this parameter are indicated for the corresponding signal differences. Mösting C, the most prominent anomaly on our list, has an indicated value for this parameter of  $\sim 20$ , which is characteristic of bare rock. Therefore, this crater probably represents a young feature which has been exposed for a relatively short time to the erosional and blanketing processes which are responsible for the highly insulating properties of the older lunar surface.

The interpretation of these thermal features on the eclipsed moon is outside the scope of this report, but several puzzling aspects of the problem can be mentioned. First, although the hot spots are associated with bright features, conversely, there are bright features which are not thermally anomalous; thus there does not appear to be a direct relationship between visual brightness and the thermal response. For example, the ray craters Menelaus and Dionysius have the same brightness and appearance at full moon, but during the eclipse Menelaus was only 11°K warmer than its environs, whereas Dionysius was 44°K warmer. Further, the ray crater Euclides was only slightly warmer than its environs during totality, even though other craters similar in size and appearance gave large thermal responses. Second, the fact that ray craters show enhanced radar returns as well as anomalous eclipse cooling has been cited as showing that the surface of these features is denser or rougher, or both. However, a comparison of the radar reflectivity contours (6) with our infrared eclipse data shows there is no simple correlation between the measurements. For instance, enhanced radar returns are found on Eratosthenes and Posidonius, but these craters do not show anomalous eclipse cooling. Also, the radar contours for the ray craters Copernicus and Tycho are decidedly different from their isotherms during totality. It is interesting to note that the uplands give a higher radar return than the maria; the reverse is generally true for the infrared eclipse data. Since the two experiments do not measure the same parameters, it may not really be surprising that there is some lack of correspondence, and, in fact, the differences between them may be as interesting the similarities. Finally, the as nonrandom distribution of hot spots (there is a concentration in Mare Tranquillitatis) can be understood in terms of a random impact origin of the craters if the original surface in which

they were formed had local properties which in some way enhanced and prolonged the anomalous thermal behavior. On the other hand, this nonrandom distribution might not be surprising if the craters were formed by internal processes. In fact, internal processes may explain the anomalies observed on the "white areas," which give the appearance of being caused by a deposition of some material of higher albedo over areas large compared to their sources on the surface.

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## **Anomalous Dispersion Method:** Its Power for Protein Structure Analysis

Abstract. The phases of x-ray reflection of a crystal can be measured, except for an ambiguity, if the crystal contains heavy atoms which scatter x-rays anomalously. Theoretical studies show that the method of resolving this ambiguity by choosing the phases closer to those of the heavy atoms has a good potentiality for solving complicated structures, in which the average heavy atom contribution to the intensity is as low as 10 percent.

The use of anomalous dispersion effects for resolving the phase ambiguity in the isomorphous replacement method was first pointed out by Bijvoet (1). The theory was tested and proved in a known structure, that of L-ephedrine hydrochloride, by Ramachandran and Raman (2). The method leads to two ambiguous values for the phase, one of which will be closer to that of the heavy anomalous scatterers than the other. By choosing the phase closer to that of the heavy atoms as suggested (2), Raman (3) determined an unknown structure, that of L-lysine monohydrochloride dihydrate, using this technique with projection data. Since then, this method (which may be called the quasi-anomalous method) has been applied for two more structure analyses, that of Factor V 1a of Dale et al. (4) and of methyl melaleucate iodoacetate (5). The relevant data regarding these are given in Table 1, in which  $\sigma_1^2$  is the mean fractional contribution to the intensity by the known anomalous scatterers and is equal to the ratio

$$\sum_{\substack{j=1\\j=1}}^{P} f_j^2 \Big/ \sum_{\substack{j=1\\j=1}}^{N} f_j^2$$

where P represents the known atoms, N all the atoms, and  $f_i$  is the scattering factor of the atom i (6).

It has been shown by theory (7) that the fractional number of reflections  $n_{90^{\circ}}$ , for which the phase differs by less than 90° from that of the heavy atoms, is a function of  $\sigma_1^2$ , which increases by an increment of  $\sigma_1^2$ . The number of reflections also changes slightly depending on the number of heavy atoms in the unit cell. Thus,  $n_{90^{\circ}}$  is 94 percent when  $\sigma_1^2$  is equal to 0.8, is as high as 82 percent even when  $\sigma_1^2$  is 0.4, and is 62 percent when  $\sigma_1^2$ is 0.1 (Table 2), with two heavy atoms in the unit cell. Therefore the quasianomalous method would work even for a value of  $\sigma_1^2$  as low as 10 percent, which is much lower than the value of 26 percent, which is the lowest for which a structure analysis has yet been made (4). With the view of testing this, the quasi-anomalous method was applied to a hypothetical case containing 24 carbon and 22 oxygen atoms in the unit cell-in the same locations as in the structure of cellobiose (8), with two additional anomalously scattering atoms, each with a scattering power of half that of a chlorine atom ( $\sigma_1^2 = 10$ percent). When the phase closer to the heavy atom was chosen, the Fourier synthesis (Fig. 1) was obtained for the c-projection. There are clear peaks of electron density at all the unknown atomic locations and very few false peaks.

In fact, the diagram can be improved with a weighting function W, whereby the two ambiguous values of the phase given by the anomalous dispersion method can be given their statistical weights, depending upon the probability of their occurrence (9). The formula for W, which is a complex quantity, is

 $W = \cos \theta_0 \tanh(X \cos \theta_0) + i \sin \theta_0$ where

$$X = 2 |F_N| |F_P| / \sum_{i} f_i^2$$

and  $\theta_0$  is the acute angle out of the two possible ambiguous values of  $(\alpha_N - \alpha_P)$ , and  $F_N$  and  $F_P$  are the structure factors of the atoms N and P and  $\alpha_N$ ,  $\alpha_P$ are their phases. The synthesis obtained with the quantities  $WF_N \exp i \alpha_P$  as Fourier coefficients (the weighted anomalous synthesis) is shown in Fig 2. The ratio of the peak strengths at the unknown atoms to the known atoms improved by a factor of 1.8 on the mean.

Thus, there is a strong case for using the weighted anomalous synthesis for solving crystal structures. Since this method works with a value as low as 10 percent for  $\sigma_1^2$ , it should work with complex protein structures. With a heavy atom like mercury or platinum,  $\sigma_1^2 = 10$  percent would correspond roughly to 2400 other atoms with a mean scattering power of nitrogen, provided the heavy atom goes only to one location. Incidentally, an advantage of the anomalous dispersion method over the isomorphous replacement method for proteins is that two different crystals are not necessary, and the possibility of the two not being strictly isomorphous is eliminated. The anomalous dispersion method makes use of only the difference between the intensities of reflections h k l and  $\bar{h} \bar{k} \bar{l}$ of the same crystal (the Bijvoet difference  $\Delta I$ ).

In fact, the probable magnitude of the Bijvoet ratio  $\delta$ , equal to  $\Delta I/$  $\frac{1}{2}(I+\overline{I})$ , where I and  $\overline{I}$  are the intensities of the two inverse reflections, has also been investigated theoretically under various conditions. So far it has been possible to work out only the mean value of this quantity (Table 3) corresponding to a value of  $k(f_P''/f_P')$  of 0.2. Here  $f_{p'}$  and  $f_{p''}$  are the real and imaginary components of the scattering factor of the anomalous scatterer. The mean value is proportional to k; for comparison, we mention that the mean value of k, in the range where  $(\sin \theta)/\lambda$ is equal to 0 to 0.5 for CuK $\alpha$  radia-8 OCTOBER 1965

Table 1. Data for crystals solved by the anomalous dispersion method.

Crystal	Space group	Heavy atoms	P* atoms	$Q^{\dagger}$ atoms	$(=\sigma_P^{\sigma_1^2}/\sigma_N^2)$	Data	
L-lysine monohydro- chloride dihydrate	<i>P</i> 2 <sub>1</sub>	Cl	2	24	0.49	2-D, G.M. counter	
Factor V 1a (4)	<i>P</i> 2 <sub>1</sub>	Со	2	154	0.26	3-D, photo- graphic	
Methyl melaleucate iodoacetate	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	I	4	160	0.83	3-D, photo- graphic	

\* P atoms are the heavy, anomalously scattering atoms.  $\dagger Q$  atoms are the light atoms (excluding hydrogen).



Fig. 1. The quasi-anomalous synthesis for  $\sigma_1^2 = 10$  percent. The contour interval  $= 2 \text{ e}\cdot A^{-2}$  and the first contour is at  $2 \text{ e}\cdot A^{-2}$ . The unknown atoms are represented by crosses (x) and the known by full circles ( $\bigcirc$ ).



Fig. 2. The weighted anomalous synthesis for  $\sigma_1^2 = 10$  percent. The symbols are the same as in Fig. 1. Note the increased ratio of heights of the unknown to the known peaks.



Fig. 3. Cumulative function  $N(\delta)$  of the Bijvoet ratio  $\delta$  (equal to  $|\Delta I|/I$ ) for  $\sigma_1^2 =$ 0.5 and k = 0.2. The mean value is marked by a vertical line.

Table 2. Fractional number of reflections  $n_{00}$ . with phase difference less than 90° from the heavy atom contribution, as a function of  $\sigma_1^2$ .

$\sigma_1^2$		$P^*$ equal to (%):					
	1	2	M.A.	M.C.			
0.2	76.0	73.0	72.0	69.5			
.4	88.5	82.3	81.5	77.5			
.6	96.0	89.0	88.5	83.0			
.8	99.8	93.5	94.5	89.0			

\* P = 1 for one heavy atom in the unit cell; \* P = 1 for one heavy atoms in the unit cert, P = 2 for two heavy atoms; P = M.A. for many heavy atoms with an acentric distribution; and, P = M.C. for many heavy atoms with a centric distribution.

Table 3. Mean value of the Bijvoet ratio  $\delta$  as a function of  $\sigma_1^2$ . The value of k is taken to be 0.2. The other symbols are as in Table 2.

${\sigma_1}^2$		P equal to						
	1	2	M.A.	M.C.				
0.1	0.2796	0.2460	0.2400	0.2098				
.2	.3831	.3289	.3200	.2738				
.3	.4478	.3750	.3666	.3094				
.4	.4824	.3951	.3919	.3290				
.5	.4857	.3928	.4000	.3370				
.6	.4529	.3704	.3919	.3354				
.7	.3795	.3315	.3666	.3237				
.8	.2720	.2800	.3200	.2993				
.9	.1609	.2129	.2400	.2514				

tion, is approximately equal to 0.28 for cobalt, 0.21 for iodine, 0.15 for platinum, and 0.17 for mercury. The anomalous effects are most prominent for  $\sigma_1^2 = 0.5$ ; in this case, the mean value of  $\delta$  (for k = 0.2) is as high as 40 percent, so that it should be possible to measure them quite accurately by means of counters. Although a theoretical formula is not available, the cumulative function  $N(\delta)$  of  $\delta$  for this example is shown in Fig. 3, as obtained from a practical case. Although the median value is about 40 percent, the number of reflections for which  $\delta >$ 100 percent is as high as 15 percent (Fig. 3).

Thus, the anomalous dispersion method is one that deserves further consideration by protein crystallographers. In fact, as early as 1958, Blow (10) used this technique in conjunction with isomorphous replacement in his study of hemoglobin, and similar combined studies have since been made by others. Thus, provided that the errors of measurement of intensities can be reduced, the anomalous effects by themselves could be used for phase determination since the errors due to nonisomorphism are avoided.

Another difficulty with proteins, but often not with ordinary crystals, is that the reflections become very weak about 2 Å, resulting in poor data. With the large unit cells which occur in proteins, the foregoing distribution functions are valid, except for about a hundred reflections with small values of  $(\sin \theta)/\lambda$ . Thus, the smallness of the total number of reflections in relation to the number of atoms, which occurs with proteins, does not affect the phasedetermining technique as such, but it certainly affects the clarity of the final diagram, even with correct phases.

Incidentally, even for finding out the positions of the heavy atoms in a protein structure, a combined application of the isomorphous crystal method and the anomalous dispersion method is the most fruitful one (11). This result was reached by application of the theoretical approaches to problems studied earlier (12). In fact, a combination of the isomorphous crystal and anomalous dispersion method has been used in the study of the structure of the protein lysozyme (13).

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## Messenger RNA in Early Sea-Urchin **Embryos: Cytoplasmic Particles**

Abstract. Three structures containing messenger RNA can be demonstrated in the cytoplasm of early sea-urchin embryos: (i) particles that sediment more slowly than ribosomes and contain newly synthesized DNA-like RNA, (ii) light polyribosomes, which also contain this newly synthesized RNA, and (iii) heavy polyribosomes, which seemingly contain only already existing or "maternal" messenger RNA and account for the bulk of the synthesis of protein.

A newly described class of ribonucleoprotein (RNP) particles, which sediment more slowly than ribosomes, occurs in fish embryos (1, 2). Because they have a unique buoyant density, about 1.42 to 1.45 g/cm<sup>3</sup>, as determined with CsCl gradients (2, 3), these particles are distinct from ribosomal subunits, which have a buoyant density of 1.52 g/cm<sup>3</sup>. Evidence for the existence of messenger RNA (mRNA) in these particles was drawn from their apparent ability to stimulate incorporation into protein in a cellfree ribosomal preparation (2). We report here evidence for the existence of similar particles in early sea-urchin embryos and demonstrate that their RNA is mRNA because it forms hybrids with DNA in high proportion.

The sea-urchin embryo synthesizes new mRNA before the eight-cell stage (4, 5). We show now that this new RNA appears in the cytoplasm, not only in the above-mentioned RNP particles, but also as a complex with ribosomes, in the form of polyribosomal structures. However, the significance of the synthesis of cytoplasmic mRNA by the cleavage-stage embryo is not immediately apparent, since there is varied evidence that such a synthesis need not contribute to embryonic development