A COMPARISON OF THE IRON BONDING IN ANHYDROHEMOGLOBIN AND ANHYDROMYOGLOBIN

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ABSTRACT Mössbauer effect measurements show that the ferrous ions in dehydrated deoxymyoglobin are in the high spin state while those in dehydrated deoxyhemoglobin (AHb) are equally distributed between high and low spin states. It is concluded that the two spin states present in AHb are associated with the iron site difference of the α- and β-chains.

INTRODUCTION

In an earlier paper (1) we reported that the removal of water in deoxyhemoglobin causes about half of the ferrous ions present in the molecule to be converted from the high to the low spin state. Two possible explanations were proposed to account for this observation; namely, that either the high and low spin states were associated with ferrous ions bonded to the α- and β-globin chains in anhydrohemoglobin (AHb), or that a thermal equilibrium between the two spin states (of the type discussed by George, Beetlestone, and Griffith [2]) might exist.

Hemoglobin (Hb) and myoglobin (Mb) are closely related molecules with Hb consisting of four Mb-like subunits. The immediate environment of the iron atoms in Mb is very similar to that in Hb; however, Mb has only a single type of globin chain. Thus, to indicate which of the above two explanations is responsible for the high and low spin states in AHb, we have obtained Mössbauer spectra of deoxymyoglobin and anhydromyoglobin (AMb).

EXPERIMENTAL

A specimen of purified sperm whale Mb was kindly supplied to us by Professor F. R. N. Gurd. Deoxymyoglobin and AMb were prepared by the same technique used previously to prepare deoxyhemoglobin and AHb (1). Briefly, a nitrogen-flushed aqueous solution of Mb was treated with an excess of sodium hydrosulfite, $Na_2S_2O_4$, to obtain deoxymyoglobin and subsequently, AMb was produced by vacuum distillation of the frozen deoxymyoglobin solution. The dehydration process was continued for three days after visual inspection indicated that the sample was dry.

The Mössbauer measurements were obtained with the equipment previously described in reference (1). All spectra and data are reported relative to a 23°C ^{57}Co in Cu source; the isomer shift of this source relative to $Na_2Fe(CN)_5NO \cdot 2H_2O$ (23°C) is −0.484 mm/sec (3).

RESULTS

In Fig. 1 we show Mössbauer spectra obtained with absorbers of (*a*) deoxymyoglobin (8°K), (*b*) AMb (10°K), (*c*) AMb (80°K), and (*d*) human AHb (40°K). The quadrupole splitting (peak separation), ΔE_Q, and isomer shift (average of peak positions), δ, obtained for these materials are given in Table I.

The ΔE_Q and δ observed for deoxymyoglobin (Fig. 1 *a*) are in accord with those observed for deoxyhemoglobin (1, 4, 5) (although ΔE_Q may be slightly smaller). This agreement is consistent with the assumed similarity of the ferrous-ion environment in the two molecules. As with deoxyhemoglobin, the δ of deoxymyoglobin indicates that the ferrous ion is in the high spin state. The dehydration of deoxymyoglobin has no observable effect on δ or ΔE_Q within the present experimental limits of error and thus all the ferrous ions in AMb remain in the high spin state. For comparison, we show in Fig. 1 *d* the Mössbauer spectrum of adult AHb. This spectrum was obtained in conjunction with an earlier study (1). The low isomer shift of the

TABLE I

QUADRUPOLE SPLITTINGS AND ISOMER SHIFTS OF SOME HEMOGLOBIN AND MYOGLOBIN COMPOUNDS

Absorber	Absorber temperature	ΔE_Q*	δ*, ‡
	°K	*mm/sec*	*mm/sec*
Deoxy-Mb	8	2.24	+0.72
	80	2.18	+0.73
AMb	10	2.25	+0.70
	80	2.13	+0.68
Human AHb	9	2.17	+0.74
		1.11	+0.20
	40	2.20	+0.74
		1.11	+0.19
	80	2.11	+0.75
		1.18	+0.29

* Approximate uncertainties ± 0.1 mm/sec.

‡ Relative to a ^{57}Co in Cu source at 23°C.

quadrupole split doublet with the smaller splitting in AHb is characteristic of low spin ferrous ion as has been established by susceptibility measurements (1). The ΔE_Q and δ associated with the high spin ferrous ions in AHb are, within experimental error, identical to those observed in AMb.

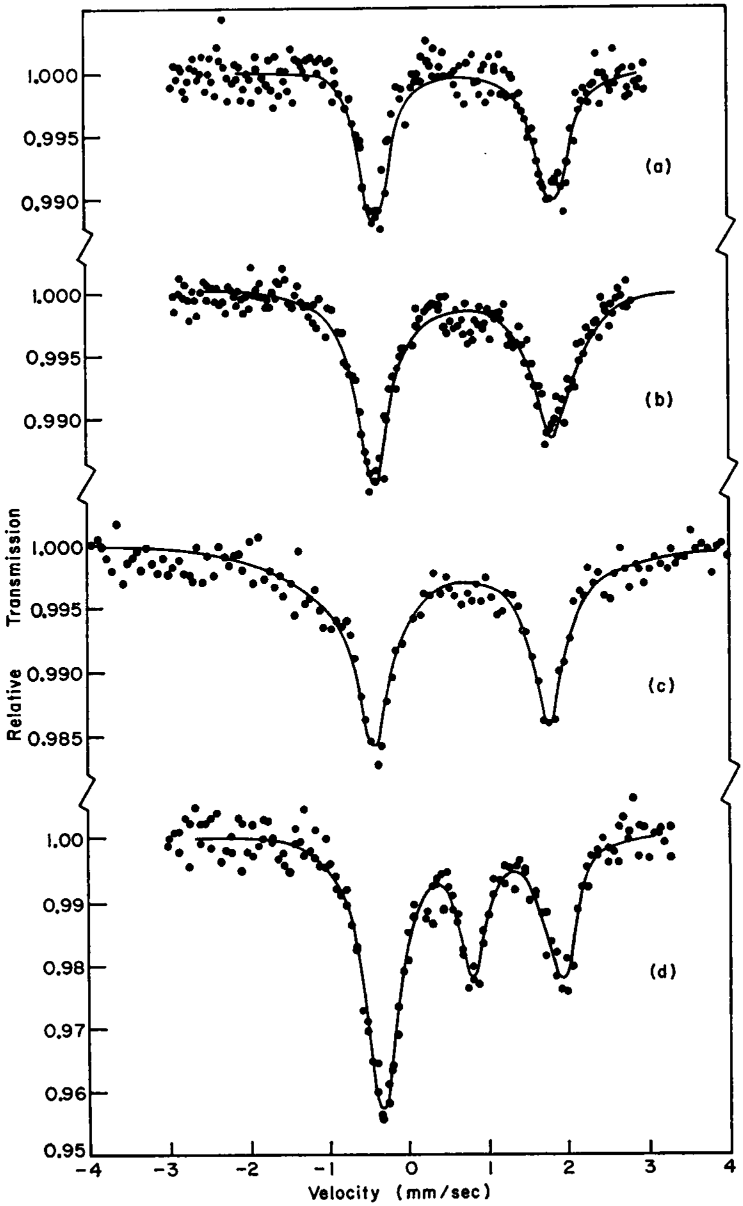

FIGURE 1 Mössbauer spectra relative to a ^{57}Co in Cu source (at 23°C) and absorbers of (*a*) deoxymyoglobin 8°K, (*b*) AMb (10°K), (*c*) AMb (80°K), and (*d*) human AHb (40°K).

DISCUSSION

The amino acid sequence of the globin chain in several hemoglobins and myoglobins is known (6). Approximately 140–155 amino acids comprise the globin chains; only seven sites in those globin chains which have been studied (6, 7) are always occupied by the same residue. Nonetheless, with minor variations, the configuration of the polypeptide chain is believed to be characteristic of the hemoglobin and myoglobin of all vertebrates (8), even though the complete structure of the molecules has been worked out in only a few cases (see, for example, references 7, 9, and 10). The seven amino acids which are apparently immutable are located mainly near the site of the attachment of the iron-heme group to the globin chain and in particular include histidine groups at positions E7 and F8 (notation of Kendrew et al. [11]). The proximal histidine at F8 is coordinated to the heme group while the distal histidine at E7 is in close proximity on the opposite side of the heme plane. Additional evidence for the similarity of the iron environment in hemoglobins of various species is provided by Mössbauer studies of human and rat hemoglobin which yield the same spectra to within experimental error (1, 4, 5).

The sequence of amino acids in Mb is not the same as in either the α- or β-globin chains in normal adult hemoglobin and, in fact, the difference between Mb and the α-chain is about the same as the difference between Mb and the β-chain (6, 12). However, from an evolutionary standpoint, the α-chain is believed to be more closely related to Mb than is the β-chain (13).

The main conclusion that can be drawn from the present study is that the high and low spin iron ions in AHb are associated with the two different iron sites in the molecule (i.e. in the α- and β-globin chains) and are not associated with a thermal equilibrium of the two spin states at each iron site in the molecule.[1] This conclusion is supported by the absence of low spin iron (at both 10 and 80°K) in AMb, which has only one globin chain, and the presence in AHb of high and low spin iron in roughly equal amounts. The previously well-known similarity of the iron environment in deoxymyoglobin and deoxyhemoglobin and the presently established similarity in AMb and one of the two sites in AHb (as indicated by the nearly equal isomer shifts and quadrupole splittings which are observed), make it very unlikely that a thermal equilibrium of high and low spin iron exists at each iron site in AHb and not in AMb.

If low spin iron is associated with the formation of a sixth covalent bond to the iron atom as, for example, is apparently the case in O_2— and CO—Hb, then the removal of H_2O from deoxyhemoglobin may result in a conformational change that brings a ring nitrogen from the distal E7 histidine group into coordination with the iron similar to the bond formed on the proximal side of the heme plane. Since the α-

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[1] After the completion of this work, we learned that H. Eicher and A. Trautwein have also obtained Mössbauer spectra of AMb and have drawn conclusions similar to ours regarding the iron bonding in AHb (talk presented at the "Symposium on Mössbauer Spectroscopy in Biological Systems," March 17–18, 1969, University of Illinois, Urbana).

chain may be somewhat more closely related to Mb than the β-chain, we speculate that if the above mechanism is correct, the six-coordination would most likely occur in the β-chain. In deoxymyoglobin (14) the iron is five-coordinated and no water or other molecule occupies the sixth position on the distal side of the heme plane. Nobbs, Watson, and Kendrew (14) conclude that the same situation, i.e., no water or other molecule is present in the sixth coordination position, must exist in deoxyhemoglobin. The nearly equal Mössbauer spectra of deoxymyoglobin, AMb, and deoxyhemoglobin indicate the similarity of all iron sites in these molecules and also support this conclusion. In particular, the Mössbauer spectrum of deoxyhemoglobin suggests that water is not present at the iron site in either the α- or β-chains. Thus, the removal of water from deoxyhemoglobin must produce an indirect conformational change in the Hb molecule which causes the iron in one of the two iron sites to become covalently bonded.

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