A crystallographic study of the oxidation of lysozyme by iodine

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The crystallographic examinations of the lysozyme molecule, and of the complexes of lysozyme with competitive inhibitors, have resulted in the identification of the active site and in a plausible hypothesis for its activity as described in the previous papers at this meeting (Blake, Mair, North, Phillips & Sarma, p. 365; Blake, Johnson, Mair, North, Phillips & Sarma, p. 378). The active site includes three tryptophanyl residues (62, 63 and 108) which appear to be involved in the binding of the substrate. Modification of some or all of these residues may provide further evidence of their function in the activity of the enzyme (see figure 19*a* of Blake *et al.*, p. 384).

Hartdegen & Rupley (1964) showed that the action of small amounts of triiodide produced two modified lysozymes. One product, containing iodine, was enzymically active, while the other which contained no iodine was inactive. Enzymic hydrolysis of the latter product revealed that it had one less tryptophanyl residue than the native enzyme. Hartdegen & Rupley were not able, however, to show which of the six tryptophanyl residues in the lysozyme molecule was the one uniquely reactive to iodine, but they were able to show that it was part of, or near, the active site.

In order to find the position, and possibly the function of the active tryptophanyl residue, a crystallographic investigation of the iodination reaction has been begun. Kendrew and his colleagues (Streyer, Kendrew & Watson 1964; Nobbs, Watson & Kendrew 1966) have shown that it is possible to detect very small modifications to proteins, once the structure is known, by standard crystallographic techniques: Perutz & Matthews (1966) have shown that this is possible even at low resolution. The method has the advantage over conventional biochemical techniques that it can reveal a modification *in situ*, together with any associated conformational change in the protein.

The expected reaction of tryptophan with iodine is shown in figure 46. The changes that are likely to be observable crystallographically are the substitution of the oxygen atom on the δ_1 carbon and the change at the γ carbon from trigonal to tetrahedral hybridization. The latter change will result in a perturbation of the whole side chain.

The reaction was carried out in the crystalline state by adding solutions of iodine to crystals of lysozyme at pH 4·7. Two concentrations of iodine were used, 1 mole iodine per mole of enzyme and 5 moles per mole of enzyme. Because of extensive

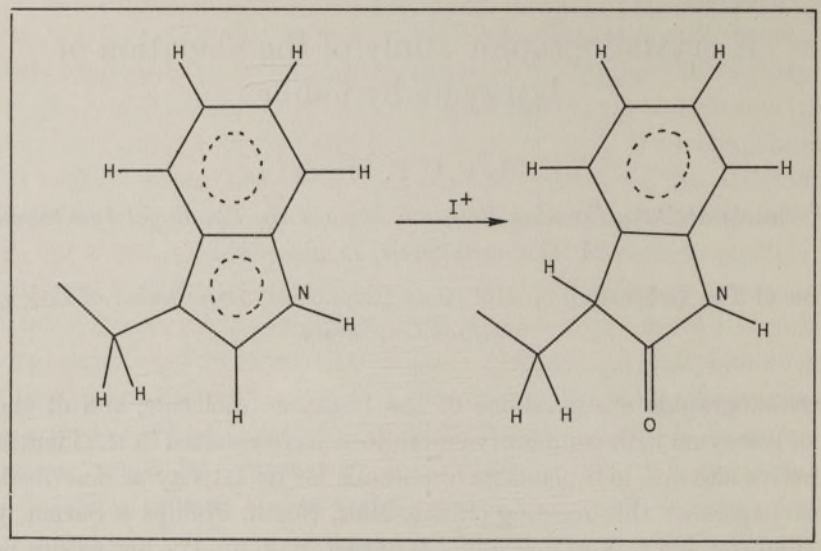

FIGURE 46. The oxidation of the indole ring of tryptophan to oxindole by iodine.

precipitation of the lysozyme still in solution in the mother liquor, however, the actual concentrations of iodine were probably lower. The crystals were then left for 2 weeks in the iodine solution to reach equilibrium. One crystal from each experiment was used to collect 6 Å three-dimensional data on the linear diffractometer and the data were used to calculate two difference electron density maps.

The most significant sections from the 5:1 and the 1:1 difference maps are shown in figure 47, on which the indole ring of Trp 108 and the carboxyl group of

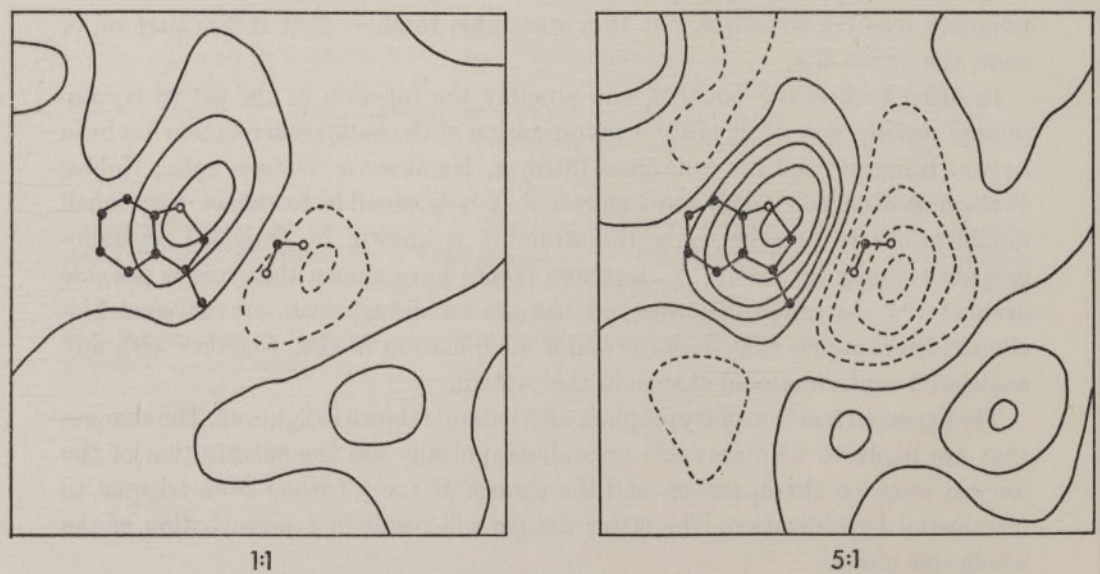

FIGURE 47. The most significant sections of the 5:1 and 1:1 difference maps. Positive contours are shown by full line, negative contours by broken line. The indole ring of Trp 108 and the carboxyl group of Glu 35 have been superimposed.

Glu 35 have been superimposed. It can be seen that the difference density in each map is of the same kind, although the 5:1 experiment produces the more marked change. A detailed interpretation is not possible at low resolution, but the changes in electron density are not inconsistent with the modification of the indole ring of Trp 108 to oxindole. The oxygen substituted on the δ_1 carbon atom of the ring of the oxindole derivative will be placed near the carboxyl group of Glu 35; this might result in a hydrogen bond being formed and account for the indication that the carboxyl group of Glu 35 moves towards Trp 108.

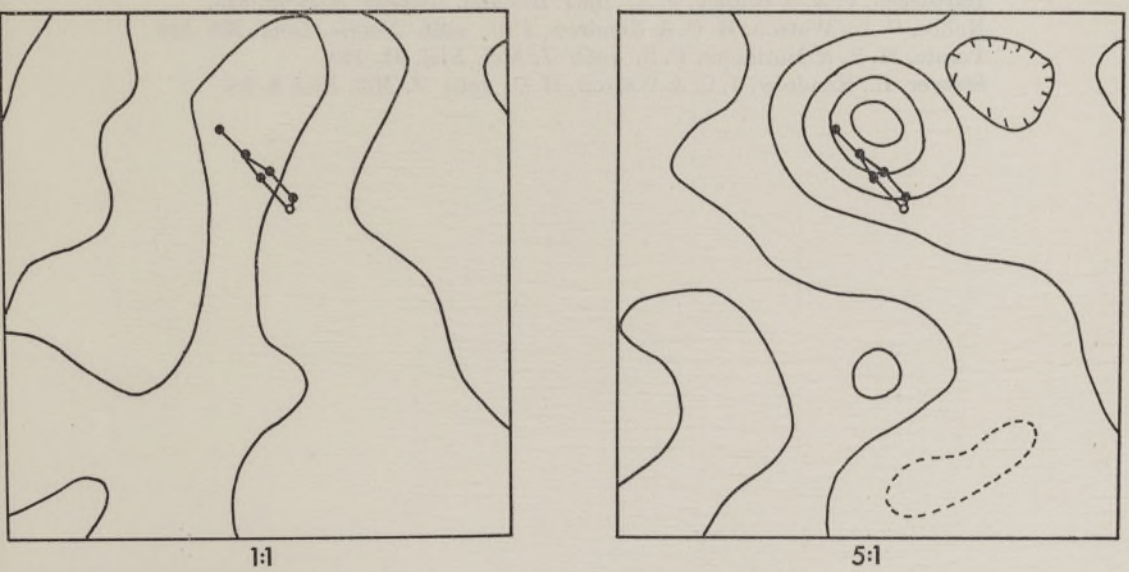

FIGURE 48. The second most significant section of the 5:1 difference map and the corresponding section of the 1:1 map. Positive contours are shown by full line, negative contours by broken line. Part of the indole ring of Trp 62 has been superimposed.

The second most significant feature in the 5:1 map is shown in figure 48 together with the corresponding section from the 1:1 map. Part of the indole ring of Trp 62 is superimposed. Thus the 5:1 map is consistent with the modification of this indole ring also to oxindole: the detail of the change cannot be directly obtained from the map but the increased electron density at the tryptophanyl position could represent the additional oxygen atom of the oxindole product. The 1:1 map, on the other hand, contains no significant indication of modification.

Thus the action of iodine on lysozyme at equimolar concentrations results in the partial modification of Trp 108 and a concommitant shift in the position of Glu 35. Increasing the concentration of iodine to 5 moles per mole of enzyme brings the reaction of Trp 108 to completion and leads also to the modification of Trp 62. There was no indication in these experiments of the presence of the product containing iodine described by Hartdegen & Rupley.

It now seems probable that the inactive product isolated by Hartdegen & Rupley is similar to that obtained from the 1:1 iodination (Rupley, this volume,

p. 416), but because both Trp 108 and Glu 35 appear to be affected at the same time, it is not possible to ascribe the loss of activity to the modification of either of these groups separately.

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