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Location of Iron and Sulfur Atoms in Myohemerythrin from Anomalous-Scattering Measurements

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Abstract

Anomalous scattering due to the iron and sulfur atoms in myohemerythrin has provided a check on the iron-iron distance and the location of the sulfur atoms which is independent of our model obtained by restrained least-squares refinement. By anomalous-scattering techniques we find an iron-iron distance of 3.35 (5) Å which compares with a distance of 3.23 Å obtained from restrained least-squares refinement. We have also used anomalous-scattering information to confirm the placement of Cys35S γ , which required a sequence change during the refinement, and to establish our tentative identification of two solvent molecules as sulfate ions. Methods used here might also help in locating metal centers and finding the sites of minor anomalous scatterers in other macromolecular structures.

Introduction

The use of anomalous scattering has allowed a model-independent assessment of the location of iron and sulfur atoms in the structure of myohemerythrin, an oxygen-carrying protein from sipunculan worms. We have used Bijvoet-difference Patterson maps (Rossmann, 1961), refinement against 'large' observed Bijvoet differences (Hendrickson, Klippenstein & Ward, 1975), Bijvoet-difference Fourier maps (Kraut, 1968) and the 'imaginary' Fourier synthesis (Hendrickson & Sheriff, 1987) in order to investigate the structure of anomalous scatterers in myohemerythrin.

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Bijvoet-difference Patterson maps

Myohemerythrin from *Themiste zostericola* crystallizes from 2.3 M (NH₄)₂SO₄ in space group *P*2₁2₁2₁ with *a* = 41.66, *b* = 80.17, *c* = 37.82 Å. The active center contains two iron atoms ligated directly to protein side chains. One iron is coordinated by three histidines (His 73, 77, 106) and the other by two (His 25, 54). There are three connecting groups between the two iron atoms - the carboxylates of Glu 58 and Asp 111 and an oxide ion, O₂²⁻. The remaining ligand is azide in the azidomet form of hemerythrin (Sheriff, Hendrickson & Smith, 1983; Stenkamp, Sieker, Jensen & Sanders-Loehr, 1981).

Rossmann (1961) showed that Patterson maps calculated with $|\Delta F|^2$, where $\Delta F = (|F^+| - |F^-|)$ is the Bijvoet difference, would yield the interatomic vectors of the anomalous scatterers in a structure. In Fig. 1 we show the three Harker sections from the Bijvoet-difference Patterson map at a nominal resolution of 2.0 Å. The iron self-peaks are clearly resolved in all three sections. In the *u* = *a*/2 and *v* = *b*/2 sections an iron-atom cross vector is also apparent.

Refinement of the iron positions

The Bijvoet difference can be calculated as follows for a single type of anomalous scatterer

$$\Delta F \approx -2\delta \sin(\psi - \varphi) \quad (1)$$

where the values are as defined in Fig. 1 of Hendrickson (1979): ΔF is the Bijvoet difference $|F(\mathbf{h})| - |F(-\mathbf{h})|$, $\delta \exp(i\psi)$ is the structure-factor contribution from anomalous-scattering centers, and φ is the phase for the complete structure. As $|\Delta F|$ becomes large the value of the sine factor must approach ± 1 , which reduces equation (1) to $|\Delta F| = 2\delta$, for large

$|\Delta F|$. It is therefore possible to refine the positions of the anomalous scatterers if only the large $|\Delta F|$ values are included. Such a refinement initially led to an iron-iron distance of 3.44 (5) Å (Hendrickson *et al.*, 1975). However, after application of a parameterized local-scaling procedure (Hendrickson & Teeter, 1981) to remove systematic errors from the data, an iron-iron distance of 3.34 (7) Å was obtained from ∞ -2.8 Å data (Hendrickson, 1981). Reflections were included in this calculation only if $|\Delta F| \geq 2\sigma_{\Delta F}$. The following criteria were used to exclude erroneous measurements: $|\Delta F| < 5 \text{ r.m.s.}|\Delta F|$ and $|F^+|, |F^-| > 3\sigma_F$.

New Friedel-pair data were collected from ∞ -2.0 Å and a data set was constructed using the original data from ∞ -3.5 Å and the new data from 3.5-2.0 Å. Iron-position refinements against $|\Delta F|$ data were repeated with the 2 Å data set, and the iron-iron distance was discovered to be dependent on the cut off used for inclusion (see Table 1). Despite the dependence of the iron-iron distance on the data included, these results agree reasonably well with those calculated by restrained least-squares refinement for the azidomet form of hemerythrins. We have found a distance of 3.23 Å in myohemerythrin (Sheriff *et al.*, 1983; Sheriff, Hendrickson & Smith, in preparation) and Stenkamp, Seiker & Jensen, (1984) have obtained a distance of 3.25 Å for the octameric hemerythrin from *T. dyscrita*.

Sulfur positions from refinement $\Delta(\Delta F)$ maps

Myohemerythrin contains five sulfur atoms: Cys35S γ , Met61S δ , Met62S δ , Met76S δ and Cys99S γ . Phases

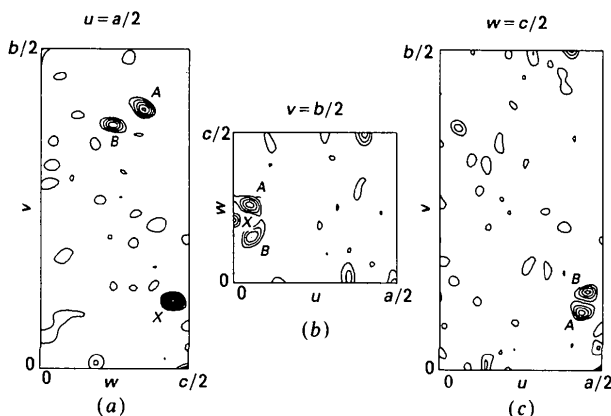


Fig. 1. The three Harker sections for the Bijvoet-difference Patterson map of native myohemerythrin. Reflections corresponding to spacing in the range ∞ -2.0 Å were included in the calculation if they met the following criteria: $|\Delta F| < 5 \text{ r.m.s.}|\Delta F|$; $|\Delta F| > 2\sigma_{\Delta F}$; $|F^+|, |F^-| > 3\sigma_F$. This yielded 2095 observations. (a) $u = a/2$; (b) $v = b/2$; (c) $w = c/2$. Self-peaks corresponding to one iron atom are labeled A, those from the other iron atom are designated as B, and cross peaks between the two iron centers are marked by an X. Centers of cross peaks are not precisely on these sections.

Table 1. Iron-iron distance determined by anomalous scattering in myohemerythrin

n , where $ \Delta F \geq n\sigma_{\Delta F}$	Dependence on σ cut off* Number of observations	Iron-iron distance [†] (Å)
1	4166	3.27 (3)
2	2095	3.35 (5)
3	934	3.39 (7)

* Data were included from ∞ -2.0 Å resolution and also met the criteria of $|\Delta F| < 5 \text{ r.m.s.}|\Delta F|$ and $|F^+|, |F^-| > 3\sigma_F$.

† Iron-iron distance determined by restrained least-squares refinement is 3.23 Å.

Table 2. Peaks associated with sulfur atoms in Bijvoet-difference difference Fourier map based on an iron-atom model

The terms used to calculate this map were $(|\Delta F| - 2\delta_{Fe}) \exp(i\psi_{Fe})$. The map was calculated with 2095 reflections in the range ∞ -2.0 Å resolution meeting the following criteria: $|\Delta F| < 5 \text{ r.m.s.}|\Delta F|$; $|\Delta F| > 2\sigma_{\Delta F}$; $|F^+|, |F^-| > 3\sigma_F$.

	Peak-to-atom distance (Å)	Rank*	Peak height, σ †	Peak proximity fraction‡
Cys35S γ	0.56	111	2.8	0.007
Met61S δ	0.66	105	2.8	0.008
Met62S δ	1.15	7	4.0	0.001
Met76S δ	1.52	149	2.6	0.084
Cys99S γ	0.50	4	4.2	0.000

* Ranking excludes ripples due to the iron atoms.

† Peaks are measured in units of standard deviations where σ is the r.m.s. density of the map ($0.027 \text{ e} \text{ \AA}^{-3}$).

‡ Fraction of total protein atoms (979) for which a peak of the indicated height is closer than the peak-to-atom distance of the peak associated with this sulfur atom. This is an estimate of the probability that by chance a peak of this height might be this close to a given atom.

based on the iron model can be used to calculate a Bijvoet-difference difference Fourier synthesis, $(|\Delta F| - 2\delta_{Fe}) \exp(i\psi_{Fe})$, to search for the sulfur-atom positions. The results of this procedure were somewhat disappointing - the fourth and seventh largest peaks could be assigned, respectively, to Cys99S γ and Met62S δ , but the three other sulfur atoms ranked much lower (Table 2).

We examined the significance of these sulfur-associated peaks in the Bijvoet-difference difference Fourier map by determining for each how many other peaks of comparable height in the map were within this distance of an atom in the entire myohemerythrin structure. These data are also listed in Table 2 for the five sulfur atoms. Plots of the distance of each atom to the nearest peak at a given significance level showed, at the 3σ level, very few atoms closer than 1.2 Å and a mean distance of about 4.5 Å. These results suggest that all of the peaks associated with sulfur atoms are significant with the possible exception of Met76S δ . When structure factors calculated from the refined protein model XVIII.3 were used in a similar Bijvoet-difference refinement, the resulting

Bijvoet-difference difference Fourier map had the four largest peaks associated with Cys35S γ , Met61S δ , Met62S δ and Cys99S γ . These peaks exceeded the largest noise peaks by small factors (1.1–1.3) but the peak associated with Met76S δ was in the noise. The peak for Met76S δ was at a height of 2.5σ whereas the height for other sulfur peaks was at 4.9 – 5.6σ ($\sigma \equiv$ r.m.s. density level). This suggests that the diffraction data are intrinsically less sensitive to Met76S δ than to the other sulfur atoms, perhaps due to the higher thermal-displacement parameter.

Kraut Bijvoet-difference and 'imaginary' Fourier syntheses

An alternative procedure to locate anomalously scattering atoms was suggested by Kraut (1968). This involves the use of phases for the whole structure in combination with the Bijvoet differences. Recently, Hendrickson & Sheriff (1987) have shown that when a model is available, one can calculate the complete 'imaginary' Fourier. Tables 3 and 4 show the results of using these procedures. The Fourier maps were calculated using model-derived phases for the ∞ – 2.0 \AA data. This model had an $R = 0.166$ for 10 – 1.3 \AA data and an r.m.s. deviation from ideal bond lengths of 0.19 \AA (Sheriff, Hendrickson & Smith, in preparation).

To ensure that we were not biasing the results by using phases calculated with sulfur atoms, we replaced all the sulfur atoms with carbon atoms and performed two cycles of refinement before calculating a map. The results of this experiment are also listed in Tables 3 and 4. Although substituting carbon for sulfur (in order to remove biasing phase influence) did not affect our ability to locate the sulfur atoms by use of these Fourier syntheses, it did have a significant impact on peak heights in the 'imaginary' Fourier map and on the relative heights of the Met76S δ peak.

With the exception of Met76S δ all of the sulfur atoms are among the highest non-iron peaks in the map. Particularly noteworthy is Cys35S γ . When we started work on myohemerythrin the sequence in that region was thought to be Cys34–Asp35 (Klippenstein, Cote & Ludlam, 1976). However, a mercury atom in the Hg(CN) $_2$ derivative of myohemerythrin bound near the current location of Cys35S γ (Hendrickson *et al.*, 1975). Early attempts to fit the protein map with an aspartate residue following the cysteine, which was located so that the sulfur and mercury could form a bond, led to unlikely geometry for the peptide chain. Placing the aspartate residue in front of the cysteine relieved the difficulties in the geometry and the model refined nicely. The present results give a model-independent confirmation that this cysteine-sulfur placement is correct.

Table 3. Peaks associated with iron and sulfur atoms in a Kraut (1968) Bijvoet-difference Fourier map at 2.0 \AA resolution

	Sulfur as sulfur			Sulfur as carbon		
	Peak-to-atom distance (\AA)	Rank*	Peak height, σ^\dagger	Peak-to-atom distance (\AA)	Rank*	Peak height, σ^\dagger
Fe1	0.14	2	36.1	0.15	2	35.5
Fe2	0.10	1	37.6	0.08	1	37.4
Cys35S γ	0.48	3	4.6	0.46	3	5.0
Met61S δ	0.31	14	3.6	0.42	23	3.4
Met62S δ	0.26	28	3.3	0.24	12	3.7
Met76S δ	0.57	144	2.6	0.52	86	2.9
Cys99S γ	0.38	6	4.1	0.45	4	4.7

* Ranking excludes ripples due to the iron atoms.

\dagger Peak heights are measured in units of standard deviations where σ is the r.m.s. density of the map. R.m.s. density of the sulfur-as-sulfur map is $0.0353 \text{ e \AA}^{-3}$ and for the sulfur-as-carbon map $0.0352 \text{ e \AA}^{-3}$.

Table 4. Peaks associated with iron and sulfur atoms in an 'imaginary' Fourier map at 2.0 \AA resolution

	Sulfur as sulfur			Sulfur as carbon		
	Peak-to-atom distance (\AA)	Rank*	Peak height, σ^\dagger	Peak-to-atom distance (\AA)	Rank*	Peak height, σ^\dagger
Fe1	0.11	2	91.9	0.10	2	92.2
Fe2	0.08	1	96.4	0.07	1	97.5
Cys35S γ	0.17	4	12.6	0.36	3	5.4
Met61S δ	0.08	6	9.9	0.23	5	4.7
Met62S δ	0.16	3	12.8	0.26	4	5.2
Met76S δ	0.06	7	8.6	0.67	83	2.9
Cys99S γ	0.15	5	11.9	0.39	6	4.6

* Ranking excludes ripples due to the iron atoms.

\dagger Peak heights are measured in units of standard deviations where σ is the r.m.s. density of the map. R.m.s. density of both the maps is $0.0352 \text{ e \AA}^{-3}$.

Both in the sulfur-as-sulfur and in the sulfur-as-carbon Bijvoet-difference Fourier maps (Kraut, 1968) there is a peak ($\sim 3.4\sigma$) near the side chain of the current Asp34 (see Table 5). However, it is nearly twice the distance ($\sim 3.4 \text{ \AA}$) from the location of the C β as would be expected for a carbon-sulfur bond. There are no significant peaks near Asp34 in either 'imaginary' Fourier map. Furthermore, in tests with calculated structure factors a similar peak was present in the Kraut Bijvoet-difference Fourier map, suggesting that it is a function of the data included, rather than a real peak. Finally, Kraut Bijvoet-difference Fourier maps calculated at an earlier stage of refinement show no significant peak in the vicinity of Asp34.

During restrained least-squares refinement of the myohemerythrin structure, we found two solvent sites which kept refining to occupancies greater than 1.0. Inspection of $2F_o - F_c$ and $F_o - F_c$ maps suggested considerable clouds of density in the vicinity of these two sites. On this basis we decided to build the model with sulfate anions at these positions. However, the sulfate ions did not refine very well by two criteria: (a) sulfate oxygen to ligand distances became too short, and (b) sulfate oxygen occupancies became

Table 5. Peaks near model solvent positions in 'imaginary' Fourier map using sulfur-as-sulfur model for phases

	Peak-to-atom distance (Å)	Rank*	Peak height, σ^\dagger
Azil38N2	0.51	25	3.2
Azil46N2	0.86	38	3.0
Wat2030	0.83	56	2.9
Wat2810	0.97	32	3.2

* Ranking excludes ripples due to the iron atoms.

† Peak heights are measured in units of standard deviations where σ is the r.m.s. density of the map.

very unequal. Since the electron density at these positions was fairly linear, we decided to substitute azide for sulfate at these solvent sites. However, when we searched either 'imaginary' or Kraut Bijvoet-difference Fourier maps for peaks corresponding to solvent atoms, only four peaks met the criteria of being within 1.0 Å of a solvent site and having a peak height of at least 2.75 times the r.m.s. density. The two sulfate positions were among these (Table 5). The third position, which was the weakest, was about 3.1 Å from one of the two sulfates and did not correspond to any density that could be attributed to a sulfate. The fourth position was the farthest from a solvent site. This site had been modeled as an 'either/or' disordered site with another water, but it was so weak that it was removed from the model at a later stage. On the basis of these results we have returned to our interpretation of two positions as sulfate anions.

In conclusion we find the 'imaginary' Fourier to be very useful when the information is available for

its calculation. However, one must be cautious about interpreting such maps due to the considerable bias in the phases (Hendrickson & Sheriff, 1987). Without this bias, the 'imaginary' Fourier is still better than the Kraut Bijvoet-difference Fourier, largely because the noise level is below that of the Kraut function.

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Peptide Chain Structure Parameters, Bond Angles and Conformational Angles from the Cambridge Structural Database

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Abstract

An analysis of the bond angles and conformational angles in the oligopeptide crystals accumulated in the Cambridge Structural Database has been carried out to obtain precise information essential to protein structural studies. The peptide torsion angles ω distribute asymmetrically around 157-201°; $\omega < 170^\circ$ occurs with probability 7%, but $\omega > 190^\circ$ less than 3%. The $\tau(C^\alpha C'N)$ and $\tau(C'NC^\alpha)$ angles do not

depend on the structures of the main and side chains. But the $\tau(NC^\alpha C')$ angles depend on ψ as well as the side-chain shapes, being in the wide range 103-117°. For each amino-acid residue the mean $\tau(NC^\alpha C')$ angle in the folded-chain region with $\psi = -50-30^\circ$ is about 4° larger than that in the extended-chain region with $\psi = 100-210^\circ$; in the same ψ region the angle of Gly is the largest and that of Val, Ile and Thr is the smallest, their difference being about 3°. The side-chain conformations of $(N^\alpha-C^\alpha-C^\beta-C^\gamma)$ are strongly