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DIFFERENT PROPERTIES OF FERROUS IRON IN DEOXYGENATED HEMOGLOBIN CHAINS

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Résumé. — On a étudié par spectrométrie Mössbauer, entre 4,2 K et 200 K, de l’hémoglobine déoxygénée, enrichie en $^{57}$Fe, d’un homme adulte (HbA), ses chaînes isolées $a_nH$ et $b_nH$ et de l’hémoglobine totale $a_2(Fe^{57})$ $b_2(Fe^{57})$ partiellement enrichie en $^{57}$Fe. Compte tenu de la précision expérimentale ($\pm 0.006$ mm s$^{-1}$) l’effet quadrupolaire des HbA des chaînes $a_nH$ et $b_nH$ varie entre 100 K et 4,2 K ; de plus l’effet quadrupolaire de HbA n’est pas la moyenne des valeurs trouvées pour les chaînes isolées. L’effet quadrupolaire de $a_2(Fe^{57})$ $b_2(Fe^{57})$ est pratiquement le même que celui de HbA. Les mêmes tendances sont retrouvées sur les valeurs du déplacement isomérique. Les largeurs de raies ont des valeurs très voisines pour tous les composés.

Abstract. — Iron 57-enriched, deoxygenated human (adult) hemoglobin (HbA), its isolated $a_nH$- and $b_nH$-chains, and the partially iron 57-enriched, fully functional hemoglobin $a_2(Fe^{57})$ $b_2(Fe^{57})$ were investigated by Mössbauer spectroscopy in the temperature range 4.2 K-200 K. According to our experimental accuracy (± 0.006 mm s$^{-1}$) the temperature dependent quadrupole splittings $\Delta E_Q(T)$ of deoxygenated HbA, $a_nH$- and $b_nH$-chains are not identical from about 100 K down to 4.2 K, and $\Delta E_Q$ of HbA is not the average value of the $\Delta E_Q$-values of the isolated chains. The quadrupole splitting of $a_2(Fe^{57})$ $b_2(Fe^{57})$ turns out to be practically the same as that of HbA. These findings are also reflected by the isomer shifts. Line widths are comparable for all compounds.

1. Introduction. — All mammalian hemoglobins possess four subunits and hence four oxygenation sites (ferrous iron ions). Cooperative interactions between these sites play an important role upon oxygen uptake: The oxygen affinity is low in the fully deoxygenated molecule and increases towards the so called « high-affinity-state » . Associated with this the molecular conformation of these sites switches between two corresponding structures, namely the initial out-of-plane iron ions move towards the center of the porphyrin plane and change their electronic properties [1, 2]. The specific oxygenation characteristic of hemoglobin is attributed to the presence of two different types of subunits, the $x$- and $b$-chains, the aminoacid sequences of which slightly differ in their total number of residues and in several local substitutions. When isolated, $x$- and $b$-chains bind molecular oxygen without any conformational change; this is interesting especially under the aspect that $b$-chains spontaneously aggregate to four tetramers [3].

From low-temperature magnetic susceptibility measurements [4] there is evidence, that inter-subunit interactions result in a change of the iron environment when going from isolated to associated chains; (such influences are undetectable by crystallographic methods at the present level of resolution). Mössbauer-spectroscopic investigation was expected to corroborate these results, however, recently Huynh et al. [5] reported that hemoglobin and its isolated chains are undistinguishable from their Mössbauer spectra.

2. Experimental. — Isomer shift, $\delta$, and quadrupole splitting, $\Delta E_Q$, has been measured between 4.2 K and 200 K for several preparations of deoxygenated Fe$^{57}$-enriched human (adult) hemoglobin, HbA, and its native and isolated chains with free SH-groups, $a_nH$ and $b_nH$. The sample preparation is described in detail in ref. [6]. All the samples used in our study were found to possess normal electrophoretic behavior, normal optical spectra, and normal cooperative oxygenation behavior for reconstituted HbA (Hill-coefficient of about 2.7 is comparable to 2.7-2.9 for native HbA).

Another type of sample has been measured, in which two Fe$^{57}$-enriched $x$-chains are associated to two unenriched $b$-chains (mass hybrids). The Mössbauer spectrum of this $a_2(Fe^{57})$ $b_2(Fe^{56})$ sample, of course, reveals the properties of iron in a $x$-chain, which is part of a normal and fully functional HbA molecule. Thus the investigation of mass-hybrids with Mössbauer spectroscopy opens the possibility to correlate electronic properties of iron in the different subunits with their suggested functional inequivalence [1].
3. Results and discussion. — The experimental quadrupole splittings, isomershifts and line widths of deoxygenated HbA, $\alpha_{\text{Hb}}$, $\beta_{\text{Hb}}$, and $\alpha_{\text{(Fe}^{57}\text{)}}$, $\beta_{\text{(Fe}^{57}\text{)}}$ are listed in Table I. The absolute values of $\delta(T)$ are comparable to those found by other authors [7-11] for deoxygenated hemoglobin or myoglobin. They confirm that iron in these proteins is in the ferrous high-spin state.

### Table I

**Experimental quadrupole splittings, $\Delta E_Q(T)$, isomershifts, $\delta(T)$, and line widths, $\Gamma(T)$, for various Fe$^{57}$-enriched human hemoglobin compounds**

<table>
<thead>
<tr>
<th>Temperature ($T$, K)</th>
<th>$\Delta E_Q$ ($\text{mm s}^{-1}$)</th>
<th>$\delta$ ($\text{mm s}^{-1}$)</th>
<th>$\Gamma$ ($\text{mm s}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>2.392 ± 0.004</td>
<td>0.928 ± 0.010</td>
<td>0.378 ± 0.006</td>
</tr>
<tr>
<td>15.2</td>
<td>2.394 ± 0.006</td>
<td>0.927 ± —</td>
<td>0.438 ± —</td>
</tr>
<tr>
<td>25.8</td>
<td>2.386 ± 0.004</td>
<td>0.925 ± —</td>
<td>0.380 ± —</td>
</tr>
<tr>
<td>35.0</td>
<td>2.386 ± 0.004</td>
<td>0.923 ± —</td>
<td>0.347 ± —</td>
</tr>
<tr>
<td>45.0</td>
<td>2.375 ± 0.005</td>
<td>0.924 ± —</td>
<td>0.322 ± —</td>
</tr>
<tr>
<td>55.0</td>
<td>2.366 ± 0.005</td>
<td>0.923 ± —</td>
<td>0.296 ± —</td>
</tr>
<tr>
<td>64.2</td>
<td>2.353 ± 0.004</td>
<td>0.921 ± —</td>
<td>0.353 ± —</td>
</tr>
<tr>
<td>74.0</td>
<td>2.330 ± 0.005</td>
<td>0.920 ± —</td>
<td>0.347 ± 0.007</td>
</tr>
<tr>
<td>98.5</td>
<td>2.278 ± 0.004</td>
<td>0.919 ± —</td>
<td>0.351 ± —</td>
</tr>
<tr>
<td>146.5</td>
<td>2.138 ± 0.004</td>
<td>0.903 ± —</td>
<td>0.362 ± —</td>
</tr>
</tbody>
</table>

**Deoxy hemoglobin**

<table>
<thead>
<tr>
<th>Temperature ($T$, K)</th>
<th>$\Delta E_Q$ ($\text{mm s}^{-1}$)</th>
<th>$\delta$ ($\text{mm s}^{-1}$)</th>
<th>$\Gamma$ ($\text{mm s}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>2.381 ± 0.005</td>
<td>0.938 ± 0.010</td>
<td>0.286 ± 0.006</td>
</tr>
<tr>
<td>77.0</td>
<td>2.319 ± —</td>
<td>0.930 ± —</td>
<td>0.304 ± —</td>
</tr>
<tr>
<td>120.0</td>
<td>2.239 ± —</td>
<td>0.923 ± —</td>
<td>0.322 ± —</td>
</tr>
</tbody>
</table>

(* enrichment with Fe$^{57}$ is > 90%.

3.1 ISOLATED $\alpha_{\text{sh}}$- AND $\beta_{\text{sh}}$-CHAIRNS. — Figure 1 illustrates that the quadrupole splittings of the deoxy-

![Fig. 1. — Temperature dependence of experimental quadrupole splittings, $\Delta E_Q(T)$ of Fe$^{57}$-enriched and deoxygenated human hemoglobin and its chains.](image-url)

The experiments by Huynh et al. were performed with unenriched samples which, in spite of their drastically high sample concentration led to relatively large experimental errors ($\pm 0.02 \text{ mm s}^{-1}$), so that the error bars assigned to the measured values overlap.

(i) Huynh et al. limited their investigations to the temperature range 80 K-200 K, whereas the inequi-
valence of our $\Delta E_Q(T)$-values of different chains is emphasized at lower temperatures.

(iii) Huynh et al. also reported values of the isomershifts (relative to metallic iron) which show a systematic deviation of about 0.1 mm s$^{-1}$ with respect to values reported by other authors [7-10] for deoxygenated hemoglobins and myoglobins.

(iv) Finally their published spectra reveal the presence of about 20% of impurity species, which they consider as unavoidable consequence of the preparational procedure. In contradiction to this we succeeded in preparing samples free of such impurity species (Fig. 2); the nature and formation of such impurity species is discussed elsewhere [4, 6].

3.2. HbA. — An interesting result of our study is the fact that the quadrupole splitting of deoxygenated HbA is not only different from that of any of the two chains in the temperature range of 4.2 K-100 K, but that it is also different from the average value

$$\frac{1}{2}[\Delta E_Q(\gamma_{\text{b}})] + \Delta E_Q(\beta_{\text{b}})].$$

This finding is in agreement with magnetic susceptibility measurements [4] and confirms that the electronic properties of iron do measurably change upon chain association.

The absolute $\Delta E_Q(T)$-values we found for HbA are comparable with those of Huynh et al. [5] within the range of errors, however, deviate from those of Eicher et al. [10] systematically by about 0.05 mm s$^{-1}$, and from those of Spartalian et al. [11] by 0.02 mm s$^{-1}$. Here again we find it worth to notice that our spectra are free from impurity components in our HbA samples.

3.3 Mass Hybrids. — The Mössbauer investigation of the mass hybrid $\alpha_2(Fe^{57}) \beta_2(Fe^{56})$ allows the inspection of the $\alpha$-chains alone in the fully functional hemoglobin. Its quadrupole splitting is almost the same as that observed for the completely enriched HbA: $\alpha_2(Fe^{57}) \beta_2(Fe^{57})$. From this, and from the discussion above (3.2) we conclude that the $\alpha$-$\beta$ association is accompanied by a measurable ligand field modification of the heme iron. More extensive discussion of these last data will appear elsewhere.

References