MOSSBAUER STUDIES OF ANHYDROUS HEMOGLOBIN AND ITS SUBUNITS

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Summary

The similarities in the Mossbauer spectra of Hb and its α and β subunits, both in the oxygenated and deoxygenated states, were observed. The relative intensities of the absorption dips in the Mossbauer spectra of ^{57}Fe in anhydrohemoglobin (AHb) and anhydrous separated alpha (Aα) and beta (Aβ) chain samples were found to be very sensitive to the conditions of samples preparation. This indicates the formation of hemochromogen as an impurity in the absorbers during the process of preparation. When contributions due to the impurity in the samples were subtracted, Mossbauer spectra of ^{57}Fe in AHb, Aα, and Aβ remained similar. Therefore, the electronic structure of the iron cation is the same for Hb and its subunits in the anhydrous state also.

Introduction

Recoilless resonance absorption (1) of 14.4 kev gamma ray of ^{57}Fe is a powerful tool for studies of hemoglobin. From the Mossbauer spectrum of ^{57}Fe in anhydrohemoglobin (AHb) Grant et al (2) postulated the coexistence of ferrous iron in high and low spin states. They offered two alternative explanations for the observed spectra; either alpha (α) and beta (β) globin chains went through different conformational changes upon dehydration of Hb resulting in two different electronic states of the iron cations in the two chains, or a thermal equilibrium between the two

states in AHb existed as the energy difference between them was very small. From comparison of Mossbauer spectra of AHb, and anhydromyoglobin (AMb) and bispyridine hemin (BPH), Trautwein et al (3), (4) and Grant et al (5), concluded that the second interpretation was unlikely. Trautwein et al further suggested that alterations of the 3d electron configuration of the iron cations, produced as a result of dehydration of deoxyhemoglobin, were different in the α and β chains.

In a preliminary investigation of direct Mossbauer measurements of separated anhydrous alpha (Aα) and anhydrous beta (Aβ) chains, Papaefthymiou et al (6) concluded that the direct sum of the Mossbauer spectra of ^{57}Fe in Aα and Aβ chains did not reproduce the Mossbauer spectrum of ^{57}Fe in AHb. They suggested that the interaction between α and β chains in Hb upon dehydration produced different structural rearrangements near the heme for different subunits. However, there was no explicit evidence in literature for such a structural change upon subunit interaction. The extensive x-ray diffraction investigations of Perutz et al (7), (8) showed that the contacts between the peripheral porphyrin and the polypeptide chain were different in α and β subunits. Yet the sum of the circular dichroism spectra of alpha and beta chains at a wavelength of 260 mu reproduced that of hemoglobin formed by α and β chains added in stoichiomatic amounts (9). Therefore, further investigations of dehydrated human Hb and its subunits were undertaken by us, and our preliminary results have already been briefly reported (10).

Sample Preparation

Samples of deoxyhemoglobin were prepared by mixing hemoglobin solutions with a small amount of sodium dithionite in a nitrogen gas atmosphere as suggested in reference 2. AHb samples were obtained by vacuum distillation of the frozen deoxyhemoglobin samples for twenty-four hours in a sealed, evacuated U-tube with one end immersed in liquid nitrogen. When the process of dehydration was completed, the AHb sample was packed under nitrogen gas atmosphere in an airtight lucite holder enveloped with oxygen-free copper. The α and β chains of human Hb were separated by the PMB (p-mercuribenzoate) method as described in the literature (9), (11). The solutions of the separated α and β chains were first concentrated by vacuum dialysis and then by an Amicon concentrator. Samples of concentrated α and β chains were deoxygenated and dehydrated in exactly the same manner as for Hb.

Experimental Results

Mossbauer spectra were measured with a standard Mossbauer spectrometer operating at temperatures ranging from 77K to 300K. The measured spectra of ^{57}Fe in oxygenated Hb and its subunits at 85K showed the usual quadrupole doublet. The source used was ^{57}Co doped in Pd lattice. The characteristics of the Mossbauer spectra were found to be very sensitive to the conditions of sample preparation for deoxygenated as well as dehydrated hemoglobin absorbers. Therefore the following conditions were carefully controlled: α and β subunits as well as Hb absorbers were prepared from the same blood sample;

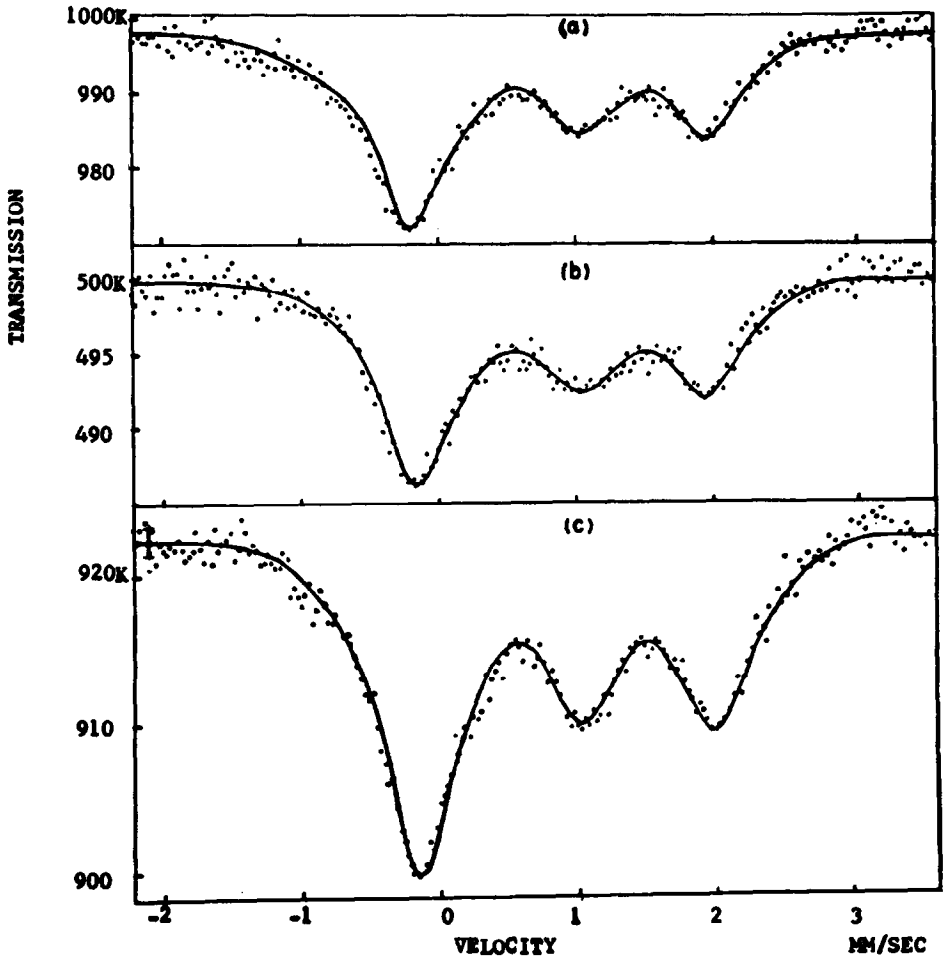

Figure 1. Mossbauer Spectra of Samples of Anhydrous (a) Alpha, (b) Beta Subunits and (c) Hemoglobin at 85K

concentrations of Hb, α and β solutions were kept equal as monitored by optical spectrometer, and the samples of Hb, α and β were dehydrated simultaneously in the same equipment after deoxygenation by sodium dithionite. Mossbauer spectra of these samples taken at 85K and 200K are shown in Figures 1 and 2, respectively. The solid lines were obtained by least square fits to the measured points, adopting a Lorentzian line shape for the Mossbauer absorption line and the results are given

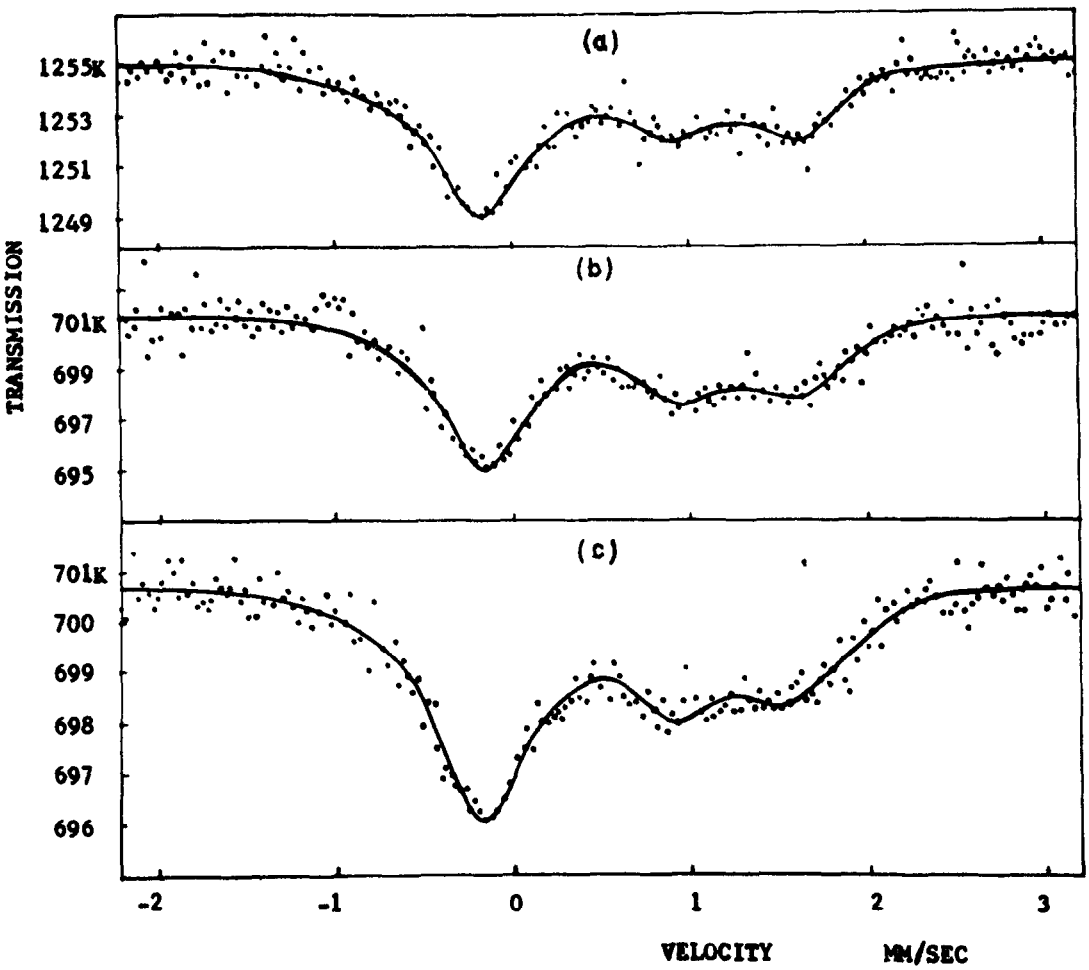

Figure 2. Mossbauer Spectra of Samples of Anhydrous (a) Alpha, (b) Beta Subunits and (c) Hemoglobin at 200K

in Table 1. The direct sum of the Mossbauer spectra of Aα and Aβ obtained by point by point addition reproduced the spectrum of AHb within experimental errors.

Discussion

In the absence of a magnetic interaction, the Mossbauer spectrum of an atom in a unique chemical site is characterized by two paramenters, the isomer shift δ, and the quadrupole splitting ΔE_Q which depends on the electronic structure of the iron ion. The Mossbauer

ABSORBER	T K	Large Quadrupole Doublet		Small Quadrupole Doublet (Impurity)	
		ΔE_Q ±.05 MM/SEC	I.S.* ±.05 MM/SEC	ΔE_Q ±.05 MM/SEC	I.S.* ±.05 MM/SEC
AHb	85	2.19	.89	1.10	.40
	200	1.85	.80	1.09	.40
Aα	85	2.19	.88	1.10	.39
	200	1.85	.80	1.08	.38
Aβ	85	2.18	.87	1.10	.40
	200	1.86	.80	1.09	.40
Hb	85	2.28	.88	1.10	.40
α	85	2.26	.87	1.10	.41
β	85	2.27	.86	1.09	.40

* Isomer shifts relative to metallic iron

Table 1. Mossbauer Parameters of Anhydrous Hemoglobin and Its Separated Subunits

spectra of ^{57}Fe in oxygenated Hb, α, and β chains in a state of frozen solution exhibited a quadrupole splitting, ΔE_Q = 1.95 ± .05 and an isomeric shift, δ = .40 mm/sec, which are in good agreement with the values for oxygenated hemoglobin given in the literature (12-14). The measured Mossbauer spectra of ^{57}Fe in deoxygenated hemoglobin and its separated subunits in a state of frozen solution have been fitted into a

spectrum with three absorption dips to be consistant with our measurements on anhydrous samples. However, for the deoxygenated Hb sample an alternate mode of fitting would be with two absorption dips only. Longer period of exposure during the process of preparation increased the relative intensity of the central absorption dip significantly. A similar effect was also observed for samples of AHb, Aα and Aβ. These observed phenomena led us to conclude that the deoxygenated as well as the dehydrated samples of Hb and its subunits were not pure, and hemochromogen was formed as an impurity in the process of absorber preparation. When absorbers of AHb, Aα and Aβ were prepared under identical conditions, the direct sum of the Mossbauer spectra of ^{57}Fe in Aα and Aβ was found to reproduce the spectrum of AHb, ruling out the hypothesis (6) of different structural rearrangements near the heme for α and β chains upon dehydration. Our results also rule out the conclusions of Trautwein et al (4) and Grant et al (5) regarding the three dips in the Mossbauer spectrum of AHb as resulting from sum of two different quadrupole doublets in Aα and Aβ. The electronic structure of the iron cation in Hb and its separated subunits are similar not only in the deoxygenated state (15) but also in the anhydrous state.

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