J. Am. Chem. Soc. **1984**, *106*, 618–622

Binuclear Iron Complexes in Methemerythrin and Azidomethemerythrin at 2.0-Å Resolution

Ronald E. Stenkamp,* Larry C. Sieker, and Lyle H. Jensen

Contribution from the Departments of Biological Structure and Biochemistry, University of Washington, Seattle, Washington 98195. *Received July 15, 1983*

Abstract: The binuclear Fe complexes in methemerythrin[1] and azidomethemerythin are compared and discussed. In methemerythrin, the complex consists of an octahedral Fe atom and a trigonal-bipyramidal Fe. The ligating atoms are provided by the amino acid side chains and a bridging oxygen atom. In converting to azidomethemerythrin, the pentacoordinate Fe becomes octahedrally coordinated with the azide ion as the sixth ligand. Binding of azide, in addition to changing the symmetry of the complex, causes changes in the bond lengths and angles in the metal center and conformational changes in the protein.

Hemerythrin and myohemerythrin are non-heme iron proteins that function in oxygen transport and storage in several invertebrate organisms. Hemerythrin is oligomeric, typically an octamer, and myohemerythrin is a monomer. The active center in both proteins is a binuclear iron complex.

As oxygen binding proteins with active sites distinctly different from those in hemoglobin and myoglobin, the hemerythrins are of considerable interest and have been studied by both physical and chemical techniques to identify the amino acid side chains bound to the Fe atoms and to characterize the complex in the deoxy, oxy, and met forms of the protein. The results of chemical modification, spectroscopic measurements (absorption, Mossbauer, resonance Raman), and magnetic studies of the proteins have been extensively reviewed.[2-5]

X-ray crystallographic studies have shown the secondary and tertiary structure in azidometmyohemerythrin[6] and in methemerythrin[7] to be similar. Approximately 70% of the 113 residue chain in the subunit of the hemerythrin octamer is in the form of four long, approximately parallel helices with the 20 N-terminal residues in a nonhelical section meandering along one side of the subunit. This four parallel helical structure motif is also found in cytochrome b_{562}, cytochrome c', and the ferritin and tobacco mosaic virus subunits.[8]

The binuclear active center of each subunit of methemerythrin is packed within the confines of the four helices and bound to them by amino acid side chains.[9] The two Fe atoms are joined by an oxo bridge and the carboxyl side chains of Glu-58 and Asp-106. In addition, side chains of His-73, His-77, and His-101 are coordinated to one Fe atom, making it hexacoordinate; side chains of His-25 and His-54 are coordinated to the second Fe, making it pentacoordinate (Figure 1).[10] In azidomethemerythrin, azide binds to the second Fe atom, making it hexacoordinate (Figure 2). In this report, we discuss and compare the structural parameters of the complexes in both the met and azidomet forms of hemerythrin resulting from the crystallographic refinements of the models of these proteins.

Experimental and Computational Section

The crystallographic studies leading to the parameters on which this report is based have already been published.[11,12] To aid in understanding the results and assessing the comparisons reported here, we present a brief summary of the crystallographic background.

Crystals of methemerythrin and azidomethemerythrin are isomorphous, space group *P4*, approximate unit cell parameters $a = b = 86.60$ Å, $c = 80.80$ Å. We have refined models of both structures against 2.0-Å resolution data by restrained least squares.[13-15] In the last stages of refinement, the Fe–X restraints were adjusted in each cycle to the average for each bond over the four independent subunits from the preceding cycle.[12] At convergence, relaxing the restraints in this way should give results similar to free refinement. No restraints were applied to the bond angles within the complexes. With x, y, z, and isotropic thermal parameters for each atom, the model for the met form (4296 atoms) and that for the azidomet form (4304 atoms) refined to R values ($R = \sum||F_o|-|F_c||/\sum|F_o|$) of 0.173 and 0.175, respectively.

Summary of Results

The bond lengths and bond angles for the iron complexes in each of the four independent subunits in the met and azidomet forms of hemerythrin are listed in Tables I and II along with their mean values and the standard deviations in the mean values. The mean values also appear in Figure 3.

The Fe–N_{His} bond lengths range from 2.15 to 2.31 Å for methemerythrin with a mean of 2.21 Å and from 2.13 to 2.29 Å for azidomethemerythrin with a mean of 2.23 Å. The Fe–N_{azide} is the longest of the Fe–N bonds, 2.34 Å.

The Fe–$O_{carboxy}$ bond lengths range from 2.03 to 2.28 Å for methemerythrin with a mean of 2.11 Å and from 2.16 to 2.33 Å for azidomethemerythrin with a mean of 2.23 Å. The Fe–$O_{\mu\text{-oxo}}$ bonds are all less than 2.0 Å in length, ranging from 1.64 to 1.92 Å. In both complexes one of the bonds is shorter, the other longer than values usually reported for such bonds.

In the met complex, Fe(1) is hexacoordinate (octahedral). The 12 angles that are ideally 90° range from 82 to 102°, while the three with ideal values of 180° range from 167 to 174°. Considering the coordination of Fe(2) in the met complex to be trigonal bipyramidal, the six angles that are ideally 90° range from 83 to 101°, the three with ideal values of 120° range from 103 to 143°, and the one that ideally would be 180° is 163°.

In the azidomet complex, both Fe atoms are octahedrally coordinated. The angles involving Fe(1) that are ideally 90° range from 82 to 102°; those with ideal values of 180° range from 168 to 172°. The angles involving Fe(2) that are ideally 90° range from 78 to 102°, and those with ideal values of 180° range from 154 to 174°.

The Fe–Fe distance is 3.21 Å in the met complex and 3.25 Å in the azido complex.

Precision. The problem of locating the light N and O ligand atoms in the presence of the heavier Fe atoms is difficult with

(1) In various publications from our laboratory, this form of the protein has been referred to as methemerythrin, metaquohemerythrin, methydroxohemerythrin, and aquomethemerythrin. The background to the name changes is covered in ref 10. We revert to the simpler name here since no exogenous Fe ligand has yet been identified in this form of the protein (see Figure 1).

(2) Klotz, I. M.; Klippenstein, G. L.; Hendrickson, W. L. *Science (Washington, D.C)* **1976**, *192*, 335–344.
(3) Kurtz, D. M., Jr.; Shriver, D. F.; Klotz, I. M. *Coord. Chem. Rev.* **1977**, *24*, 145–178.
(4) Klippenstein, G. L. *Am. Zool.* **1980**, *20*, 39–51.
(5) Loehr, J. S.; Loehr, T. M. *Adv. Inorg. Biochem.* **1979**, *1*, 235–252.
(6) Hendrickson, W. A.; Klippenstein, G. L.; Ward, K. B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2160–2164.
(7) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H.; Loehr, J. S. *J. Mol. Biol.* **1976**, *100*, 23–34.
(8) Weber, P. C.; Salemme, F. R. *Nature (London)* **1980**, *287*, 82–84.
(9) Stenkamp, R. E.; Jensen, L. H. *Adv. Inorg. Biochem.* **1979**, *1*, 219–233.
(10) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Inorg. Biochem.* **1983**, *19*, 247–253.
(11) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *Acta Crystallogr., Sect. B* **1982**, *B38*, 784–792.
(12) Stenkamp., R. E.; Sieker, L. C.; Jensen, L. H. *Acta Crystallogr. Sect. B* **1983**, *B39*, 697–703.
(13) Waser, J. *Acta Crystallogr.*, **1963**, *16*, 1091–1094.
(14) Konnert, J. H. *Acta Crystallogr., Sect. A* **1976**, *A32*, 614–617.
(15) Hendrickson, W. A.; Konnert, J. H. "Biomolecular Structure, Function, Conformation and Evolution"; Srinivasan, R., Ed.; Pergamon Press: Oxford, 1980; Vol. 1, 43–57.

0002-7863/84/1506-0618$01.50/0 © 1984 American Chemical Society

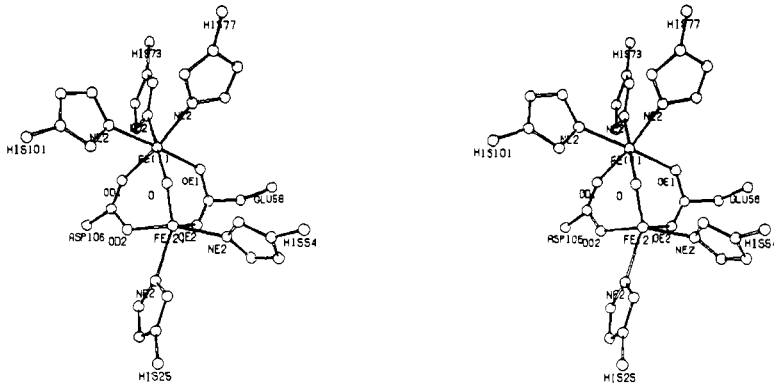


Figure 1. Stereoscopic view of the complex in methemerythrin.

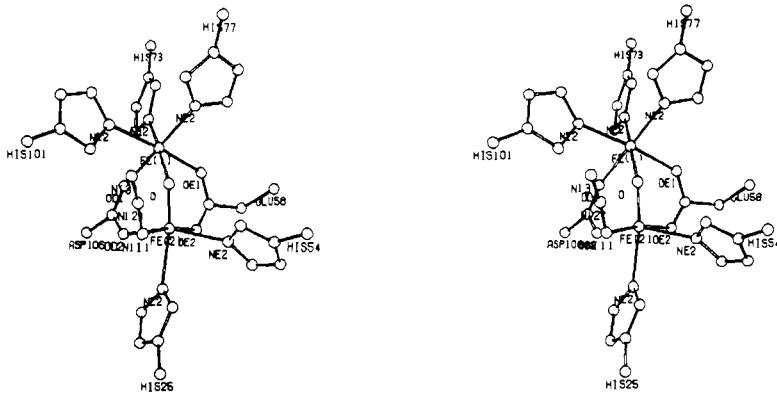
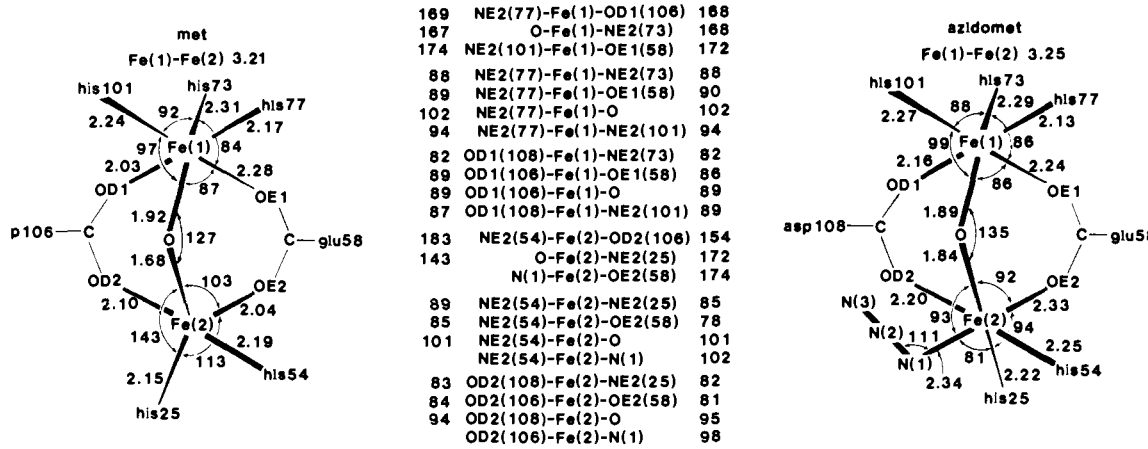


Figure 2. Stereoscopic view of the complex in azidomethemerythrin.



169	NE2(77)-Fe(1)-OD1(106)	168
167	O-Fe(1)-NE2(73)	168
174	NE2(101)-Fe(1)-OE1(58)	172
88	NE2(77)-Fe(1)-NE2(73)	88
89	NE2(77)-Fe(1)-OE1(58)	90
102	NE2(77)-Fe(1)-O	102
94	NE2(77)-Fe(1)-NE2(101)	94
82	OD1(108)-Fe(1)-NE2(73)	82
89	OD1(106)-Fe(1)-OE1(58)	86
89	OD1(106)-Fe(1)-O	89
87	OD1(108)-Fe(1)-NE2(101)	89
183	NE2(54)-Fe(2)-OD2(106)	154
143	O-Fe(2)-NE2(25)	172
	N(1)-Fe(2)-OE2(58)	174
89	NE2(54)-Fe(2)-NE2(25)	85
85	NE2(54)-Fe(2)-OE2(58)	78
101	NE2(54)-Fe(2)-O	101
	NE2(54)-Fe(2)-N(1)	102
83	OD2(108)-Fe(2)-NE2(25)	82
84	OD2(106)-Fe(2)-OE2(58)	81
94	OD2(108)-Fe(2)-O	95
	OD2(106)-Fe(2)-N(1)	98

Figure 3. Summary of bond lengths and angles in the two complexes.

the limited data available for large structures such as the octameric hemerythrins. Since both reflections of each Friedel pair were recorded, we measured a minimum of approximately 80 000 reflections for each of the 2.0-Å resolution data sets on which the present results are based. Although data at higher resolution could be collected, we have reached the practical limit of our resources. At this point, therefore, our problem is to glean as much information as possible from the present results.

If we are to assess departures from expected or ideal values of bond lengths and angles and differences between corresponding ones in the two complexes, we need an estimate of their standard deviations. The four independent subunits in the asymmetric unit of the crystal form should allow us to make such an estimate. In fact, we estimated that for the met and azidomet complexes, a difference in length of 0.12 Å between *independent* bonds is possibly significant,[12] but recognized the uncertain nature of the estimate because the Fe–X bond lengths in the complexes were restrained in the refinement. Although the estimate of the standard deviation in the bond lengths is not definitive, it is nevertheless a useful guide in comparing the complexes in different forms of hemerythrin.

In the case of the X–Fe–Y bond angles, no restraints were applied, and we may estimate σ_{mean} as we did previously for the met complex[10] when the Fe–X bond lengths were restrained to 2.0 Å. The σ_{mean} values appear in the last columns of Tables I and II. Because of the small sample size (4), the values show considerable scatter, but since we expect similar σ_{mean} values for all X–Fe–Y angles, we take the rms value of 1.6° as an estimate of the standard deviations of these angles. It is noteworthy that the present 1.6° estimate of σ is considerably less than the earlier 2.1° estimate for the angles in the met complex when 2.0 Å restraints had been imposed on the Fe–X bond lengths. Thus relaxing restraints on the bond lengths in the complex by adjusting them leads to less scatter in the resulting X–Fe–Y angles.

A difference of 2.6 σ, i.e., the 0.99 confidence level, is often taken as significant when an experimental value is compared with a fixed, errorless quantity. However, we will take a difference of (2.6)(1.6°) ≅ 4° as only *possibly significant* when comparing

Table I. Bond Lengths (Å) and Angles (deg) for the Complex in Methemerythrin[a]

Fe(1)-Fe(2)	3.20	3.21	3.18	3.24	3.21	0.022	0.012	
Fe(1)-NE2(73)	2.30	2.31	2.29	2.33	2.31	0.015	0.009	*[b]
Fe(1)-NE2(77)	2.16	2.18	2.15	2.19	2.17	0.016	0.009	*
Fe(1)-NE2(101)	2.20	2.28	2.26	2.21	2.24	0.033	0.019	*
Fe(1)-OE1(58)	2.28	2.29	2.27	2.27	2.28	0.008	0.005	*
Fe(1)-OD1(106)	2.06	1.96	2.00	2.09	2.03	0.051	0.029	*
Fe(1)-O	1.94	1.95	1.87	1.91	1.92	0.031	0.018	*
Fe(2)-NE2(25)	2.20	2.11	2.13	2.14	2.15	0.034	0.020	*
Fe(2)-NE2(54)	2.19	2.22	2.21	2.16	2.19	0.023	0.013	*
Fe(2)-OE2(58)	2.04	2.07	2.04	2.02	2.04	0.018	0.010	*
Fe(2)-OD2(106)	2.09	2.06	2.12	2.14	2.10	0.030	0.017	*
Fe(2)-O	1.67	1.68	1.73	1.62	1.68	0.039	0.023	*
NE2(73)-Fe(1)-NE2(77)	82.3	89.6	91.5	87.0	87.6	3.5	2.0	
NE2(73)-Fe(1)-NE2(101)	92.3	90.0	91.7	91.9	91.5	0.9	0.5	
NE2(73)-Fe(1)-OE1(58)	81.6	82.7	85.5	87.4	84.3	2.3	1.3	
NE2(73)-Fe(1)-OD1(106)	83.0	82.1	79.1	82.0	81.5	1.5	0.8	
NE2(73)-Fe(1)-O	168.3	166.2	168.0	164.4	166.7	1.6	0.9	
NE2(77)-Fe(1)-NE2(101)	97.0	87.3	96.8	96.2	94.3	4.1	2.3	
NE2(77)-Fe(1)-OE1(58)	87.2	91.2	88.8	87.7	88.7	1.5	0.9	
NE2(77)-Fe(1)-OD1(106)	165.2	171.6	170.0	168.9	168.9	2.3	1.4	
NE2(77)-Fe(1)-O	105.0	101.7	96.2	104.5	101.9	3.5	2.0	
NE2(101)-Fe(1)-OE1(58)	172.1	172.5	173.8	175.9	173.6	1.5	0.9	
NE2(101)-Fe(1)-OD1(106)	83.3	94.0	87.1	83.6	87.0	4.3	2.5	
NE2(101)-Fe(1)-O	95.8	98.3	96.4	97.3	96.9	1.0	0.5	
OE1(58)-Fe(1)-OD1(106)	90.9	86.4	87.0	92.3	89.1	2.5	1.4	
OE1(58)-Fe(1)-O	89.5	89.2	85.6	82.5	86.7	2.9	1.7	
OD1(106)-Fe(1)-O	89.6	86.4	92.5	86.5	88.7	2.5	1.5	
NE2(25)-Fe(2)-NE2(54)	92.0	88.1	90.1	86.1	89.1	2.2	1.3	
NE2(25)-Fe(2)-OE2(58)	111.4	115.6	112.9	109.9	112.5	2.1	1.2	
NE2(25)-Fe(2)-OD2(106)	80.1	85.8	80.7	87.0	83.4	3.0	1.8	
NE2(25)-Fe(2)-O	144.1	137.5	147.0	145.0	143.4	3.6	2.1	
NE2(54)-Fe(2)-OE2(58)	83.9	90.4	85.7	79.0	84.8	4.1	2.4	
NE2(54)-Fe(2)-OD2(106)	162.3	169.1	163.5	156.0	162.7	4.6	2.7	
NE2(54)-Fe(2)-O	100.5	96.0	96.3	111.6	101.1	6.3	3.6	
OE2(58)-Fe(2)-OD2(106)	84.3	84.0	85.3	81.9	83.8	1.2	0.7	
OE2(58)-Fe(2)-O	103.4	106.7	99.9	103.1	103.3	2.4	1.4	
OD2(106)-Fe(2)-O	95.0	94.6	98.9	86.6	93.8	4.5	2.6	
Fe(1)-O-Fe(2)	125.0	124.1	123.7	133.0	126.5	3.8	2.2	

[a] Values tabulated are for each of the four independent subunits, their mean value, the rms deviation from the mean, and the estimated standard deviation in the mean. [b] Asterisks denote restraints used in the refinement and corresponding underestimates of the standard deviation in the mean. Care should be taken in comparing standard deviations from restrained and unrestrained refinements. See ref 12.

X–Fe–Y angles with ideal values, e.g., when considering distortions from ideal coordination. When comparing *independent* angles, we take a diference of $(2^{1/2})(4°) \cong 6°$ as possibly significant.

The absolute values of bond lengths and angles reported here may be subject to unknown systematic errors for which we cannot correct. (For a discussion of possible errors, see ref 12). Differences between the two complexes are more reliable, however, and will be unaffected by systematic errors to the extent that such effects are the same in both models.

Discussion and Comparison of Structures

Bond Lengths. As noted above and as evident in Figures 1 and 3, Fe(1) is hexacoordinate in both complexes whereas Fe(2) is pentacoordinate in the met complex and hexacoordinate in the azidomet complex. Thus parameters involving Fe(1) in the two complexes can serve to monitor the results from the refinements of these models. In fact, in comparing corresponding parameters in the two complexes, we find better agreement between those involving Fe(1) than between those involving Fe(2).

Table IIIa lists the differences in bond lengths of corresponding Fe(1)–X bonds in the two complexes. Bonds in five of the six pairs do not differ in length by more than 0.04 Å, with an rms difference of 0.033 Å, but bonds in the sixth pair, Fe(1)–OD1(106) differ by 0.13 Å, a difference that would be classed as possibly significant. The present uncertainty in the standard deviations in the bond lengths, however, suggests caution in accepting differences of this magnitude as real.

Table IIIb lists the differences in lengths of corresponding Fe(2)–X bonds. We note the differences in lengths of bonds involving Fe(2) are considerably larger on the average than those involving Fe(1). Nevertheless, four out of five pairs of bonds do not differ by more than 0.10 Å and cannot be said to differ significantly in length. Bonds in the fifth pair, however, Fe(2)–OE2(58), differ by 0.29 Å, a value sufficiently large that it suggests a significant difference.

In Table IIIb four of the five differences are negative, i.e., the Fe(2)–X bonds involving the pentacoordinate Fe in the met complex are shorter than the corresponding bonds involving the hexacoordinate Fe in the azidomet complex. This is consistent with the expectation of shorter bonds for pentacoordinate than for hexacoordinate Fe.

The Fe(2)–N_{azide} bond at 2.34 Å in the azidomet complex is the longest of the Fe–N bonds and has no counterpart in the met complex.

The range of lengths we observe for bonds of a given type, particularly the 2.03- to 2.33-Å range for the Fe–$O_{carboxy}$ bonds and the 1.64- to 1.92-Å range for the Fe–$O_{\mu\text{-}oxo}$ bonds, emphasizes the need for caution in applying statistical criteria to apparent differences in bond lengths. For example, we note that in both complexes, the Fe–$O_{\mu\text{-}oxo}$ bond lengths show the same asymmetric pattern, one longer, the other shorter than the 1.76- to 1.83-Å range commonly reported for such bonds in small molecules, including the recently reported model compounds for the hemerythrin center.[16,17] The difference in the lengths of the bonds in each complex, 0.24 Å in the met complex and 0.25 Å in the azidomet complex, appear to be significant, but the fact that the lengths of these bonds are highly correlated in each complex and the difficulty of locating accurately a single O atom in the vicinity of two Fe atoms suggest caution in accepting the asymmetry of

(16) Armstrong, W. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 4837–4838.
(17) Wieghardt, K.; Pohl, K.; Gebert, W. *Angew. Chem.* **1983**, *95*, 739–740.

Table II. Bond Lengths (Å) and Angles (deg) for the Complex in Azidomethemerythrin[a]

Fe(1)-Fe(2)	3.27	3.25	3.24	3.26	3.25	0.011	0.006	
Fe(1)-NE2(73)	2.28	2.33	2.27	2.30	2.29	0.023	0.013	*[b]
Fe(1)-NE2(77)	2.12	2.13	2.16	2.13	2.13	0.015	0.009	*
Fe(1)-NE2(101)	2.26	2.27	2.26	2.30	2.27	0.016	0.009	*
Fe(1)-OE1(58)	2.22	2.29	2.20	2.24	2.24	0.033	0.019	*
Fe(1)-OD1(106)	2.19	2.11	2.15	2.19	2.16	0.033	0.019	*
Fe(1)-O	1.91	1.84	1.86	1.92	1.89	0.033	0.019	*
Fe(2)-NE2(25)	2.25	2.21	2.19	2.25	2.22	0.026	0.015	*
Fe(2)-NE2(54)	2.27	2.22	2.23	2.27	2.25	0.023	0.013	*
Fe(2)-OE2(58)	2.33	2.35	2.30	2.35	2.33	0.020	0.012	*
Fe(2)-OD2(106)	2.19	2.19	2.22	2.20	2.20	0.012	0.007	*
Fe(2)-O	1.64	1.64	1.66	1.63	1.64	0.011	0.006	*
Fe(2)-N(1)	2.37	2.33	2.36	2.32	2.34	0.021	0.012	*
NE2(73)-Fe(1)-NE2(77)	88.0	86.6	87.8	87.5	87.5	0.5	0.3	
NE2(73)-Fe(1)-NE2(101)	91.6	83.0	89.9	88.6	88.3	3.2	1.9	
NE2(73)-Fe(1)-OE1(58)	83.8	84.6	89.0	87.7	86.3	2.2	1.2	
NE2(73)-Fe(1)-OD1(106)	80.8	83.5	79.9	81.9	81.5	1.3	0.8	
NE2(73)-Fe(1)-O	166.7	166.8	169.7	169.8	168.2	1.5	0.9	
NE2(77)-Fe(1)-NE2(101)	97.7	88.8	96.6	91.0	93.5	3.7	2.2	
NE2(77)-Fe(1)-OE1(58)	88.7	88.8	88.8	94.1	90.1	2.3	1.3	
NE2(77)-Fe(1)-OD1(106)	168.4	169.1	166.9	169.4	168.4	1.0	0.6	
NE2(77)-Fe(1)-O	101.9	103.4	100.5	100.4	101.5	1.2	0.7	
NE2(101)-Fe(1)-OE1(58)	171.9	167.4	174.5	173.6	171.9	2.7	1.6	
NE2(101)-Fe(1)-OD1(106)	85.7	94.4	87.9	88.3	89.1	3.2	1.9	
NE2(101)-Fe(1)-O	95.9	105.5	95.2	97.6	98.5	4.1	2.4	
OE1(58)-Fe(1)-OD1(106)	87.1	85.9	86.6	85.9	86.4	0.5	0.3	
OE1(58)-Fe(1)-O	87.5	87.1	85.0	85.3	86.2	1.1	0.6	
OD1(106)-Fe(1)-O	88.8	85.8	91.3	90.2	89.0	2.1	1.2	
NE2(25)-Fe(2)-NE2(54)	84.6	89.4	85.5	78.5	84.5	3.9	2.3	
NE2(25)-Fe(2)-OE2(58)	91.6	95.9	95.6	91.6	93.7	2.1	1.2	
NE2(25)-Fe(2)-OD2(106)	79.7	78.8	81.3	86.9	81.7	3.1	1.8	
NE2(25)-Fe(2)-O	172.4	169.8	173.5	173.8	172.4	1.6	0.9	
NE2(25)-Fe(2)-N(1)	83.1	78.3	81.9	82.4	81.4	1.8	1.1	
NE2(54)-Fe(2)-OE2(58)	76.0	85.1	79.5	73.0	78.4	4.5	2.6	
NE2(54)-Fe(2)-OD2(106)	150.8	159.4	155.2	150.1	153.9	3.7	2.2	
NE2(54)-Fe(2)-O	102.0	99.5	99.2	102.1	100.7	1.4	0.8	
NE2(54)-Fe(2)-N(1)	100.1	99.9	102.8	105.9	102.2	2.4	1.4	
OE2(58)-Fe(2)-OD2(106)	79.9	79.5	81.0	81.6	80.5	0.9	0.5	
OE2(58)-Fe(2)-O	93.5	89.9	89.8	94.4	91.9	2.1	1.2	
OE2(58)-Fe(2)-N(1)	173.7	172.3	176.4	174.0	174.1	1.5	0.9	
OD2(106)-Fe(2)-O	95.7	94.0	95.9	95.2	95.2	0.7	0.4	
OD2(106)-Fe(2)-N(1)	102.2	94.3	96.0	97.7	97.5	3.0	1.7	
O-Fe(2)-N(1)	92.1	95.1	92.6	91.6	92.8	1.4	0.8	
Fe(1)-O-Fe(2)	133.8	138.4	133.4	132.4	134.5	2.3	1.3	

[a] Values tabulated are for each of the four independent subunits, the mean value, the rms deviation from the mean, and the estimated standard deviation in the mean. [b] Asterisks denote restraints used in the refinement and corresponding underestimates of the standard deviation in the mean. Care should be taken in comparing standard deviations from restrained refinements. See ref 12.

Table III. Differences in Lengths (Å) of Corresponding Fe-X Bonds in the Met and Azidomet Forms of Hemerythrin

	bond	diff in length (met - azidomet)
(a)	Fe(1)-NE2(73)	0.02
	-NE2(77)	0.04
	-NE2(101)	−0.03
	-O	0.03
	-OE1(58)	0.04
	-OD1(106)	−0.13
(b)	Fe(2)-NE2(25)	−0.07
	-NE2(54)	−0.06
	-O	0.04
	-OD2(106)	−0.10
	-OE2(58)	−0.29

these bonds as real. Accordingly, we take the average of the two bonds, 1.80 Å in the met complex and 1.76 Å in the azidomet complex, as the best values for the Fe–$O_{\mu\text{-oxo}}$ bond lengths at this point.

It should be pointed out, however, that the same pattern of Fe–$O_{\mu\text{-oxo}}$ distances appears in eight protein subunits in two separate structure determinations, arguing that the differences between the Fe–$O_{\mu\text{-oxo}}$ bonds might be real and caused by the asymmetry of the surrounding protein. Higher resolution studies of the molecules will be necessary to clarify this issue.

Bond Angles. The coordination octahedra of Fe(1) in both complexes appear to be somewhat distorted. Three of the twelve X–Fe(1)–Y bond angles in each complex differ by 6 to 12° from the ideal value of 90°, and all three angles between opposite bonds, again in each complex, differ by 6 to 13° from the ideal value of 180°. The differences are enough greater than the 4° estimate for possible significance that we consider these angles to differ from the ideal values. This assertion is strengthened by the fact that the pattern of distortion in the two complexes is similar, as evident by comparing the X–Fe(1)–Y angles in Tables I and II.

The extent of agreement is indicated by the 1.6° rms difference between corresponding angles in the complexes, the maximum difference being 3.2°. In fact, the agreement is better than we have a right to expect, since the independent determinations of the angles in the two complexes are both subject to error. Thus, in view of the 1.6° estimate of the standard deviations in the X–Fe–Y angles made above, we expect a mean difference between angles in the two complexes to approximate $2^{1/2}$ (1.6°) = 2.3°, a value considerably greater than that observed.

The coordination of Fe(2) in the met complex may be considered as square pyramidal or trigonal bipyramidal, in either case highly distorted. For comparative purposes, we choose the latter. Thus three of the six angles that are ideally 90° differ from that value by 6 to 11°, all three that are ideally 120° differ from that value by 7 to 23°, and the one that is ideally 180° differs from it by 17°.

Table IV. Angles (deg) Involving Azide in Azidomethemerythrin[a]

Fe(2)-N(1)-N(2)	108.0	111.7	115.0	107.5	110.5
Fe(2)-N(1)-N(3)	117.0	119.4	121.9	120.2	119.6
Fe(1)-Fe(2)-N(1)	111.5	110.5	108.4	110.3	110.2

[a] Values tabulated are for each of the four independent subunits, and the mean value.

The coordination of Fe(2) in the azidomet complex is octahedral and considerably more distorted than the similarly coordinated Fe(1) in either complex. Seven of the twelve X-Fe(2)-Y angles differ from the ideal 90° by 6 to 12°, and all three angles between opposite bonds differ by values ranging from 6 to 26° from the ideal 180°.

Comparing the angles involving Fe(2), we note that despite the difference in coordination in the two complexes, five of the ten corresponding X-Fe(2)-Y angles differ by no more than 4°, but for the remaining five, differences are quite large, ranging from 7 to 29°.

The values we observe for the Fe(1)-O-Fe(2) angles, 127° in the met complex and 135° in the azidomet complex, probably differ significantly and are considerably less than the 139-171° range of values that have been reported for a number of small molecular complexes.[18-20] However, the Fe-$O_{\mu\text{-oxo}}$-Fe angles found in the model complexes for the azidomet center are 123.5 (1)°[16] and 118.3 (5)°,[17] values smaller than found in the proteins.

Fe-Fe Distances. The Fe-Fe distance is 3.21 Å in the met complex and 3.25 Å in the azidomet complex. While the difference is not large, it is nominaly significant and can be justified on the basis of the different geometry of Fe(2) in the two complexes. The distances we observe can to be compared with the value of 3.34 (±0.07) Å reported by Hendrickson et al.[21] for the Fe-Fe distance in the azidomet complex of myohemerythrin, based on the anomalous scattering differences in the X-ray diffraction data. Values of the Fe-Fe distance derived from EXAFS data are 3.49[22] and 3.38 (±0.05) Å,[21] these both being measurements of the Fe-Fe distances in azidomethemerythrin from *P. gouldii*. The Fe-Fe distances found in the model compounds are less than those observed in the proteins, being 3.145 (1)[16] and 3.064 (5) Å.[17]

Changes on Binding of Azide. The structural changes in the complex that occur upon addition of the azide ion to methemerythin are substantial and must require at least some adjustment of the polypeptide structure to accommodate them. A preliminary investigation indicates that most of the conformational changes are located in the three or four residues surrounding His-25 and the C terminus of the polypeptide from residues 109 to 113. There are also indications of slight structural changes near the perchlorate binding site identified earlier,[23] consistent with the ability of perchlorate to destabilize ligated forms of the complex, both oxy and thiocyanatomet[24,25] forms of the protein. The perchlorate site is more restricted in the ligated forms, and the addition of a bound oxyanion at that site would cause the conformational change in the protein to reverse and stabilize the unligated forms.

The mode of binding of N_3 in the azidomet complex is of considerable interest and has been extensively studied spectroscopically.[26-28] The model of the complex in Figure 2 shows N_3 to be bound only to one Fe atom, in conformity with one of the models based on resonance Raman and polarized absorption spectroscopy, and to be aligned approximately parallel to the Fe(2)-$O_{\mu\text{-oxo}}$ bond, the Fe-N-N bond angle being 111° as seen in Table IV. The spectroscopic evidence has long indicated that the complex in azidomet is analogous to that in oxyhemerythrin, and preliminary results of a crystallographic investigation of oxyhemerythrin at 2.2 Å are consistent with this view.

Comparison with Results of 2.0-Å Fe-X Restraints. It is instructive to compare the present bond lengths of the methemerythrin complex with the ones from the earlier restrained least-squares refinement in which 2.0-Å restraints were imposed on the Fe-X lengths.[10] In that refinement, the Fe-X bond lengths ranged from 1.84 Å for the Fe(2)-$O_{\mu\text{-oxo}}$ bond to 2.16 Å for the Fe(1)-NE2(73) bond compared to 1.68 and 2.31 Å, respectively, reported here. It should be noted that adjusting the restraints led to relatively large changes in most of the Fe-X bond lengths[12] and a decrease in the differences between the four subunits. The latter effect is an indication that the adjusted restraints are more appropriate for these complexes than are the 2.0-Å restraints imposed on the Fe-X bonds in the earlier study.[10]

In the case of the bond angles in the complexes, restraints were not applied either in the earlier refinement with fixed 2.0-Å restraints on the Fe-X bond lengths or in the refinement leading to the present parameters. Comparing the 25 X-Fe-Y angles from the two refinements of the met model, we find a maximum difference of 4° and an rms difference of 2.2°. It is readily shown that the latter is less than we would expect on the basis of the estimated standard deviations of the sets of angles. Thus we observe no significant changes in the X-Fe-Y angles of the met complex by using different restraints for the Fe-X bonds.

The Fe(1)-O-Fe(2) angle, however, is particularly sensitive to the values of the Fe-X restraints.[12] This angle changed by 6° on adjusting the restraints and illustrates the effects of what can be termed systematic error in the model, namely, imposing improper restraints on the length of the Fe-X bonds.

Conclusion

In general, the Fe-N and Fe-$O_{carboxy}$ bonds in both complexes are greater in length than usually reported for such bonds. However, the Fe-N bonds tend to be longer than the Fe-$O_{carboxy}$ bonds, which are longer than the Fe-$O_{\mu\text{-oxo}}$ bonds, a pattern also found in small molecule Fe complexes. Because of the uncertainty in the estimated standard deviations in the bond lengths noted above and the possible operation of systematic error in the data or in the model, the departures of bond lengths from accepted values should only be regarded as suggestive.

In the case of angular parameters, we find remarkable agreement between corresponding X-Fe(1)-Y angles in the two complexes. The coordination octahedra are somewhat distorted in both complexes, but in the same sense in both and to a similar extent.

The coordination of Fe(2) in the two complexes is different, pentacoordinate (trigonal bipyramidal or square pyramidal) in the met complex and hexacoordinate (octahedral) in the azidomet complex. Whatever the coordination polyhedron of Fe(2), it is highly distorted. The fact that corresponding angles involving Fe(1) in the two complexes agree so well lends credence to the differences we observe for the angles involving Fe(2).

The subunits of hemerythrin are large, linked structure with a certain rigidity imposed by the extensive helical structure present in the subunits. The helices limit the ability of the amino acid side chains to take positions accommodating ideal, symmetric complexes. Because of the molecular rigidity and the asymmetric environment of the complexes, we must allow for differences in bond lengths and angles in the binuclear Fe complex beyond what we would expect based on model compounds.

Acknowledgment. We wish to thank Joann Sanders-Loehr for many helpful comments. This work has been supported by Grant GM-10828 from the National Institutes of Health and equipment Grant PCM 76-20557 from the National Science Foundation.

Registry No. Iron, 7439-89-6.

(18) Gerloch, M.; McKenzie, E. D.; Towl, A. D. *J. Chem. Soc. A* **1969**, 2850-2858.
(19) Coggen, P.; McPhail, A. T.; Mabbs, F. E.; McLachlan, V. N. *J. Chem. Soc. A* **1971**, 1014-1019.
(20) Davies, J. E.; Gatehouse, B. M. *Acta Crystallogr., Sect. B* **1973**, *B29*, 1934-1942.
(21) Hendrickson, W. A.; Co, M. S.; Smith, J. L.; Hodgson, K. O.; Klippenstein, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6255-6259.
(22) Elam, W. T.; Stern, E. A.; McCallum, J. D.; Sanders-Loehr, J.. *J. Am. Chem. Soc.* **1982**, *104*, 6369-6373.
(23) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1978**, *126*, 457-466.
(24) DePhillips, H. A., Jr. *Arch. Biochem. Biophys.* **1971**, *144*, 122-126.
(25) Gorman, E. G.; Darnall, D. W. *Biochemistry* **1981**, *20*, 38-43.
(26) Gay, R. R.; Solomon, E. I. *J. Am. Chem. Soc.* **1978**, *100*, 1972-1973.
(27) Dunn, J. B. R.; Shriver, D. F.; Klotz, I. M. *Biochemistry* **1975**, *14*, 2689-2695.
(28) Kurtz, D. M. Jr.; Shriver, D. F.; Klotz, I. M. *J. Am. Chem. Soc.* **1976**, *98*, 5033-5035.