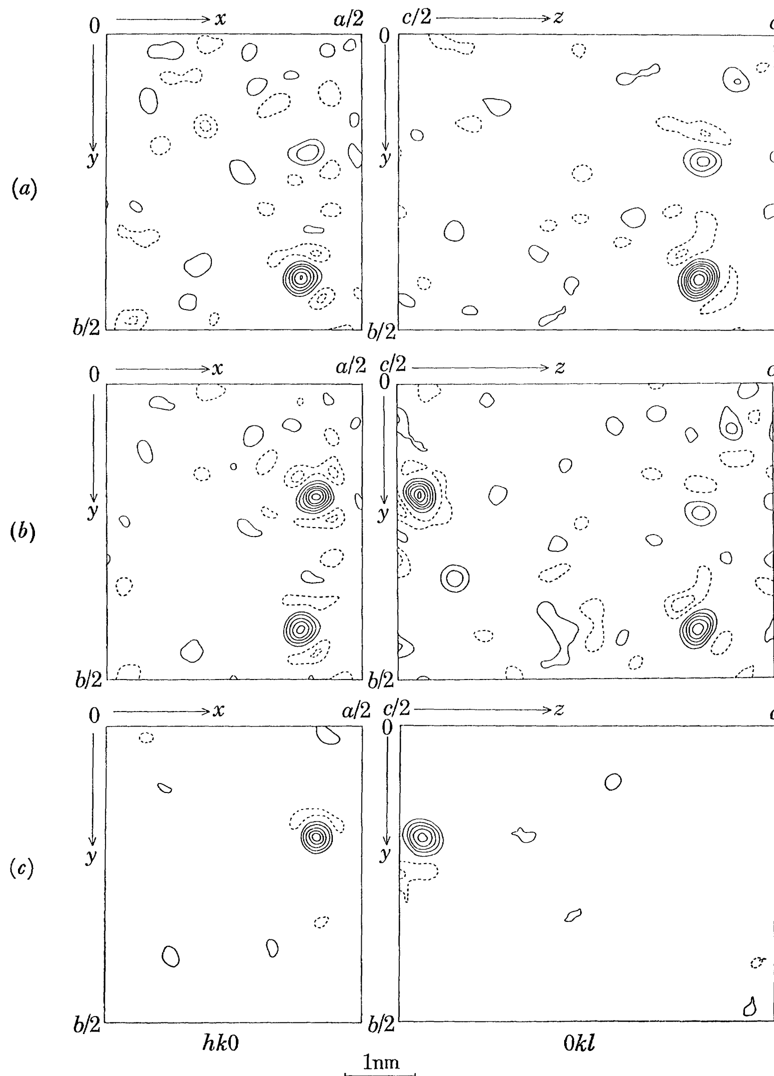

FIGURE 3. $hk0$ and $0kl$ difference Fourier projections calculated at a resolution of 0.35 nm, using tosyl elastase as the parent structure, (*a*) of uranyl tosyl elastase, with phases calculated using the PCMBS elastase mercury atom position, (*b*) of uranyl PCMBS elastase, with phases calculated using the PCMBS elastase mercury atom position, (*c*) of PCMBS elastase, with phases calculated using the uranyl tosyl elastase uranyl ion position. Zero contours omitted.

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3. The three-dimensional data collection and Fourier calculation

Atoms which are separated by more than the Van der Waal radii are resolved by an accurate structure determination at 0.35 nm resolution. It was therefore decided to determine the structure of tosyl elastase at this resolution, in the hope that this would permit a meaningful comparison to be made with the known structure of α-chymotrypsin (Matthews, Sigler, Henderson & Blow 1967).

Preliminary investigations showed that crystals of elastase derivatives are extraordinarily stable to X-irradiation and that three-dimensional data could be collected to a resolution of 0.35 nm from a single crystal of each derivative, without introducing errors in the data due to radiation damage. The use of a single crystal of each derivative eliminated several sources of error inherent in the normal practice of collecting three-dimensional data from several crystals, including variability in isomorphism, salt concentration and heavy atom site occupancies between crystals, and scaling errors introduced when data from several crystals are combined.

The 2700 Friedel related pairs of X-ray reflexions were measured from each derivative by an ordinate analysis procedure using a Hilger and Watts four-circle diffractometer connected on line to a Ferranti Argus computer (Watson, Shotton, Muirhead & Cox 1970). The X-ray intensity data were corrected for Lorentz, polarization and absorption factors, and the data from the heavy atom derivatives were then scaled to those of tosyl elastase. The heavy atom parameters obtained from the two-dimensional studies were first improved by the calculation of three-dimensional difference Patterson functions and then by using alternate cycles of phase determination and least squares refinement (Dickerson, Kendrew & Strandberg 1961). Subsequent difference Fourier calculations confirmed that PCMBS elastase was a single site mercury derivative and that those derivatives containing uranyl ions possessed one major uranyl site, of occupancy comparable to that of the mercury site, and one secondary site which, while significant, was considered to have too low an occupancy to warrant inclusion in the final phase determination. After including the anomalous dispersion data of the three heavy atom derivatives, the mean error in phase angle, averaged over all reflexions, was 28°.

The electron density distribution of tosyl elastase was then calculated, using the centroid weighting scheme of Blow & Crick (1959), in sections perpendicular to the a axis, at intervals of $a/60$, $b/60$ and $c/80$. These sections were displayed in contour form on an oscilloscope screen and recorded on 35 mm film (Gossling 1967). Fourier sections were then enlarged to the appropriate size and drawn on to perspex sheets.

4. The interpretation of the electron density map

The electron density map of tosyl elastase shows the following features. The molecular boundaries of the elastase molecules in the unit cell are clearly visible, each molecule being an oblate elipsoid having approximate dimensions 5.3 nm along the a axis, 4.0 nm along the b axis and 3.8 nm along the c axis. Neighbouring molecules have relatively few points of contact, and are separated by wide interstices filled with salt solution, in which no strong features appear in the electron density map. In contrast, the density associated with the atoms of tosyl elastase stands out clearly. The course of the single polypeptide chain can be traced unambiguously from one end to the other as a column of continuous high density. At regular intervals along the chain side chains can be seen branching off in various directions (see figure 4). The peaks of the main

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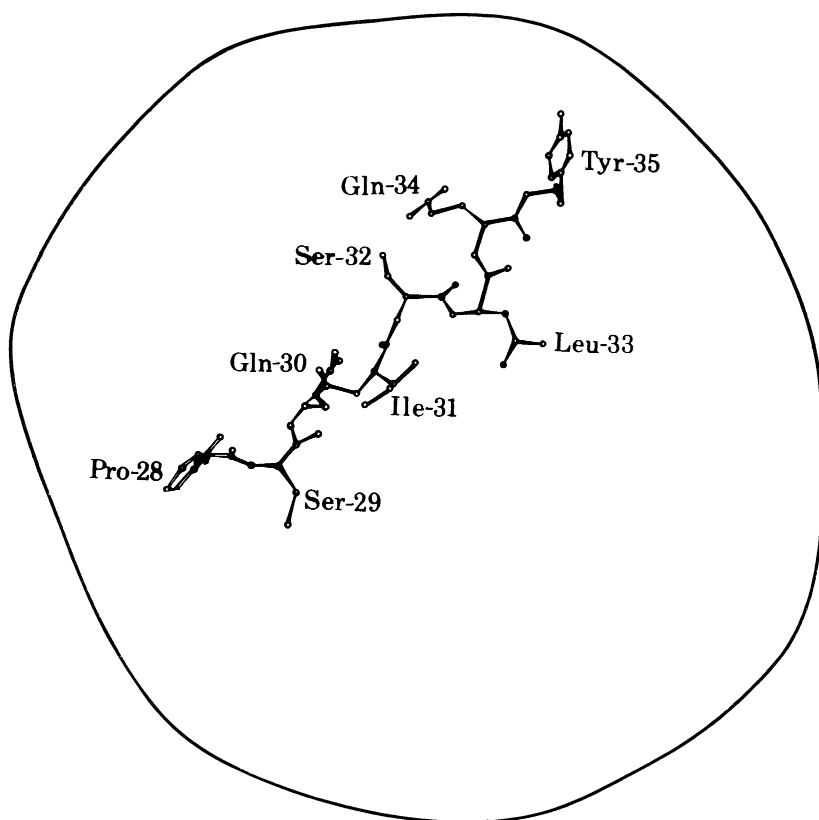

Overlay to figure 4, p. 115.

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chain density are located at those branch points, which correspond to the α-carbon atoms of the amino acid residues, and not at the positions of the peptide carbonyl groups, a feature of higher resolution electron density maps. At no point in the map is the density less well defined than at any other, except perhaps for one small loop near the amino terminal end of the chain, which protrudes from the molecular surface and which has no contacts with neighbouring molecules.

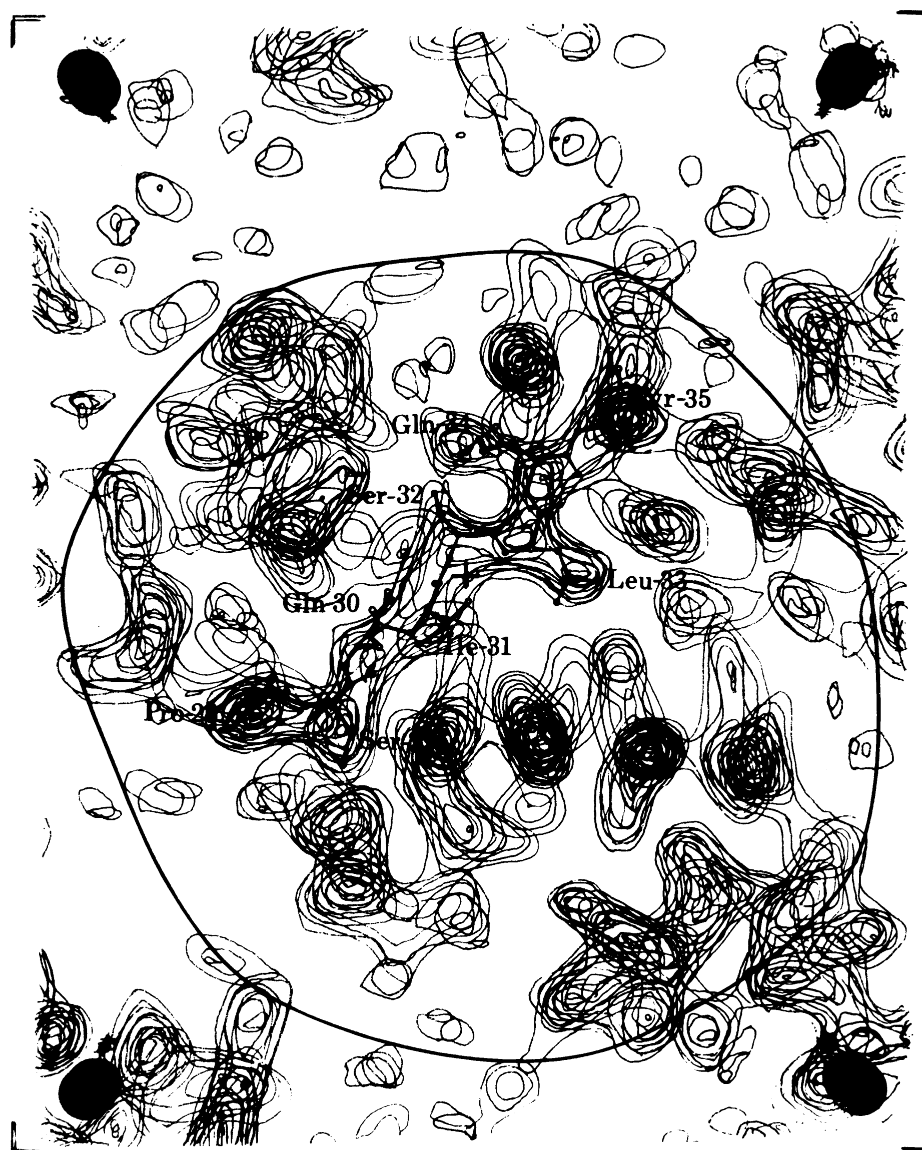

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FIGURE 4. A photograph of seven sections from the complete three-dimensional electron density map of tosyl elastase. The overlay indicates the molecular boundary and also the positions of the atoms in a region of the polypeptide chain which lies in the plane of these sections. The amino acid sequence in this region is -Pro-Ser-Gln-Ile-Ser-Leu-Gln-Tyr-. The side chain of Gln-30 lies below the plane of the sections.

Several of the amino acid side chains are sufficiently well defined to enable one to identify them unambiguously. The four cystine disulphide bridges are each clearly visible as rods of high density linking two parts of the main chain, while both methionine residues can be recognized from their dense sulphur peaks, as can the tosyl group attached to the active centre serine

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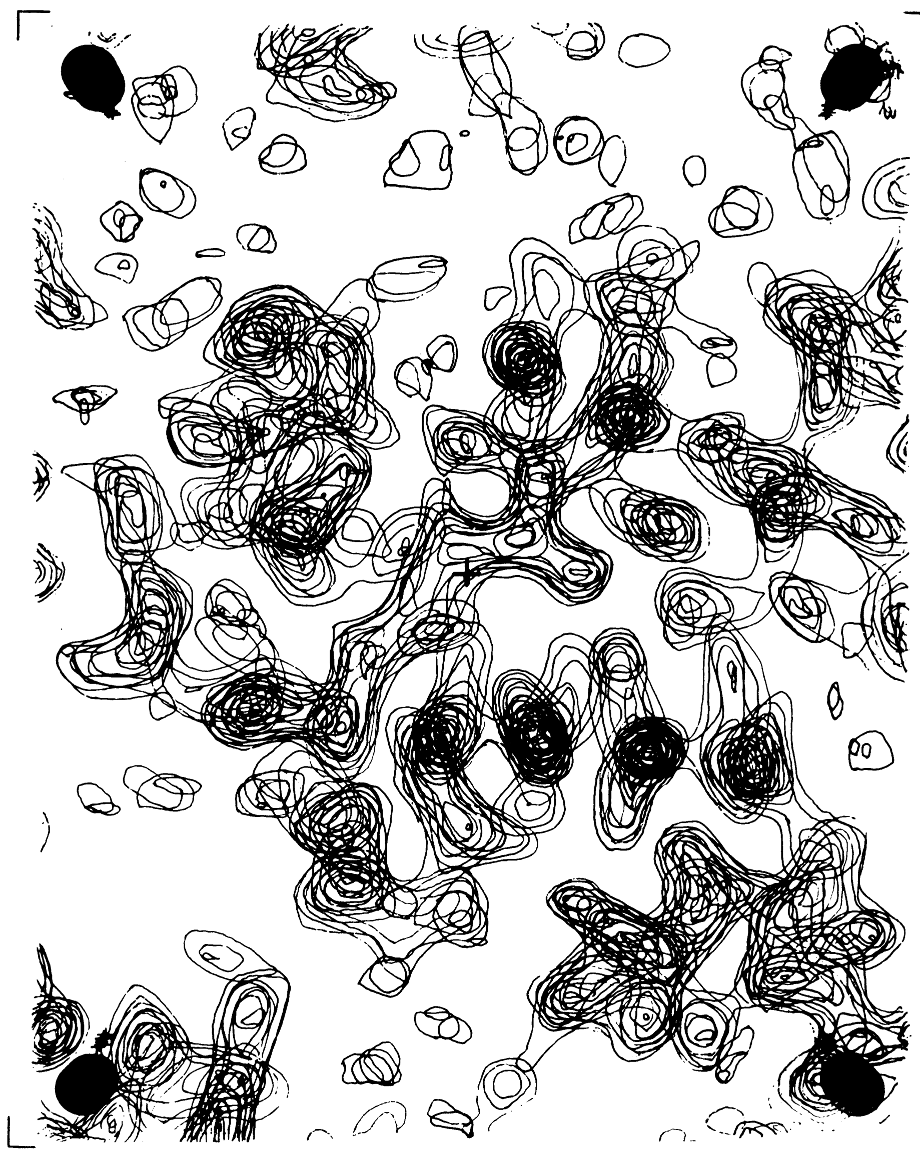

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residue. The positions and orientations of the aromatic rings of tryptophan, tyrosine, phenylalanine and histidine side chains are in most cases clearly defined. Glycine residues are usually distinguishable from residues bearing side chains. The side chains of other residues appear as stalks or bulges of various sizes, and are not identifiable from the electron density alone. Figure 4 shows a stack of seven sections from the complete three-dimensional electron density map. The molecular boundary and the position of the several pieces of the main chain which traverse these sections can be seen. The overlay shows the molecular boundary and the detailed conformation of one region of the polypeptide chain, from Pro-28 to Tyr-35, which lies in the plane of these sections.†

For the detailed interpretation of the map we relied heavily on our almost complete knowledge of the amino acid sequence of elastase (D. M. Shotton & B. S. Hartley, unpublished results).

With the aid of a half-silvered mirror device similar to that described by Richards (1968), which increased the speed and accuracy of model building, we constructed a skeletal model of tosyl elastase, on the scale 20 cm to 1 nm, using parts designed by one of us (H.C.W.) in conjunction with Dr J. C. Kendrew and now supplied by Cambridge Repetition Engineers. This model satisfactorily accounts for all the electron density in the map, except that side chains are omitted where the amino acid sequence information is still incomplete.

5. The comparison with α-chymotrypsin

5.1 *General*

In order to compare the structure of elastase with that of α-chymotrypsin it was necessary to transport the molecular model of elastase to the Medical Research Council Laboratory of Molecular Biology, Cambridge, to make a direct comparison with the skeletal model of α-chymotrypsin built by Blow and his co-workers. The comparison showed the tertiary structure of the two enzymes to be very similar. For example, the N-terminal valine residue of elastase, like the N-terminal isoleucine of the B chain of α-chymotrypsin, has its amino group buried in the interior of the molecule in a position where it can form an ion pair with the carboxyl group of Asp-194, which is adjacent to the active centre serine residue. Similarly the C-terminal end of the polypeptide chain forms three turns of right-handed α-helix. Figure 5, plate 55 shows the skeletal model of elastase tilted to bring out this similarity, and should be compared with the diagram of the conformation of the chains of α-chymotrypsin in the paper of Sigler *et al.* (1968).

Description of plate 55

Figure 5. An overall view of the skeletal model of tosyl elastase. The model is tilted to facilitate a comparison with the published diagram of the main chain configuration of α-chymotrypsin. The red tubing indicates the course of the polypeptide chain. The sulphonyl group attached to the active centre serine residue is represented by the black and grey balls.

Figure 6. A stereophotograph of the skeletal model of tosyl elastase showing the stereochemical arrangement of groups at the catalytic site.

Figure 7. A stereophotograph of the skeletal model of tosyl elastase showing the stereochemical arrangement of groups around the 'tosyl hole'.

† To aid in the comparison between various serine proteinases we have, in this paper, used the numbering of the amino acid residues in the chymotrypsinogen A sequence to describe the position of amino acid residues in these homologous enzymes (see Hartley, this volume, p. 77).

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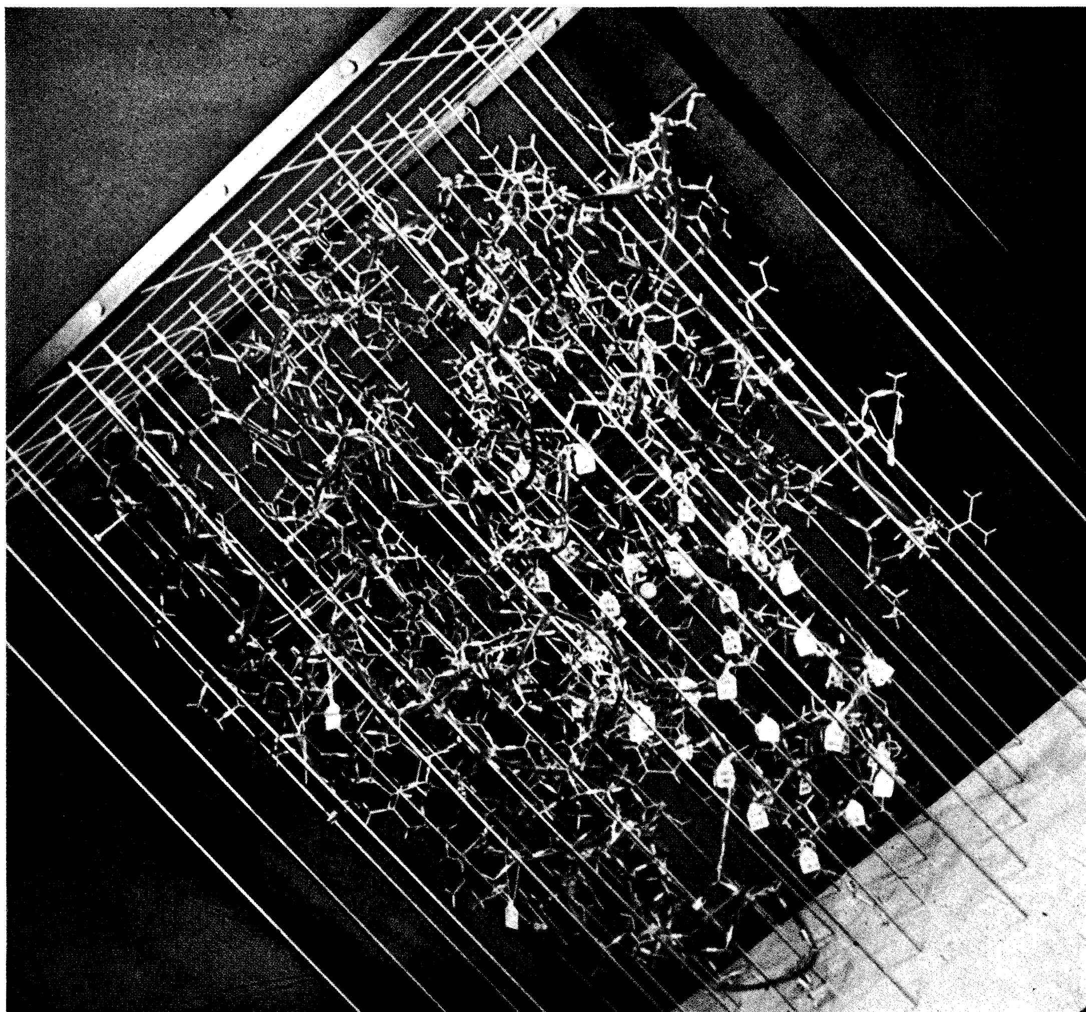

Figure 5

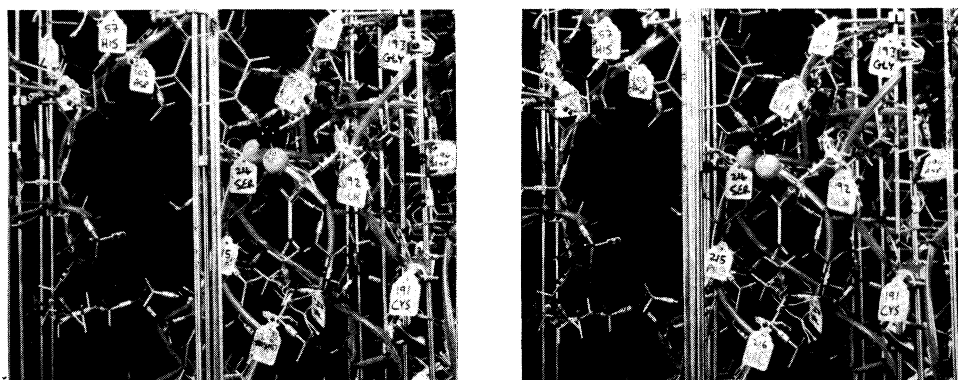

Figure 6

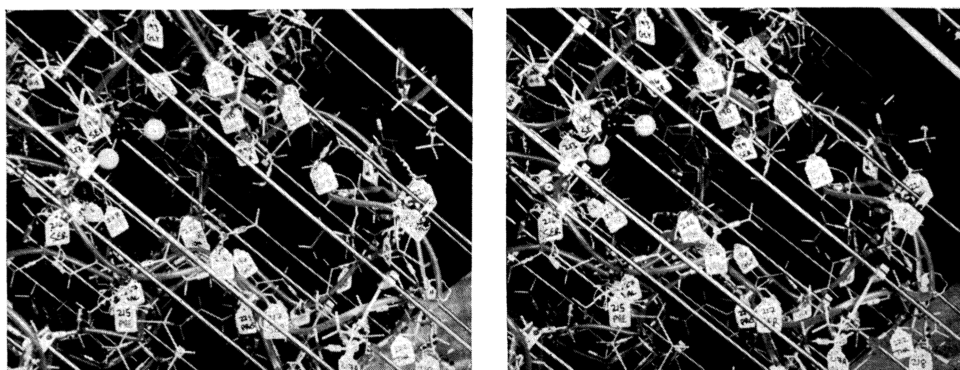

Figure 7 (for legends see facing page)

(*Facing p.* 116)

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For details of the overall configuration of the elastase polypeptide chain the reader is referred to this paper. Not only does the conformation of the main chain closely resemble that of the B and C chains of α-chymotrypsin, but also the orientation of the side chains in the two enzymes appears to be very similar, especially in the interior of the molecules.

Differences between the local conformations of the chains do occur, however, where elastase has insertions or deletions of amino acids with respect to the chymotrypsin chains. These all occur on the outside of the elastase molecule, usually at the ends of external loops, and do not disturb the identity of conformation of those parts of the chains common to both enzymes.

5.2 *The catalytic site*

Elastase is known to contain all the amino acid residues implicated in the catalytic mechanism of α-chymotrypsin (Blow, Birktoft & Hartley 1969; Birktoft, Blow, Henderson & Steitz, this volume, p. 67), and believed to be common to all serine proteinases. In the tosyl elastase molecule the active centre Ser-195 and Asp-102 have the same relative conformation as those in tosyl α-chymotrypsin. However, the tosyl group attached to Ser-195 of tosyl elastase does not occupy the same position in the molecule as that of tosyl α-chymotrypsin, for reasons which will be discussed below. Instead it interacts with the imidazole side chain of His-57, presumably displacing it from its native configuration in which, by analogy with α-chymotrypsin, it could form a charge relay system with Asp-102 and the active centre serine residue identical to that found in α-chymotrypsin. If this proves to be the case, it would provide an explanation in molecular terms for the common catalytic mechanism which these two enzymes exhibit.

It should be noted that, unlike the situation found in crystals of α-chymotrypsin, the active centre region of elastase is not blocked by intermolecular contacts with neighbouring molecules, but faces one of the largest interstices in the crystal structure.

Figure 6, plate 55 shows the spatial arrangement of the groups in the catalytic site of tosyl elastase.

5.3 *The specificity site*

Blow and his co-workers have shown that *N*-formyl-L-tryptophan binds to α-chymotrypsin in the 'tosyl hole', the site occupied by the tosyl group in tosyl α-chymotrypsin. They have concluded, therefore, that this hole is occupied by the aromatic side chains of chymotrypsin substrates (Birktoft *et al.*, this volume, p. 67). This hole is also present in the three-dimensional structure of tosyl elastase, but its mouth is partially occluded by a valine residue at position 216, which in chymotrypsin and trypsin is a glycine residue. Furthermore, in tosyl elastase the bottom of the hole is partly filled by a threonine residue at position 226, replaced in both trypsin and chymotrypsin by a second glycine residue. This offers an explanation for the difference in orientation of the tosyl group of tosyl elastase with respect to that of tosyl α-chymotrypsin and, more important, offers a possible explanation for the differing specificities of these two enzymes, since bulky aromatic groups will be sterically prevented from entering the 'tosyl hole' of elastase. This is illustrated in figure 7, plate 55, which also shows clearly Ser-189, referred to by Hartley (this volume, p. 77) as being replaced by an aspartic acid residue in trypsin, and thus implicated in the substrate specificity of that enzyme for basic amino acid side chains.

6. Summary

The three-dimensional structure of tosyl elastase has been determined in sufficient detail to allow a molecular model to be built. Comparison of this preliminary model with the model

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of the homologous enzyme, α-chymotrypsin, has shown the conformation of the polypeptide chains of these two enzymes to be very similar, bearing out the predictions referred to in the introduction. Furthermore, by relying heavily on the studies of α-chymotrypsin by Blow and his co-workers, it has been possible to offer an explanation for the common catalytic mechanism and differing substrate specificities of these two enzymes. These results also support the proposals of Hartley concerning the three-dimensional structure and specificity of trypsin.

The elastase crystallographic system has been shown to possess many inherent technical advantages over those of other proteins whose structures have been determined. These will be most useful in our proposed future studies of elastase, which include an extension to very high resolution of the structure determination of tosyl elastase, a detailed study of the slight conformational differences which exist between tosyl elastase and the native enzyme, and an attempt to exploit the accessibility of the active centre of elastase in crystallographic studies of enzyme–substrate complexes. We will then be in a better position to answer in more detail questions concerning the structure, activity and specificity of this enzyme.

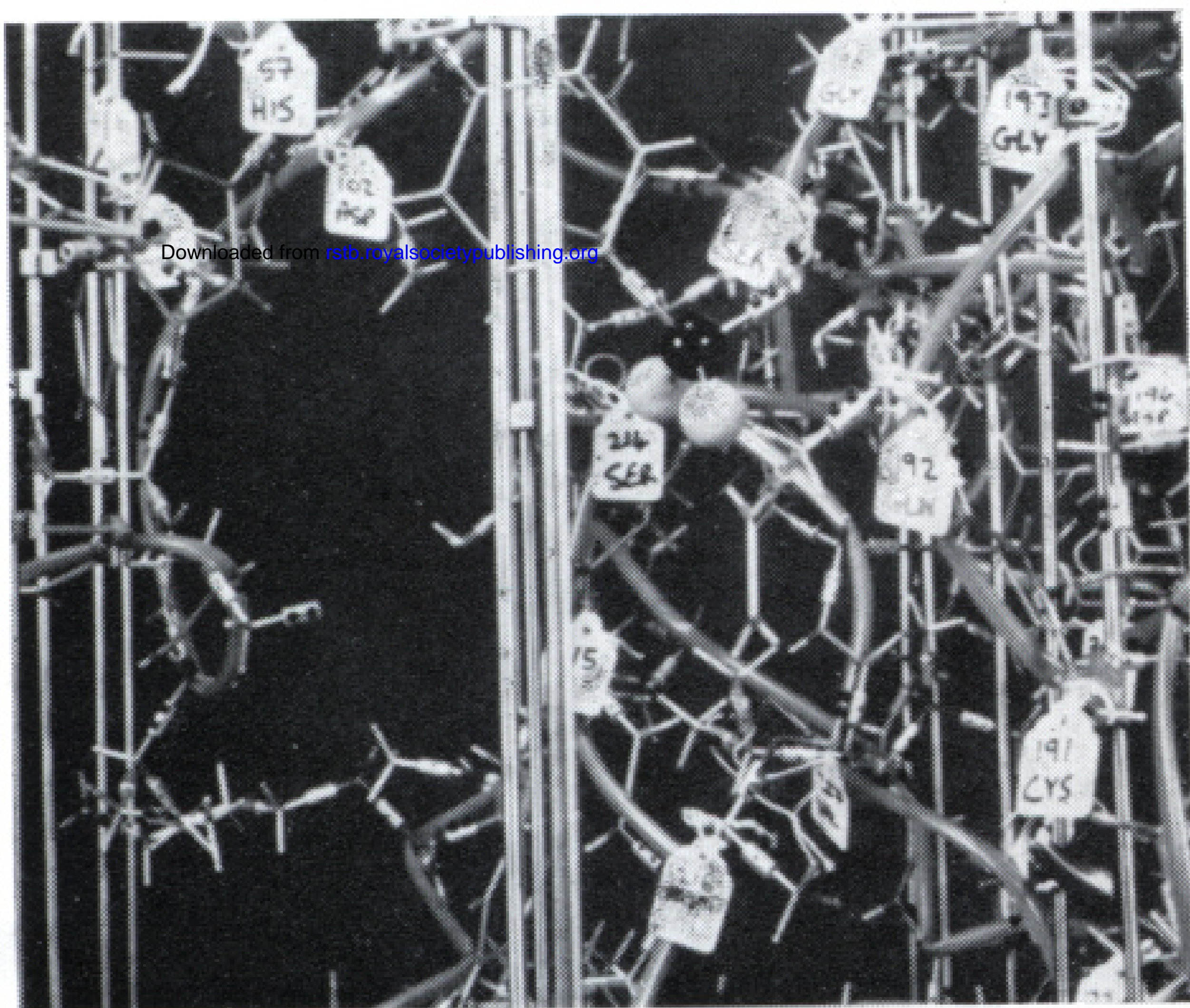
The data collection and calculations reported in this paper were carried out while the authors were members of the Medical Research Council Laboratory of Molecular Biology, Cambridge. Progress with this project would not have been so rapid had it not been for the help of our numerous colleagues, to whom we are very grateful. We would like to express our special thanks to Dr B. S. Hartley for suggesting this project and for his continued advice and encouragement; to Dr P. B. Sigler for the gift of the sulphonyl fluoride inhibitors; and to Dr H. Muirhead and Miss J. M. Cox, who carried out most of the three-dimensional data processing procedures, including the phase refinement and Fourier calculations. The Fourier interpretation and model building were carried out in the Molecular Enzymology Laboratory of the Department of Biochemistry, University of Bristol, using funds and equipment generously provided by the Science Research Council. We would like to thank Mr N. White, Dr P. Wendell and Mrs S. K. Watson for helping us to complete the model in time for this meeting.

One of us (D.M.S.) expresses thanks for the financial support of a Medical Research Council Scholarship and a Beit Memorial Medical Research Fellowship received during the course of this work.

References (Shotton & Watson)

Arndt, U. W., Crowther, R. A. & Mallett, J. M. W. 1968 *J. scient. Instrum.* (Series 2), **1**, 510.
Blake, C. C. F., Koenig, D. F., Mair, G. A., North, A. C. T., Phillips, D. C. & Sarman, V. A. 1965 *Nature, Lond.* **206**, 757.
Blow, D. M., Birktoft, J. J. & Hartley, B. S. 1969 *Nature, Lond.* **221**, 337.
Blow, D. M. & Crick, F. H. C. 1959 *Acta Crystallogr.* **12**, 794.
Dickerson, R. E., Kendrew, J. C. & Strandberg, B. E. 1961 *Acta Crystallogr.* **14**, 1188.
Gossling, T. H. 1967 *Acta Crystallogr.* **22**, 465.
Green, D. W., Ingram, V. M. & Perutz, M. F. 1954 *Proc. Roy. Soc. Lond.* A, **225**, 287.
Matthews, B. W., Sigler, P. B., Henderson, R. & Blow, D. M. 1967 *Nature, Lond.* **214**, 652.
Naughton, M. A. & Sanger, F. 1961 *Biochem. J.* **78**, 156.
Richards, F. M. 1968 *J. molec. Biol.* **37**, 225.
Shotton, D. M., Hartley, B. S., Camerman, N., Hofmann, T., Nyburg, S. C. & Rao, L. 1968 *J. molec. Biol.* **32**, 155.
Sigler, P. B., Blow, D. M., Matthews, B. W. & Henderson, R. 1968 *J. molec. Biol.* **35**, 143.
Sigler, P. B., Jeffery, B. A., Matthews, B. W. & Blow, D. M. 1966 *J. molec. Biol.* **15**, 175.
Sigler, P. B., Skinner, H. C. W., Coulter, C. L., Kallos, J., Braxton, H. & Davies, D. R. 1964 *Proc. natn. Acad. Sci. U.S.A.* **51**, 1146.
Watson, H. C., Shotton, D. M., Muirhead, H. & Cox, J. M. 1970 To be published.

Figure 5



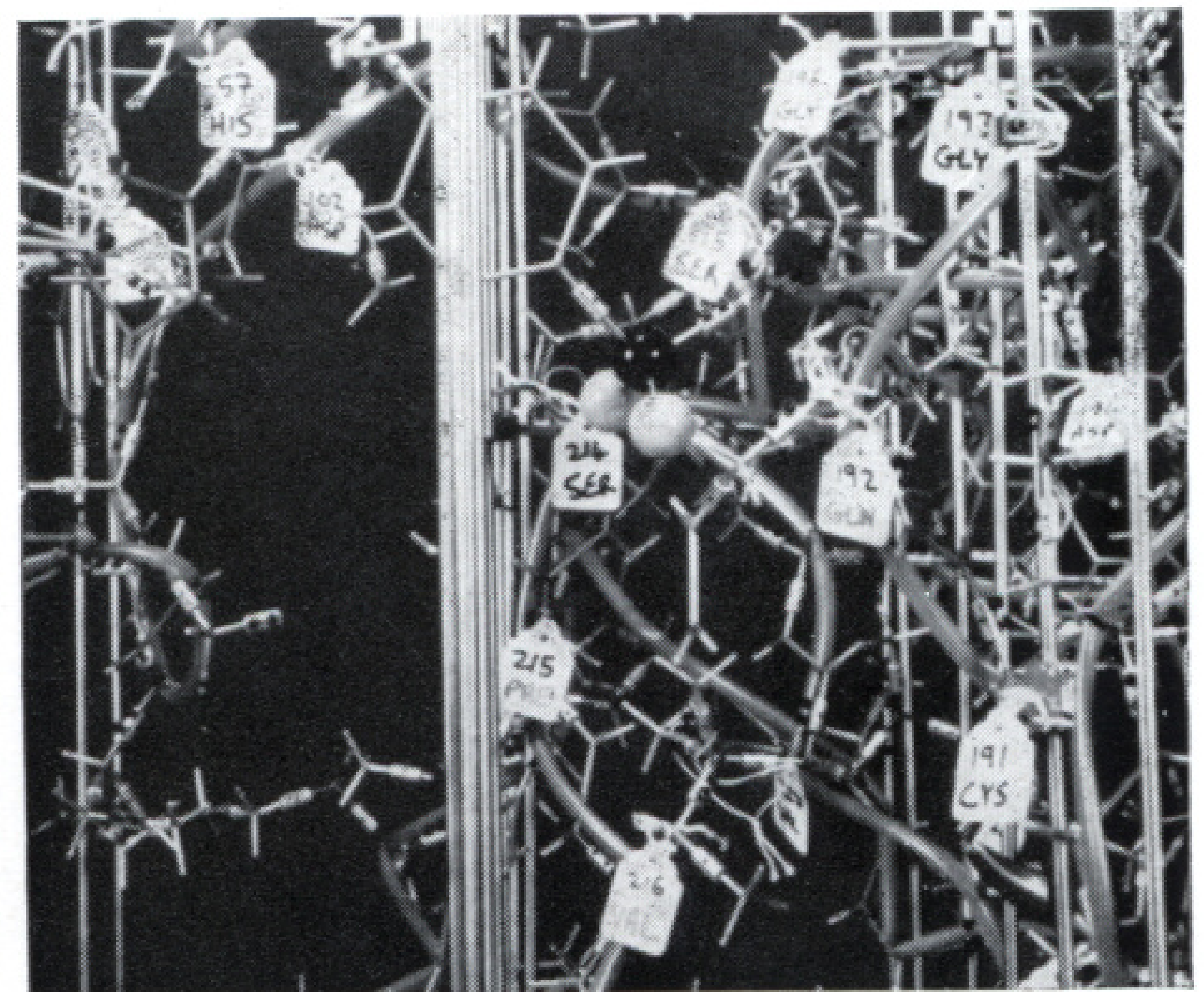
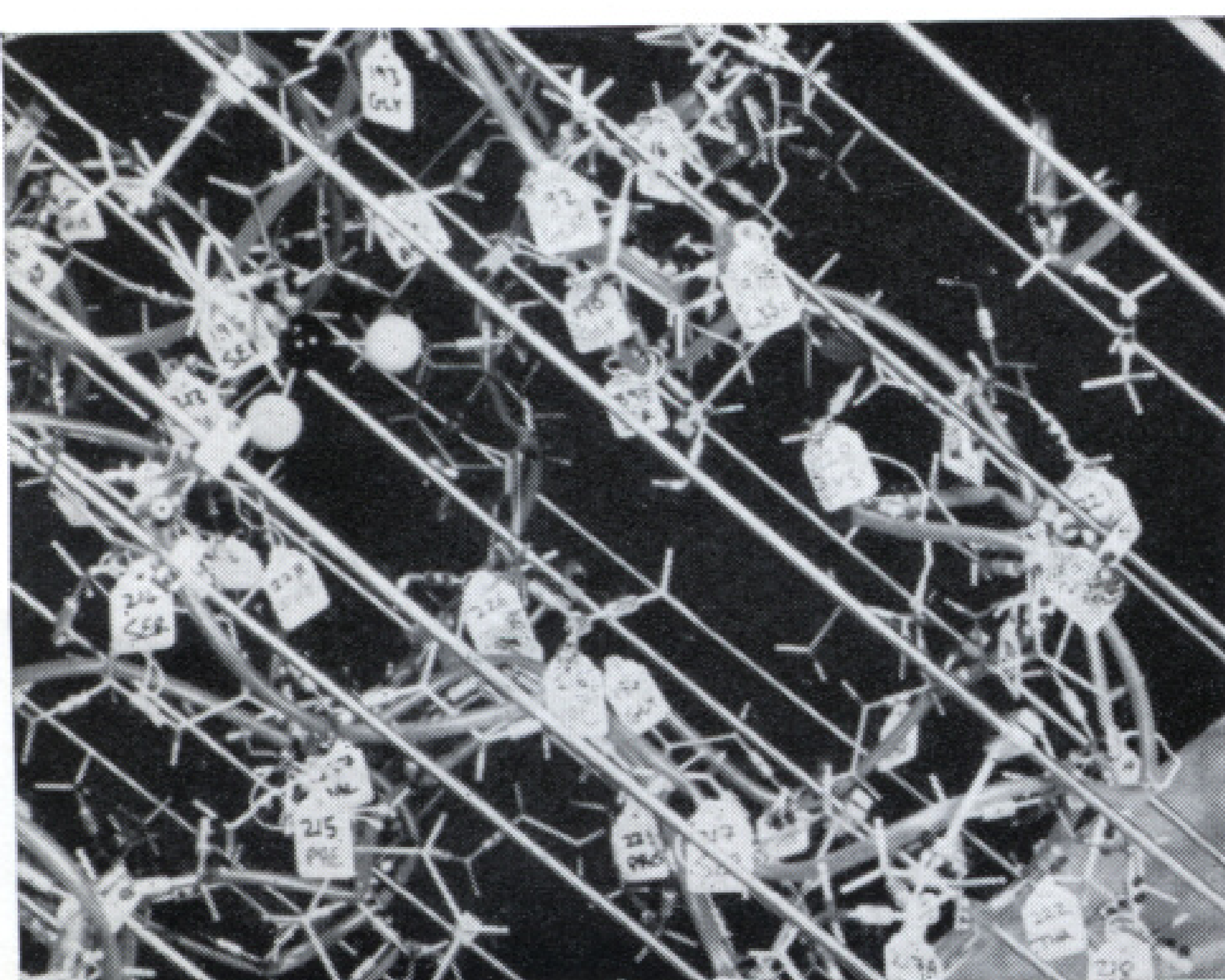


Figure 6



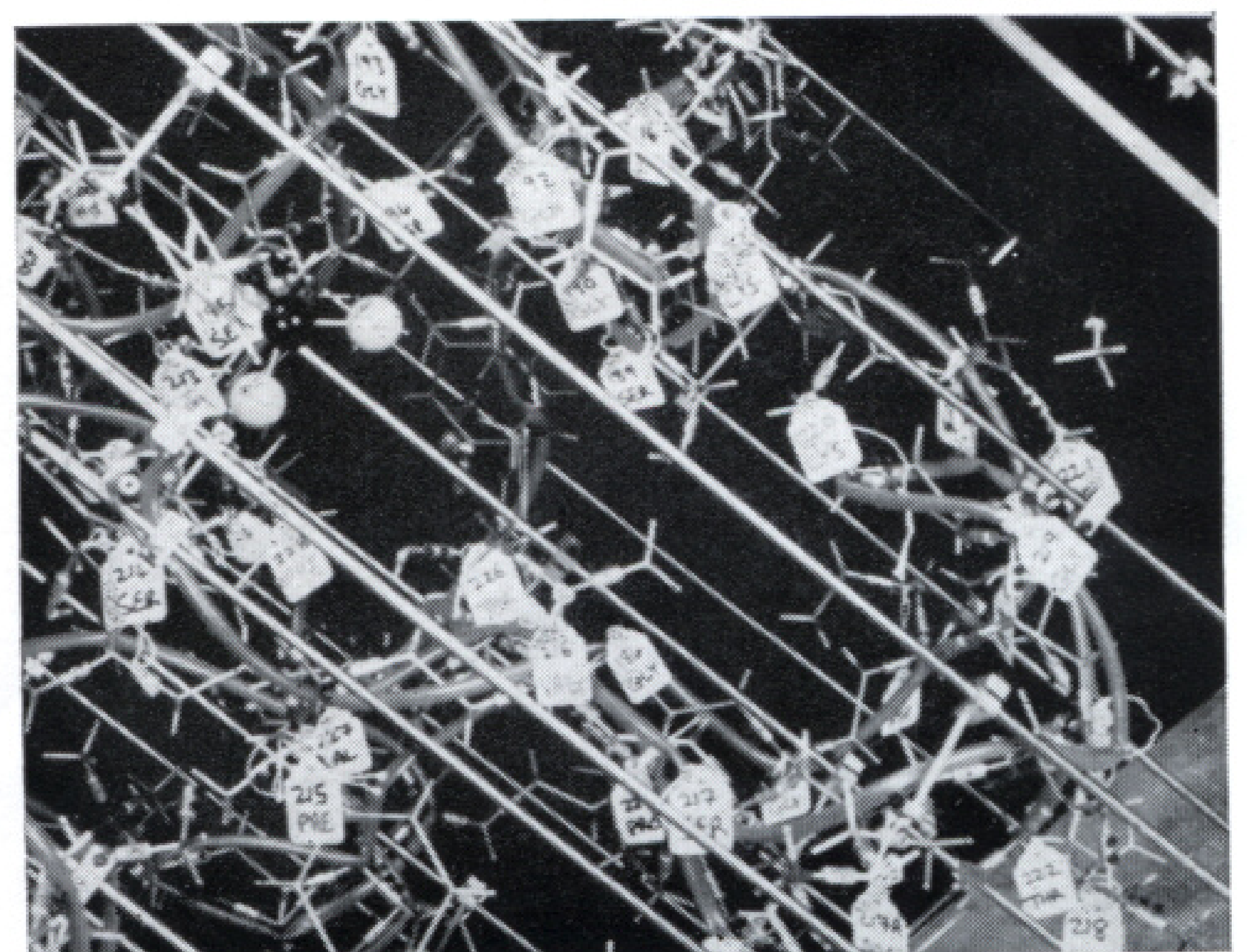


Figure 7 (for legends see facing page)

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