The strategies adopted in the refinement of occupancies, temperature factors and coordinates of the anomalously scattering atoms will be considered. Attention will be given to the expected statistical distribution of Bijvoet differences.

01.2–4 DIRECT DETERMINATION OF SAS PHASE INFORMATION. By D.S.C. Yang, Z.B. Xu, W. Furey Jr. and B.C. Wang, Biocrystallography Laboratory, P.O.Box 12055, VA Medical Center, Pittsburgh, PA 15240, U.S.A. and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

When dealing with Single-wavelength Anomalous Scattering data (SAS), the SAS-vector F_{SAS} is defined as the projection of the protein structure factor vector F_P onto the anomalous component ($F_{H''}$) of the anomalous scatterer's contribution to the total structure factor. The associated phase angle, Φ_{SAS} is numerically equal to the average value of a pair of true and false phase solutions in the conventional SAS treatment. Recently we found a relationship for identifying triplet phase invariants with values near $\pi/2$ or $-\pi/2$ linking these SAS phases. That is

$$\phi_{hS} + \phi_{kS} + \phi_{(\bar{h}+\bar{k})S} \sim -\pi/2 \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) > 0 \tag{1}$$

$$\phi_{hS} + \phi_{kS} + \phi_{(\bar{h}+\bar{k})S} \sim \pi/2 \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) < 0$$

where S denotes SAS phases and ΔF_h denotes $|F_h|-|F_{\bar{h}}|$. Equation (1) is very similar to the rule $R_{ANO,1}$ of Karle (Paper 12, Int. Sch. on Cryst. Comp., Japan, 1983). However, the above relationship is considerably more reliable than $R_{ANO,1}$ which links protein phases.

A calculation with experimentally obtained anomalous scattering data, collected from an Au-derivative of Bence Jones Protein Rhe (Wang, et al, J.M.B. 129, 657. 1979) produced the following results:

#Refl. of largest $\|\Delta F\|$	#Triplets	Ave. Error (^{o}) $R_{ANO,1}$	Equation 1
50	42	68.9	23.3
100	343	65.9	35.7
150	1062	67.0	38.9
200	2476	71.6	41.6
300	5000	71.7	43.3
500	5000	71.7	40.1

The errors were calculated using phases computed from the refined Rhe structure (Furey, et al., J.M.B. 167,661,1983) and the heavy atom parameters previously reported. More results and discussions will be presented.

01.2–5 PROGRESS REPORT ON THE STRUCTURE DETERMINATION OF Cd, Zn METALLOTHIONEIN By W. Furey, A.H. Robbins and C.D. Stout Biocrystallography Laboratory, P.O. Box 12055, VA Medical Center, Pittsburgh, PA 15240, U.S.A. and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

Single crystals of Cd, Zn metallothionein (isoform II) from rat liver were grown by seeding using sodium formate as a precipitant (Melis, et al., J.B.C. 258,6255, 1983). The unit cell is tetragonal with space group $P4_12_12$ (or $P4_32_12$) and cell constants a = b = 31.0, c = 120.04 Å. There is one molecule per asymmetric unit. Assays of the single crystals are consistent with those of the "as isolated" protein which contains 5 Cd and 2 Zn per metallothionein molecule. Native data to 2.3 Å resolution has been collected by oscillation photography with a rotating anode x-ray source. The merging R factor (based on F) is 0.032 for reflections equivalent by point group symmetry and 0.041 for Bijvoet pairs. Data to 2.3 Å resolution were also collected for a potential isomorphous derivative (Tungsten). The R factor between the native and derivative data is 0.139. In addition to the traditional isomorphous replacement method, we are trying to develop protein phases from the values of 3-phase structure invariants estimated by the direct methods procedures of Hauptman (Acta Cryst. A38, 289, 1982 and Acta Cryst. A38, 632, 1982). Results of the study will be presented. This work is supported by NIH grant GM-32913.

01.2–6 DIRECT DETERMINATION OF SIR PHASE INFORMATION BY AN EXTENSION OF KARLE'S RULE. By Z.B. Xu, D.S.C. Yang, W. Furey Jr., M. Sax, J. Rose and B.C. Wang Biocrystallography Laboratory, P.O. Box 12055, VA Medical Center, Pittsburgh, PA 15240, U.S.A. and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

Karle (Acta Cryst. A39, 800, 1983) recently introduced a simple rule for identifying triplet phase invariants with values near 0 or π from single isomorphous replacement (SIR) data. The rule states that:

$$\phi_{hp} + \phi_{kp} + \phi_{(\bar{h}+\bar{k})p} \sim 0 \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) > 0$$

$$\phi_{hp} + \phi_{kp} + \phi_{(\bar{h}+\bar{k})p} \sim \pi \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) < 0$$

where ΔF is $|F_{ph}|-|F_p|$; F_{ph} and F_p are structure factor amplitudes for the derivative and native data respectively. The rule is valid for protein phases only when reflections with the largest $|\Delta F|$ in the data set are used.

Recently we applied Karle's rule to Au-SIR data of Bence Jones protein Rhe (Wang, et al., J. Mol. Biol. 129, 657, 1979). Using 280 reflections with the largest $|\Delta F|$ values (top 11%) and by means of symbolic addition we obtained all 280 individual phases. When the phases were compared with those computed from the refined protein structure (Furey, et al., J. Mol. Biol. 167, 661, 1983) we found average phase errors of 2.8^o and 34.3^o for the 131 centric and 149 acentric reflections respectively. However when the new phases were compared with the SIR phases (calculated from refined heavy atom positions) the average phase errors were 4.1^o and 16.7^o respectively for centric and acentric reflections. This observation and other considerations led us to discover that the approximations used in Karle's derivation fit well with the idea of assuming an artificial structure factor vector which is the projection of the F_p vector onto the F_H vector.

Therefore we conclude that when the ΔF cutoff is relaxed, Karle's rule will give SIR phase information instead of protein phase information. That is:

$$\phi_{hS} + \phi_{kS} + \phi_{(\bar{h}+\bar{k})S} \sim 0 \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) > 0$$

$$\phi_{hS} + \phi_{kS} + \phi_{(\bar{h}+\bar{k})S} \sim \pi \quad \text{if } (\Delta F_h)(\Delta F_k)(\Delta F_{\bar{h}+\bar{k}}) < 0$$

where S denotes SIR phases. More results and discussion of the above equations will be presented.

01. 2–7 A METHOD FOR RESOLVING PHASE AMBIGUITY IN SIR OR SINGLE-WAVELENGTH ANOMALOUS SCATTERING DATA.

By Bi-Cheng Wang Biocrystallography Laboratory, Box 12055, V A Medical Center, Pittsburgh, Pa 15240, U.S.A. and Department of Crystallography, University of Pittsburgh, Pa 15260, U.S.A.

The use of the gross molecular image, revealed by a new and automated procedure, together with a Fourier "image-enhancement" process has been found to be highly effective for resolving phase ambiguity in single isomorphous replacement (SIR) data or single-wavelength anomalous scattering (SAS) data. When the method was tested using diffraction data for Bence Jones protein Rhe (Wang, et al, JMB 129, 657, 1979) the results were comparable with those obtained by the MIR process. The MIR phases (3Å) obtained previously from two sets of isomorphous and one set of anomalous data had an average phase error of 32.3 degrees. However, the new process using only one set of isomorphous data (no anomalous data), gave an average phase error of 31.8 degrees. The method has been used for the determinations of several previously unknown macromolecular structures, including the crystal structures of two immunoglobulin light chain dimers and a DNA-protein complex.

The method and its applications to known and unknown structures will be presented. In addition, an integrated program package for carrying out the various steps in the method will be introduced. This program package can also be used for extending phase information and improving MIR phases.

01. 3–1 Structural Refinement of a [2Fe-2S] Ferredoxin from Aphanothece sacrum by the Molecular replacement method. By T.Tsukihara, M.Mizushima, K.Fukuyama, and Y.Katsube, Faculty of Engineering, Tottori University, Tottori, 680, JAPAN

A molecular structure of [2Fe-2S] ferredoxin from Spirulina platensis has been determined at 2.5A resolution(Fukuyama et al.,Nature(1980) 286,522). Since the crystal of S. platensis ferredoxin was too thin in thickness to give a good intensity data set, the crystal was not used for a higher resolution analysis. Another [2Fe-2S] ferredoxin from A. sacrum whose amino acid sequence is different from that of S. platensis by about 30% was crystallized in a barnet-shape. We have determined the main chain fold of this ferredoxin by the single isomorphous replacement method at 2.5A resolution (Tsutsui et al.,J.Biochem.(1983)94,299). These ferredoxins are similar to each other in the main chain structure. As four molecules of A. sacrum ferredoxin were in an assymmetric unit, their structures were refined by the molecular replacement method in the present study.

The space group is $P4_1$, and the unit cell dimensions are a=b=92.2 and c=47.6A. Rotational and translational parameters of four independent molecules named A, B, C, and D were determined by a least-squares method on the electron density calculated by the single isomorphous replacement method at 2.5A resolution. Correlation coefficients of the electron density, $C=\sum(\rho-\bar{\rho})(\rho_A-\bar{\rho}_A)/[\sum(\rho-\bar{\rho})^2(\rho_A-\bar{\rho}_A)^2]^{1/2}$, were 0.50, 0.38, and 0.38 for molecules B, C, and D, respectively. Electron density of the solvent region was left unchanged during the first two cycles of the molecular replacement refinement. In the further refinement that of the solvent region was replaced by an averaged electron density of the corresponding region at the previous cycle. The R-value, $R=\Sigma||F_o|-|F_{MR}||/\Sigma|F_o|$, was reduced from 0.45 to 0.36, and the correlation coefficient of structure factors, $C=\Sigma(F_o-F_o)(F_{MR}-F_{MR})/[\Sigma(F_o-F_o)^2\Sigma(F_{MR}-F_{MR})^2]^{1/2}$, was 0.70 after the six cycles of the refinement. Figs.1 a and b show electron density maps of the same section for the first and the final cycles, respectively. The feature of electron density distribution was obviously improved by the refinement. Phase extension from 2.5A to 2.2A resolutions is in progress by the molecular replacement method.

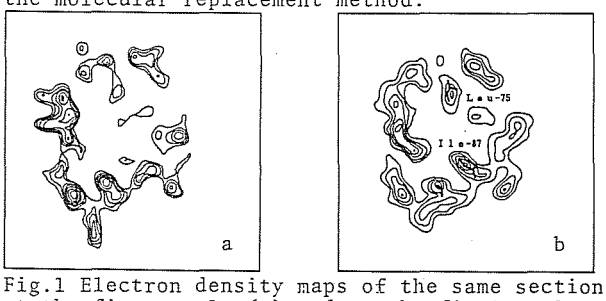

Fig.1 Electron density maps of the same section at the first cycle (a) and at the final cycle (b) of the present refinement. In (b) the side chains of Leu-75 and Ile-87 are drawn on the electron density contours.