COPPENS, P. & HAMILTON, W. C. (1970). *Acta Cryst.* A**26**, 71–83.
FUJINO, K., SASAKI, S., TAKÉUCHI, Y. & SADANAGA, R. (1981). *Acta Cryst.* B**37**, 513–518.
FUKAMACHI, T. (1971). Tech. Rep. B12. Institute of Solid State Physics, Univ. of Tokyo.
GHOSE, S., OKAMURA, F. P., WAN, C. & OHASHI, H. (1974). *EOS Trans. Am. Geophys. Union Abstr.* **55**(4), 467.
International Tables for X-ray Crystallography (1968). Vol. III. Birmingham: Kynoch Press.
International Tables for X-ray Crystallography (1974). Vol. IV. Birmingham: Kynoch Press.
LAGER, G. A. & MEAGHER, E. P. (1978). *Am. Mineral.* **63**, 365–377.
MARUMO, F., ISOBE, M. & AKIMOTO, S. (1977). *Acta Cryst.* B**33**, 713–716.
MARUMO, F., ISOBE, M., SAITO, Y., YAGI, T. & AKIMOTO, S. (1974). *Acta Cryst.* B**30**, 1904–1906.
MATSUI, M. & MATSUMOTO, T. (1982). *Acta Cryst.* A**38**, 513–515.
MORIMOTO, N., TOKONAMI, M., WATANABE, M. & KOTO, K. (1974). *Am. Mineral.* **59**, 475–485.
RAJAMANI, V., BROWN, G. E. & PREWITT, C. T. (1975). *Am. Mineral.* **60**, 292–299.
SASAKI, S., FUJINO, K., TAKÉUCHI, Y. & SADANAGA, R. (1980). *Acta Cryst.* A**36**, 904–915.
SASAKI, S., TAKÉUCHI, Y., FUJINO, K. & AKIMOTO, S. (1982). *Z. Kristallogr.* **158**, 279–297.
SHANNON, R. D. & PREWITT, C. T. (1969). *Acta Cryst.* B**25**, 925–946.
TAMADA, O. (1977). Abstr. Annu. Meet. of the Mineral. Soc. Japan, B**17**, 65.
TAMADA, O. (1980). *Mineral. J.* **10**, 71–83.
TOKONAMI, M. (1965). *Acta Cryst.* **19**, 486.
ZACHARIASEN, W. H. (1967). *Acta Cryst.* **23**, 558–564.

Acta Cryst. (1983). B**39**, 697–703

Adjustment of Restraints in the Refinement of Methemerythrin* and Azidomethemerythrin at 2·0 Å Resolution

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Abstract

Restrained least-squares refinement of the met and azidomet forms of hemerythrin has been carried out at 2·0 Å resolution. Average values for the Fe–ligand bond distances from the four subunits in the asymmetric unit were used as restraints in the following refinement cycle. The process was repeated until the restraints and Fe–X bond distances no longer changed significantly. Considerable variation is observed in each type of Fe–*X* bond, the Fe–N and Fe–$O_{carboxy}$ bonds being longer than 2·0 Å, the Fe–$O_{\mu\text{-oxo}}$ bonds being shorter. Systematic errors caused by absorption, anomalous scattering, and the limited resolution of the diffraction data do not account for the variation and lead to the tentative conclusion that the observed bond lengths are characteristic of the binuclear Fe complexes found in these proteins.

Introduction

In the restrained least-squares refinement (Waser, 1963; Konnert, 1976; Hendrickson & Konnert, 1980) of novel metalloprotein structures, the choice of restraints to apply to the metal–ligand distances is not trivial. These distances are of great interest and should be free of bias introduced by the use of inappropriate values. If no model compounds are known, one is unsure of the appropriate distances for use as restraints. Even if model compounds can provide initial target values, the question remains whether these would be appropriate for a metal complex bound to a protein matrix. In particular, in metalloproteins, one must allow for possible differences in angular and distance parameters induced by the protein.

In the case of methemerythrins from *Themiste dyscritum* (Stenkamp, Sieker, Jensen & Sanders-Loehr, 1981; Stenkamp, Sieker & Jensen, 1983), we are unaware of appropriate model compounds for the binuclear, non-heme Fe complex in this oxygen transport protein. While some EXAFS distances are available for the metal complex (Elam, Stern, McCallum & Sanders-Loehr, 1982; Hendrickson, Co, Smith, Hodgson & Klippenstein, 1982), they are not consistent enough to provide definitive restraint information. In the earlier restrained least-squares refinement of the met form of the protein, we simply restrained all Fe–O and Fe–N distances to 2·0 Å. The resulting bond lengths (Stenkamp, Sieker & Jensen, 1983) ranged from 1·84 to 2·16 Å. Comparison of the bonds suggested possible differences in length within bonds of

* This form of the protein has been referred to as met-, metaquo-, methydroxo- and methemerythrin. The background to the name changes is covered elsewhere (Stenkamp, Sieker & Jensen, 1983). We revert to the simpler name since no exogenous Fe ligand has yet been identified in this form of the protein.

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a given type as well as general differences between Fe–N and Fe–O distances. We report here the details of refining models for both met and azidomet forms of hemerythrin with Fe–X restraints adjusted for each kind of bond and discuss the validity of the resulting bond-length parameters.

Method and data

To eliminate the bias of the 2·0 Å Fe–X restraints used initially in refining the models (Stenkamp, Sieker & Jensen, 1983), we adjusted the restraints in the present refinements so that ultimately the value used for each kind of Fe–X bond in a given cycle was the average value of the bond length from the preceding cycle obtained from the four subunits in the asymmetric unit. Since the restraint used for each kind of bond is an average over the four subunits in the asymmetric unit, this procedure in effect increases the overdetermination of the problem. Restraints were not applied to the angles in the complex involving the Fe atoms nor were restraints imposed on the noncrystallographic symmetry relating the four subunits because we did not wish to suppress any possible differences among them. Since the metal complexes are located within each subunit, averaging of the bond lengths for the complexes should not be sensitive to any possible inequivalence of the four subunits in the asymmetric unit.

The 2·0 Å data set for methemerythrin used in this study is the same as that described earlier (Stenkamp, Sieker & Jensen, 1982), and the initial model was from the earlier refinement and was deposited in the Brookhaven Protein Data Bank, but identified at that time as hydroxymethemerythrin (Stenkamp, Sieker & Jensen, 1982). For the initial model, R ($= \sum ||F_o| - |F_c|| / \sum |F_o|$) was 0·187 for the 31 860 observed reflections [$I > 2\sigma(I)$] from 10·0 to 2·0 Å resolution.

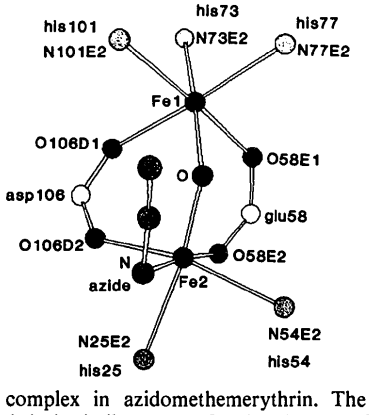

Fig. 1. The complex in azidomethemerythrin. The complex in methemerythrin is similar except for the absence of the azide or any other small-molecule ligand bound to Fe(2), making its coordination polyhedron a distorted trigonal bipyramid.

Table 1. *Data collection and processing for azidomethemerythrin*

Crystal number	1	2	3	4
Cell constants (Å)				
$a = b =$	86·62 (2)	86·62 (2)	86·60 (1)	86·45 (3)
$c =$	80·92 (1)	80·90 (2)	80·87 (1)	81·32 (2)
ω–2θ steps, No. of steps	5	5	5	5
Step size (°)	0·09	0·09	0·10	0·10
Cu $K\alpha$ wavelength (1·5418 Å)				
Maximum absorption correction	1·620	1·284	1·317	1·519
Maximum deterioration correction	1·976	1·598	1·889	2·149
R (on F) for replicates	0·028	0·038	0·022	0·079
Number of replicates	40	60	1294	766
R (on F) for Friedel pairs	0·041	0·046	0·022	0·067
Number of Friedel pairs	10006	8599	10880	4975
R (on F) against F_c for scaling	0·221	0·242	0·191	0·277

Functional form of deterioration correction where the C_i are determined by a least-squares fit of the standard reflections:

for crystals 1–3: scale $= C_1 + C_2 \times 2\theta \times \text{time} + C_3 \times (2\theta)^2 \times \text{time}^2 + C_4 \times 2\theta + C_5 \times (2\theta)^2 \times \text{time} + C_6 \times (2\theta)^2$

for crystal 4: scale $= C_1 + C_2 \times 2\theta \times \text{time} + C_3 \times (2\theta)^2 \times \text{time}^2 + C_4 \times (2\theta)^2 \times \text{time}$

Before the present study was initiated, a 2·2 Å data set for azidomethemerythrin had been collected from three crystals (Stenkamp *et al.*, 1981). When the F_o values from this data set were compared with the F_c values from the met model referred to above, R was 0·239 for the 27 131 reflections with $I > 2\sigma(I)$ from 10 to 2·2 Å resolution. R increased to 0·288 on removal of the Fe atoms, the bridging oxygen and the amino-acid side chains bonded to the Fe atoms. Two restrained least-squares refinement cycles were calculated to relieve the bias in phases caused by the met complex. At that point, an $F_o - F_c$ map clearly showed the similarity of the complexes in the two forms of hemerythrin, and the position of the bound azide ion was evident in all four subunits, Fig. 1. The Fe atoms, the bridging oxygen, the amino-acid side chains and the azide were added to the model and refined in five least-squares cycles, keeping the rest of the model fixed. With Fe–X distances restrained to 2·0 Å, R decreased to 0·207 in the five cycles. The resulting parameters were the initial values used in the present study.

The resolution of the 2·2 Å data set for azidomethemerythrin was extended to 2·0 Å for this study by collecting data from an additional crystal. The added data raised R to 0·218 for the 32 363 reflections from 10 to 2·0 Å resolution. Table 1 summarizes the data collection and processing for azidomethemerythrin.

Computation and results

The restrained least-squares program of Hendrickson (Sielecki, Hendrickson, Broughton, Delbaere, Brayer & James, 1979) was used for the refinements

reported here. Standard deviations of the restraints and the weights of the X-ray data relative to the restraints were the same as used in the final stages of the earlier refinement of methemerythrin (Stenkamp *et al.*, 1982).

Fig. 2 shows the course of the refinement for methemerythrin as a plot of the Fe–X bond lengths against model numbers. Restraints in the complexes for the first four cycles were average distances over the subunits and the type of bond, *i.e.* the twenty Fe–N bonds, the sixteen Fe–$O_{carboxyl}$ bonds, and the eight Fe–$O_{\mu\text{-oxo}}$ bonds in the four subunits. Restraints for the next six cycles were simple averages over the four subunits for each kind of bond. The ten refinement cycles reduced R from 0·187 to 0·178.

The course of refining the azidomet model with adjusted restraints is shown in Figs. 3 and 4. Two cycles of refinement were carried out with restraints set to 2·05 Å for all Fe–N bonds, 2·00 Å for Fe–$O_{carboxy}$ bonds and 1·90 Å for Fe–$O_{\mu\text{-oxo}}$ bonds. In subsequent cycles, as indicated by the solid light lines in Figs. 3 and 4, values of each kind of Fe–X bond averaged over the subunits were used as restraints. Seven refinement cycles reduced R from 0·218 to 0·181.

At this point, while ΔF maps for both met and azidomethemerythrin were being interpreted, three refinement cycles with the Fe–X restraints removed were carried out on each structure, as indicated by the dotted lines in the midsections of Figs. 2–4.

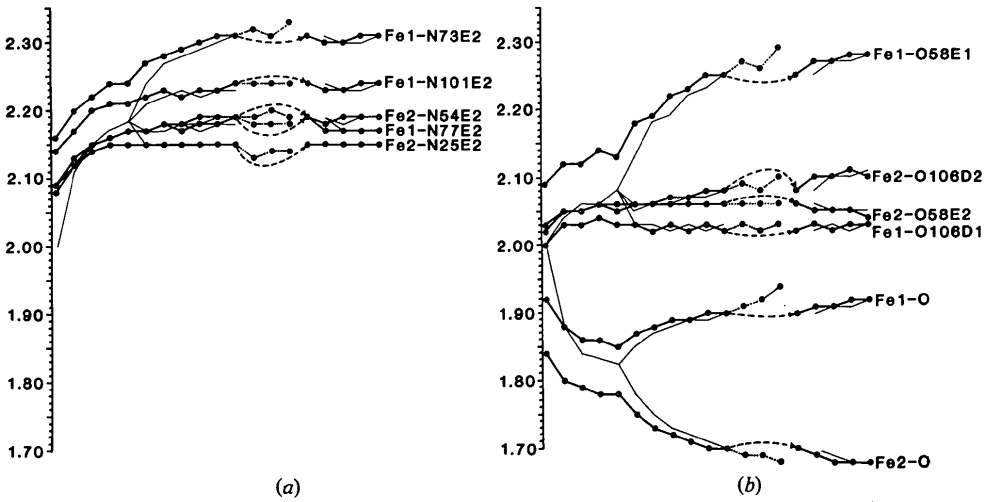

Fig. 2. Plot of Fe–X bond lengths (averaged over four subunits) and restraints for methemerythrin as a function of model number. (*a*) Fe–$N_{histidine}$ bonds. (*b*) Fe–$O_{carboxy}$ and Fe–$O_{\mu\text{-oxo}}$ bonds. —— Restrained least-squares-refinement cycles. ····· Unrestrained least-squares-refinement cycles. – – – Adjustment of model on the basis of examination of a ΔF map. —— Restraint values used to generate that model. (Distances in Å in Figs. 2–5.)

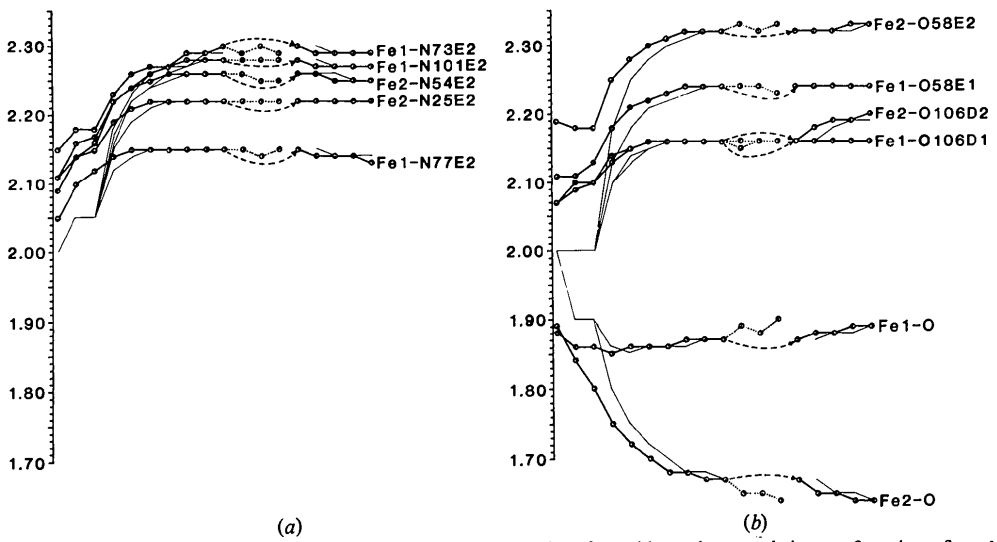

Fig. 3. Plot of Fe–X bond lengths (averaged over four subunits) and restraints for azidomethemerythrin as a function of model number. (*a*) Fe–$N_{histidine}$ bonds. (*b*) Fe–$O_{carboxy}$ and Fe–$O_{\mu\text{-oxo}}$ bonds. See legend to Fig. 2 for description of lines.

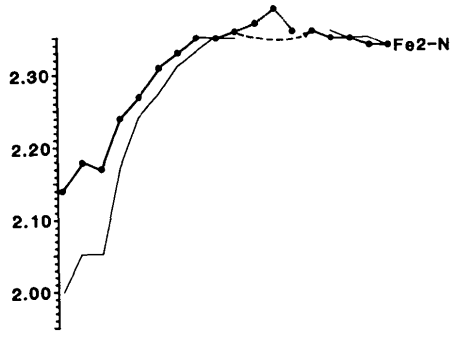

Fig. 4. Plot of Fe–azide bond lengths (averaged over four subunits) and restraints for azidomethemerythrin as a function of model number. See legend to Fig. 2 for description of lines.

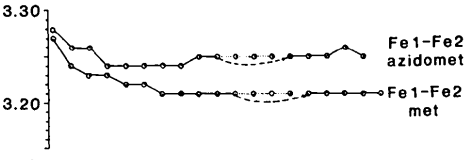

Fig. 5. Plot of Fe–Fe distances (averaged over four subunits) for methemerythrin and azidomethemerythrin. See legend to Fig. 2 for description of lines.

Use of stereochemical information, non-crystallographic symmetry and the similarity in the subunits from both met forms limited the number of corrections based on the ΔF map and made in the models. Several amino-acid side chains were repositioned, and three water molecules were added to the models. This increased R to 0·183 for methemerythrin and to 0·186 for the azidomet form.

The models were refined in four final least-squares cycles, updating the restraints after each cycle as noted above, until there was little or no change in the parameters. R decreased to 0·173 for methemerythrin and 0·175 for the azidomet form. The r.m.s. differences between the bond lengths in the polypeptide and the ideal values used as restraints were 0·027 and 0·026 Å respectively.*

Fig. 5 shows the Fe···Fe distance for both met and azidomethemerythrin over the course of refinement with adjustable Fe–X restraints.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1HMQ, 1HMZ, R1HMQSF, R1HMZSF), and are available in machine-readable form from the Protein Data Bank at Brookhaven or one of the affiliated centers at Cambridge, Melbourne or Osaka. The data have also been deposited with the British Library Lending Division as Supplementary Publication No. SUP 37011 (4 microfiche). Free copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Discussion

In Table 2(*a*), we list for both the met and azidomet complexes the average bond lengths for each Fe–X bond type in the models used to initiate the present study. These were the bond lengths resulting from refinements with 2·0 Å fixed restraints for all Fe–X bonds. Comparison of the lengths suggests differences for some bond types. Furthermore, consideration of the initial lengths of each kind of bond, the first point in each plot in Figs. 2–4, suggests possible differences within some types. The initial values range from 1·84 Å for the Fe(2)–O bond in the met complex to 2·19 Å for the Fe(2)–O(58E2) bond in the azidomet complex.

It was this variation of bond lengths that prompted us to study the possibilities of adjusting the restraints. Altering the restraints as we did in this study approaches free refinement which experience has shown may be ill-behaved for models with bond lengths near the minimum interplanar spacings of the data. Nevertheless, the plots in Figs. 2–4 indicate that the refinements behaved well, presumably because the restraints damped out the tendency of the bond lengths to oscillate or diverge, as evident for some of the bonds when the restraints were removed (see dotted-line segments near middle of Figs. 2–4). Although we cannot claim that the refinements have completely converged, only four of the 23 bonds shown in Figs. 2–4 changed by as much as 0·01 Å in the last cycles.

In Table 2(*b*), we list the average bond lengths for each bond type resulting from the refinement with adjusted restraints. Comparison with values in Table 2(*a*) indicates that bonds greater than 2·0 Å in length increased on relieving the bias of the 2·0 Å restraints and conversely for those less than 2·0 Å. In methemerythrin, final Fe–N bond lengths range from 2·15 to 2·31 Å, values considerably greater than expected for either five- or six-coordinate Fe (see Fig. 2). Three of the Fe–$O_{carboxy}$ bonds are in the range 2·03–2·10 Å with an average value of 2·06 Å, somewhat greater than expected for such bonds. The fourth Fe–$O_{carboxy}$ bond, however, Fe(1)–O(58E1), of length 2·28 Å, is much greater than expected. The two Fe–$O_{\mu\text{-oxo}}$ bonds are less than 2·0 Å as found for such bonds in small molecules, but one of them, the Fe(2)–O bond, is much shorter than expected, even for five-coordinate Fe.

Table 2. *Average* Fe–X *bond lengths* (Å) *for each bond type with different restraints*

	(*a*)		(*b*)	
Bond	2·0 Å fixed restraints		Variable restraints	
type	met	azidomet	met	azidomet
Fe–$N_{histidine}$	2·11	2·10	2·21	2·23
Fe–N_{azide}	–	2·14	–	2·34
Fe–$O_{carboxy}$	2·04	2·11	2·11	2·23
Fe–$O_{\mu\text{-oxo}}$	1·88	1·89	1·80	1·76

Table 3. Fe–*X* distances (Å)

Final Fe···Fe and Fe–*X* distances in the four subunits, the mean value, and the e.s.d. in the mean (see *Discussion*). Protein atom names are a combination of atom type, residue number and atom designation.

					Mean	E.s.d.
(*a*) Methemerythrin						
Fe(1)–Fe(2)	3·20	3·21	3·18	3·24	3·21	0·012
Fe(1)–N(73E2)	2·30	2·31	2·29	2·33	2·31	0·009
Fe(1)–N(77E2)	2·16	2·18	2·15	2·19	2·17	0·009
Fe(1)–N(101E2)	2·20	2·28	2·26	2·21	2·24	0·019
Fe(1)–O(58E1)	2·28	2·29	2·27	2·27	2·28	0·005
Fe(1)–O(106D1)	2·06	1·96	2·00	2·09	2·03	0·029
Fe(1)–O	1·94	1·95	1·87	1·91	1·92	0·018
Fe(2)–N(25E2)	2·20	2·11	2·13	2·14	2·15	0·019
Fe(2)–N(54E2)	2·19	2·22	2·21	2·16	2·19	0·013
Fe(2)–O(58E2)	2·04	2·07	2·04	2·02	2·04	0·010
Fe(2)–O(106D2)	2·09	2·06	2·12	2·14	2·10	0·018
Fe(2)–O	1·67	1·68	1·73	1·62	1·68	0·023
(*b*) Azidomethemerythrin						
Fe(1)–Fe(2)	3·27	3·25	3·24	3·26	3·25	0·006
Fe(1)–N(73E2)	2·28	2·33	2·27	2·30	2·29	0·013
Fe(1)–N(77E2)	2·12	2·13	2·16	2·13	2·13	0·009
Fe(1)–N(101E2)	2·26	2·27	2·26	2·30	2·27	0·009
Fe(1)–O(58E1)	2·22	2·29	2·20	2·24	2·24	0·019
Fe(1)–O(106D1)	2·19	2·11	2·15	2·19	2·16	0·019
Fe(1)–O	1·91	1·84	1·86	1·92	1·89	0·019
Fe(2)–N(25E2)	2·25	2·21	2·19	2·25	2·22	0·015
Fe(2)–N(54E2)	2·27	2·22	2·23	2·27	2·25	0·013
Fe(2)–O(58E2)	2·33	2·35	2·30	2·35	2·33	0·012
Fe(2)–O(106D2)	2·16	2·19	2·22	2·20	2·20	0·007
Fe(2)–O	1·64	1·64	1·66	1·63	1·64	0·006
Fe(2)–N_{azide}	2·37	2·33	2·36	2·32	2·34	0·013

After refining the azidomet form with variable restraints, the range of the Fe–N bonds, 2·14–2·29 Å, is similar to that of the met form, but one of the bonds, Fe(1)–N(77E2), is considerably shorter than the other four (see Fig. 3). The Fe–$O_{carboxy}$ bonds range from 2·16 to 2·33 Å, similar to the Fe–N range, and three of the four are longer than the corresponding bonds in the met form. The Fe–O bonds in azidomethemerythrin are similar in length to those in the met form, but the Fe(2)–O bond is very short at 1·64 Å, although the Fe atom is six coordinate. The Fe(2)–N_{azide} bond, on the other hand, is very long, 2·34 Å (Fig. 4).

In order to assess the possible significance of differences in bond lengths, we resort to an estimate based on the scatter in lengths of a given bond over the four independent subunits. We note that the estimates will be low because the restraints suppress the scatter, but they are nevertheless useful as an indication of the precision. The last column in Table 3 lists the σ_{mean} of the final values for each of the 23 bonds in Figs. 2–4 calculated from the equation

$$\sigma_{mean} = [\sum (\bar{l} - l_i)^2/n(n-1)]^{1/2},$$

where $\bar{l}$ is the mean bond length for a given bond, l_i is its length in the ith subunit and n equals 4, the number of subunits.

Because the sample size is small, the standard deviations of the mean values for each kind of Fe–*X* bond show substantial scatter as seen for the individual values in Table 3. Since we expect similar standard deviations in all Fe–*X* bond lengths, we take the r.m.s. value of 0·016 Å as a measure of σ for each kind of bond. The standard deviation in the difference between any two bonds is $\sqrt{2}(0{\cdot}016) = 0{\cdot}023$ Å, and, to be confident at the 99% level, we take (2·58)(0·023) = 0·059 Å. Because of the restraints, however, we *arbitrarily* multiply by a factor of two and consider a difference of 0·12 Å between two *independent* bonds as possibly significant. Nevertheless, in view of the nature of these refinements, we emphasize that this estimate cannot be regarded as definitive.

We now ask whether we can believe the values we have derived for the Fe–*X* bond lengths. Are they accurate or are there systematic errors that shift the relative positions of the Fe and ligand atoms, altering the bond lengths from their true values? We have corrected the data for absorption (North, Phillips & Mathews, 1968), thus minimizing errors from that source, and both reflections of each Friedel pair were collected and averaged to eliminate the effects of anomalous scattering in space group *P*4. In this context, we note that the habit and orientation differed for the met and azidomet crystals, and the crystallographic and noncrystallographic symmetry elements generate several orientations of the subunits with respect to the crystal axes.

We turn now to the matter of series termination error stemming from data limited to 2·0 Å resolution. The effects of diffraction ripples in image formation are well known and have been treated theoretically by James (1948) and numerically for Na^+ and Cl^- by Bragg & West (1930). In Fig. 6, we show plots of the radial electron density for Fe and N atoms with *B* values of

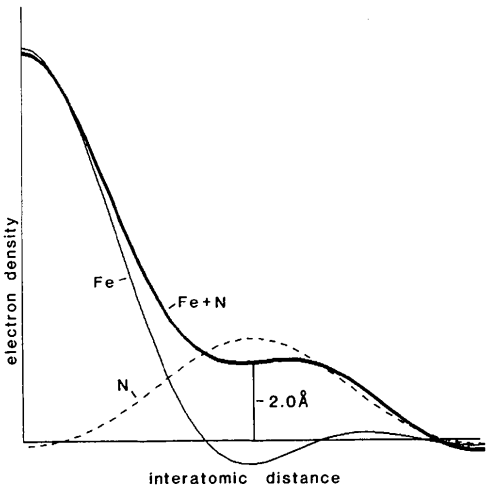

Fig. 6. Radial electron density, $\rho(r)$, in e Å^{-3} for Fe and N atoms with B = 10 Å^2, 2 Å resolution data, and the sum of $\rho(r)$ for these two atoms separated by 2·0 Å.

10 Å^2 obtained by calculating the transforms of the corresponding F_c data sets with d_{min} equal to 2·0 Å. It is evident that the first minimum for both Fe and N atoms is near 2·0 Å from the atomic center. The result of superimposing the electron densities for Fe and N atoms separated by 2·0 Å is also shown in Fig. 6. The ripple from Fe has shifted the nitrogen peak by about 0·25 Å. This is what we would observe in an F_o map under the assumed conditions.

We now ask whether similar effects carry over into least squares which was used in the refinements reported here. The ripples in Fig. 6, arising from series termination error, can be *approximately* corrected for by Fourier difference syntheses which tend to minimize $\sum(|F_o| - |F_c|)$. Restrained least squares, on the other hand, tends to minimize $\sum w(|F_o| - |F_c|)^2$, where we have set the weight, w, equal to 1·0. The similarity of the functions suggests that vestiges of series termination may remain in the least-squares results.

We tested for possible effects by truncating the met data at 2·2 Å resolution and subjecting the model to two additional least-squares cycles. For the truncated data, the first minimum of the Fe ripple will occur near 2·2 Å. Thus any ripple effect, if present, would tend to lengthen bonds longer than this value, and conversely for bonds that are shorter. For the three bonds longer than 2·2 Å [Fe(1)–N(73E2), Fe(1)–N(101E2) and Fe(1)–O(58E1), Fig. 2], the bond lengths increased an average of 0·007 Å in the two additional least-squares cycles. The eight remaining bonds are all of length less than 2·2 Å, and they decreased slightly in the two cycles by an average of 0·001 Å. In view of the fact that the additional cycles at 2·2 Å resolution did not alter any bond length by more than 0·01 Å, we conclude that the effects of series termination on the least-squares results are small.

We note that the lengths of the Fe–N bonds in both the met and azidomet forms are greater than usually reported for such bonds (1·94–1·99 Å, Sim & Sinn, 1978; 2·04–2·06 Å, Weiss & Goedken, 1979; 2·11–2·12 Å, Davies & Gatehouse, 1973) although values as great as 2·30–2·34 Å have been reported for seven-coordinate Fe^{III}–N bonds (Lind & Hoard/Hamor, Hamor & Hoard, 1964). Similarly, all of the Fe–$O_{carboxy}$ bonds in the azidomet form and one of them in the met form are longer than lengths usually reported for such bonds (1·94–2·04 Å, Calgero, Russo & Del Pra, 1980; 1·98–2·00 Å, Holt, Alcock, Sumner & Asplund, 1979; 1·94–2·13 Å, Lind & Hoard/Hamor, Hamor & Hoard, 1964).

The Fe(1)–O bond lengths are greater than values commonly reported (1·79–1·80 Å, Lippard, Schugar & Walling, 1967; 1·79 Å, Weiss & Goedken, 1979), but the Fe(2)–O lengths are considerably less. Whether the asymmetry of the complexes in these forms of hemerythrin accounts for the inequivalence of these bonds is unknown, but the averages of the two bonds in both complexes, Table 2(*b*), are within the range reported for small structures. In any case, we note that the Fe(1)–O and Fe(2)–O bonds are *not independent* and are particularly sensitive to the position of the single O atom in the presence of two nearby Fe atoms.

Other experimental evidence supporting the short Fe–$O_{\mu\text{-oxo}}$ bonds comes from EXAFS (Elam, Stern, McCallum & Sanders-Loehr, 1982; Hendrickson, Co, Smith, Hodgson & Klippenstein, 1982) and magnetic susceptibility studies (Dawson, Gray, Hoenig, Rossman, Schredder & Wang, 1972). The EXAFS experiments indicate the presence of Fe–O distances as short as 1·71 Å, and the large antiferromagnetic coupling of 134 cm^{-1} is also indicative of a short Fe–$O_{\mu\text{-oxo}}$ bond (Thich, Toby, Powers, Potenza & Schugar, 1981).

The Fe···Fe distance is an important parameter, and since only the heavy Fe atoms are involved, it should be rather accurately determined. In Fig. 5 we observe that, on adjusting the Fe–X restraints, the Fe···Fe distance decreases from 3·27 to 3·21 Å for the met form and from 3·28 to 3·25 Å for the azidomet form. The reason for the decrease can be seen by noting in Fig. 1 that all Fe–N bonds (neglecting Fe–N_{azide}) which increased in length on relaxing the restraints would pull the Fe atoms apart when restrained to 2·0 Å. Similarly, the Fe–$O_{\mu\text{-oxo}}$ bonds which decreased in length on relaxing the restraints would force the Fe atoms apart when restrained to 2·0 Å. The force exerted by the 2·0 Å restraints on the Fe–N and Fe–$O_{\mu\text{-oxo}}$ bonds would be countered only by the Fe–$O_{carboxy}$ bonds which increased on relaxing the restraints, but their expected effects are relatively less important because of the angular flexibility at the carboxyl groups. The changes in the Fe···Fe distances clearly demonstrate the systematic effects of imposing restraints.

We regard the bond lengths reported here, based on the results of restrained least squares with adjusted restraints, as representing the best we can achieve under present circumstances with the present data sets. We recognize that systematic errors beyond those we have considered may operate. On the basis of a tenuous estimate of the precision of the Fe–X bond lengths we cannot accept as definitive the large deviations from expected values for some of the Fe–X bonds. Nevertheless, our results are suggestive of possible differences imposed by the polypeptide and emphasize the need for additional studies to determine with improved accuracy the geometrical parameters of the binuclear complexes in both met and azidomethemerythrin. In addition, we wish to point out that the general applicability of adjusting restraints is not clear. No cases have been studied using this approach at various levels of resolution or in the absence of multiple copies in the asymmetric unit to increase the overdetermination. Until calculations are carried out investigating the effects of resolution and overdetermination, no firm conclusions can be made concerning

the generality of the method of choosing restraints described here.

A detailed comparison of the met and azidomet complexes is beyond the scope of this paper and will be reported elsewhere.

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References

BRAGG, W. L. & WEST, J. (1930). *Philos. Mag.* **10**, 823–841.
CALGERO, S., RUSSO, U. & DEL PRA, A. (1980). *J. Chem. Soc. Dalton Trans.* pp. 646–653.
DAVIES, J. E. & GATEHOUSE, B. M. (1973). *Acta Cryst.* B**29**, 1934–1942.
DAWSON, J. W., GRAY, H. B., HOENIG, H. E., ROSSMAN, G. R., SCHREDDER, J. M. & WANG, R.-H. (1972). *Biochemistry*, **11**, 461–465.
ELAM, W. T., STERN, E. A., MCCALLUM, J. D. & SANDERS-LOEHR, J. S. (1982). *J. Am. Chem. Soc.* **104**, 6369–6373.
HENDRICKSON, W. A., CO, M. S., SMITH, J. L., HODGSON, K. O. & KLIPPENSTEIN, G. L. (1982). *Proc. Natl Acad. Sci. USA*, **79**, 6255–6259.
HENDRICKSON, W. A. & KONNERT, J. H. (1980). *Biomolecular Structure, Function, Conformation and Evolution*, edited by R. SRINIVASAN, Vol. 1, pp. 43–57. Oxford: Pergamon Press.
HOLT, E. M., ALCOCK, N. W., SUMNER, R. H. & ASPLUND, R. O. (1979). *Cryst. Struct. Commun.* **8**, 255–259.
JAMES, R. W. (1948). *Acta Cryst.* **1**, 132–134.
KONNERT, J. H. (1976). *Acta Cryst.* A**32**, 614–617.
LIND, M. D. & HOARD, J. L./HAMOR, M. J., HAMOR, T. A. & HOARD, J. L. (1964). *Inorg. Chem.* **3**, 34–43.
LIPPARD, S. J., SCHUGAR, H. & WALLING, C. (1967). *Inorg. Chem.* **6**, 1824–1831.
NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 351–359.
SIELECKI, A. R., HENDRICKSON, W. A., BROUGHTON, C. G., DELBAERE, L. T. J., BRAYER, G. D. & JAMES, M. N. G. (1979). *J. Mol. Biol.* **134**, 781–804.
SIM, P. G. & SINN, E. (1978). *Inorg. Chem.* **17**, 1288–1290.
STENKAMP, R. E., SIEKER, L. C. & JENSEN, L. H. (1982). *Acta Cryst.* B**38**, 784–792.
STENKAMP, R. E., SIEKER, L. C. & JENSEN, L. H. (1983). *J. Inorg. Biochem.* In the press.
STENKAMP, R. E., SIEKER, L. C., JENSEN, L. H. & SANDERS-LOEHR, J. S. (1981). *Nature (London)*, **291**, 263–264.
THICH, J. A., TOBY, B. H., POWERS, D. A., POTENZA, J. A. & SCHUGAR, H. J. (1981). *Inorg. Chem.* **20**, 3314–3317.
WASER, J. (1963). *Acta Cryst.* **16**, 1091–1094.
WEISS, M. C. & GOEDKEN, V. L. (1979). *Inorg. Chem.* **18**, 819–826.

Acta Cryst. (1983). B**39**, 703–711

Analysis of the Thermal Parameters of the Water Molecule in Crystalline Hydrates Studied by Neutron Diffraction

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Abstract

A survey is given of the thermal parameters of 150 water molecules in crystalline hydrates determined by neutron diffraction. The accuracy of the thermal parameters has been examined and rigid-bond tests revealed systematic errors for approximately 25% of the molecules. Considering only the most precise and accurate studies, good agreement is obtained between vibrational amplitudes derived from diffraction and spectroscopy. The influence of the immediate environment on the vibrations of the water molecule has also been investigated. A positive correlation is found between H···O hydrogen-bond distances and librational amplitudes. The coordination geometry around the O atom is shown to influence the vibrational amplitudes of the O atom.

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Introduction

Several attempts have been made in the past to test the validity of diffraction-obtained thermal parameters. Particularly relevant is the study of hexamethylenetetramine by Willis & Howard (1975), who showed that the neutron-diffraction thermal parameters were in close agreement with those obtained independently from the phonon-dispersion curves. Their result was a very important one since it is difficult to determine the accuracy of the absolute magnitudes of neutron-diffraction-obtained vibrational amplitudes. It was also most encouraging, especially since an earlier IUCr project (Hamilton & Abrahams, 1970) had created a rather depressing picture of the accuracy of thermal parameters.

Recently, Trueblood & Dunitz (1983) have used