Acta Cryst. (1962). **15**, 423

Studies on the structure of lysozyme. I. A non-centrosymmetric projection to 4 Å resolution.

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(*Received* 30 *October* 1961)

Hen-egg white lysozyme nitrate (mol.wt. 15,000) can be induced to crystallize in triclinic space group $P1$, with one molecule per cell and with cell dimensions as follows (Steinrauf, 1959):

$a = 27{\cdot}5$ Å	$\alpha = 88{\cdot}33°$
$b = 32{\cdot}1$ Å	$\beta = 109{\cdot}0°$
$c = 34{\cdot}4$ Å	$\gamma = 111{\cdot}0°$

The $0kl$ projection has now been solved in this laboratory by isomorphous replacement methods to a resolution of 4 Å. Two heavy atom derivatives were used, a platinum chloride and a mercury iodide, the latter form in four different modifications. The derivatives were all isomorphous with the parent compound within the limits of accuracy of cell parameter measurement. Derivatives were obtained by diffusion by adding heavy atom solutions to the mother liquor after crystallization. It was found that after approximately thirty hours of irradiation there was an appreciable migration of mercury from one site to another. This factor plus the difference in site occupancy with ageing of the crystals produced four variants: fresh crystals, fresh but irradiated, aged, and aged and irradiated, which agreed in the positions of the sites but disagreed in relative occupancy. No comparable behavior was observed with the platinum derivative.

Initial heavy atom positions were found from $0kl$, $h0l$ and $h\bar{h}l$ modified Patterson projections of a type first used by Blow (1958) on horse haemoglobin. These used as Patterson coefficients the quantities $(\Delta F_H)^2$, where ΔF_H is the difference in magnitude of structure factors of heavy atom bearing derivative and native protein:

$$\Delta F_H = |F_{PH}| - |F_P| \,. \qquad (1)$$

Relative platinum–mercury coordinates were obtained from a cross-Patterson function using the coefficients:

$$2(\Delta F_{\mathrm{Pt}})(\Delta F_{\mathrm{Hg}}) \,. \qquad (2)$$

Such a function gives positive peaks representing platinum–mercury vectors on a random background. It can easily be shown to be a more convenient expression for Function 1 of Steinrauf (1962).

Initial phase circle diagrams were drawn for the 93 reflections out to 4 Å, and the set of initial protein phase angles used to refine the individual heavy atom positions. For each derivative there was calculated a Fourier synthesis with the coefficients $\Delta F_H \exp i\varphi_P$. (Here φ_P is the phase of the native protein and due allowance is made for the fact that ΔF_H can be negative.) This convenient approximation to a true difference Fourier synthesis is equivalent to the difference between the native protein Fourier synthesis and that of the heavy atom bearing derivative in which the native phases are used as first approximations to the true derivative phases. It has been shown (Steinrauf, 1962) that this function may be expected to represent the heavy atoms at the correct positions but with reduced peak heights. The resulting slightly modified heavy atom peaks were used for a second cycle of phase determination, which led to improvement in the phase diagrams. Two more refinement cycles resulted in little change in the electron density map. The $0kl$ projection of the native protein using phases of cycle 3 is shown in Fig. 1.

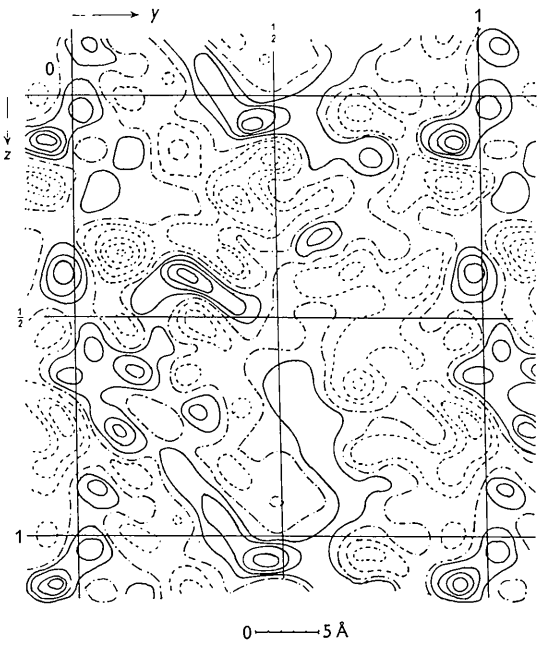

Fig. 1. $0kl$ electron density projection of triclinic hen egg white lysozyme nitrate, to 4 Å resolution. Contours at equal intervals; positive contours are solid, negative are dashed, zero contour is alternating. F_{000} term omitted. Heavy atom peaks to same scale would be 1·0 to 2·5 contours.

As all of these approximate methods are more powerful with more data and in three dimensions, it is felt that no more refinement is worthwhile in projection. Work is proceeding immediately toward the collection of 260 reflections per derivative for a three-dimensional analysis to 6 Å resolution, with the $0kl$ projection being used to screen new derivatives.

References

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