Patterson maps of the heavy-atom structures of the derivatives. These difference-Patterson maps were calculated on the MERCURY computer and even from time to time by the use of Beevers–Lipson strips – a less demanding task than it might appear, since only about 80 *hk*0 and 60 0*kl* reflections are included within the 6 Å limit.

26.1.2.4.1. *The mercuri-iodide (K_2HgI_4) derivative*

After trying several levels of substitution, RJP used the K_2HgI_4 salt at a molar concentration eight to ten times that of lysozyme. Based on the fact that lysozyme contains two methionine residues per molecule, and in keeping with a suggestion of Bluhm *et al.* (1958), RJP was expecting to see two heavy-atom sites,* but the *hk*0 difference-Patterson map was interpretable in terms of a single site of substitution. This site, however, was very close to the crystallographic twofold axis that runs along a diagonal in the [001] projection of the unit cell, and it proved necessary to correct the details of the first interpretation when phase information became available from other derivatives. It then appeared that there was one HgI_4^{2-} (or HgI_3^-) on the twofold axis between two protein molecules, but that it was best modelled by two closely spaced sites to allow for the elongated shape of the group (see Table 26.1.2.1). Several other heavy-atom salts, including K_2HgBr_4, K_2PtBr_4 and K_2AuCl_4, gave derivatives in which the heavy atom was attached to the same site as K_2HgI_4, and consequently seemed not to provide useful additional phase information.

26.1.2.4.2. *The palladium chloride (K_2PdCl_4) derivative*

An attempt to use K_2PtCl_4 to produce a useful derivative gave disordered crystals, but a substitute for it was found by soaking crystals in K_2PdCl_4 at a molar ratio of 3:1 relative to lysozyme. Despite the relatively light Pd atom, this derivative gave an easily interpretable difference-Patterson map (see Fig. 26.1.2.2) that yielded very good *R* factors,

$$R = \sum \| F_{HP} | - | F_P \| - | F_H(\text{calc}) | / \sum | F_H(\text{calc}) |,$$

where the summations are over centric reflections only.

26.1.2.4.3. *The o-mercurihydroxytoluene p-sulfonate (MHTS) derivative*

The *hk*0 difference-Patterson map of the *p*-mercuribenzene sulfonate (PCMBS) derivative was interpretable in terms of a single site of substitution at 8 Å resolution, but it was not useful beyond about 8 Å because of lack of isomorphism. RJP and RHF then explored the usefulness of MHTS as a derivative. This compound had been specially synthesized by JWHO in the hope that a small rearrangement of groups present in PCMBS would lead to an isomorphous derivative. Happily, this strategy worked, and MHTS gave a useful isomorphous derivative in which the major site overlapped that of PCMBS (Fig. 26.1.2.3).

26.1.2.4.4. *Other potential derivatives*

As is usual in protein work, RJP tried many other heavy-atom compounds (Poljak, 1963), but none gave useful results. In particular, a uranyl derivative, obtained by the use of UO_2NO_3, gave difference-Patterson maps that were difficult to interpret, and it was not taken further at this stage.

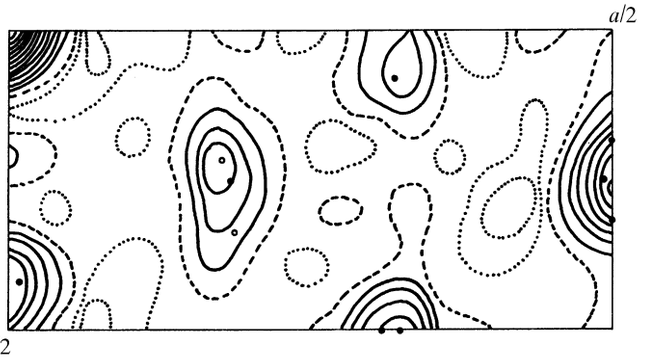

Fig. 26.1.2.2. Difference-Patterson *h*0*l* projection map for the derivative obtained with K_2PdCl_4. The ends of heavy-atom double-weight (filled circles) and single-weight (open circles) vectors are shown (R. J. Poljak, unpublished material).

26.1.2.5. *Refinement of heavy-atom parameters*

Refinement of the heavy-atom parameters was first performed by the use of Rollett's (1961) least-squares program on the MERCURY computer, using the $|\Delta F|$ values as structure amplitudes. This procedure gave satisfactory results for the K_2HgI_4, K_2PdCl_4 and MHTS derivatives described above, and they were used, therefore, in an attempt to determine the structure of the protein to 6 Å resolution in three dimensions.

26.1.2.6. *Analysis in three dimensions*

26.1.2.6.1. *X-ray intensity measurements*

We had three options for the collection of three-dimensional data. First, we could have used precession photographs and

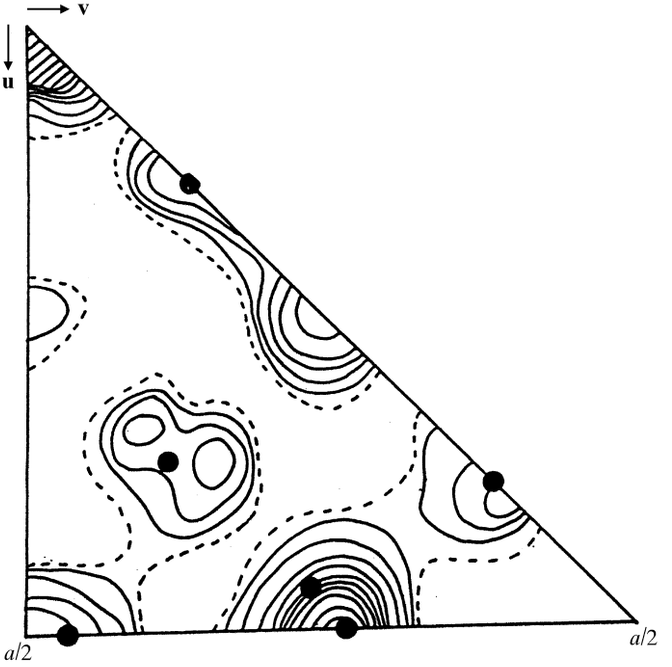

Fig. 26.1.2.3. Difference-Patterson *hk*0 projection for the derivative obtained with MHTS. The large peak at $\frac{1}{4}, \frac{1}{4}$ is not explained by this solution (Fenn, 1964).

* In order to study the nature of the ligand formed from K_2HgI_4, RHF and DCP studied the structures of two compounds, $(CH_3)_3S{\cdot}HgI_3$ and $[(CH_3)_3S]_2{\cdot}HgI_4$ (Fenn *et al.*, 1963; Fenn, 1964), which were prepared for this purpose in the laboratory by JWHO. These studies showed that HgI_3^- and HgI_4 are, respectively, planar trigonal and tetrahedral in configuration. Meanwhile, Dr Helen Scouloudi was examining the nature of the K_2HgI_4 derivative of seal myoglobin (Scouloudi, 1965) and showed that the ligand was HgI_3^- and not associated with methionine.

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