Electron Transfer Kinetics between Hemoglobin Subunits*

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The kinetics for electron transfer have been measured for samples of hemoglobin valency hybrids with initially one type of subunit, α or β, in the oxidized state. Incubation of these samples under anaerobic conditions tends to randomize the type of subunit that is oxidized. With a time coefficient of a few hours at pH 7, 25 °C, the Hb solution (0.1 mM heme) approaches a form with about 60% of β chains reduced, indicating a faster transfer rate in the direction α to β. There was no observable electron transfer for samples saturated with oxygen. The electron transfer occurs predominantly between deoxy and aquo-met subunits, both high spin species. Furthermore, electron transfer does not depend on the quaternary state of hemoglobin. Incubation of oxidized cross-linked tetramer Hb A with deoxy Hb S also displayed electron transfer, implying a mechanism via inter-tetramer collisions. A dependence on the overall Hb concentration confirms this mechanism, although a small contribution of transfer between subunits of the same tetramer cannot be ruled out. These results suggest that in vivo collisions between the Hb tetramers will be involved in the relative distribution of the methemoglobin between subunits in association with the reductase system present in the erythrocyte.

In biomolecules iron is in equilibrium between predominantly two oxidation states, the ferrous Fe(II) and the ferric Fe(III) states. In heme proteins such as Hb, the oxidation state will determine the ligands that bind to the iron of the active center heme; O₂, CO, and NO bind to the ferrous form, whereas water, OH⁻, or CN⁻ are ligands of the ferric state. Free heme in solution oxidizes on contact with oxygen, whereas the globin offers the possibility of reversible oxygen binding by prolonging the ferrous lifetime to more than 1 day. This is still low compared with the turnover of red blood cells of about 120 days, and reversal to the reduced state requires metHb reduction. Although heme proteins can be generally classed as the electron transfer cytochromes and oxygen transporters (hemoglobin (Hb), myoglobin), the latter group obviously also participates in electron transfer reactions as part of their natural function. One of the great paradoxes of aerobic life is that although O₂ is essential for respiration, it also drives oxidation reactions, in particular that of the ferrous iron into its ferric oxidation pathway, are linked to oxidative damage reactions (1, 2) such as the accumulation of heme degradation products in the membrane, which might accelerate the erythrocyte aging. Fortunately reducing systems inside the erythrocyte such as the NADH metHb reductase/cytochrome b₅ along with glutathione and ascorbic acid counterbalance the continuous in vivo autooxidation of hemoglobin, whereas catalase and superoxide dismutase, to cite the most familiar, participate in the elimination of the free radicals.

If the oxygen binding properties and the autooxidation mechanism of hemoglobin are well described in the literature, the electron transfer reactions in hemoglobin intra- or inter-tetramers have not been extensively studied. In fact more is known about the electron transfer between Hb and other oxidant or reductant molecules. Furthermore, valency exchange in hemoglobin was often attributed to heme exchange between subunits; indeed, heme loss is relatively rapid for the oxidized or degraded form of hemoglobin. However, reduction of Hb by electron transfer is a natural process, and transfer between the subunits should also occur, although possibly on a much slower time scale. Electron transfer in hemoglobin and its reductase-associated reactions are thus linked to its in vivo life span.

In the present work we study electron transfer reactions between hemoglobin molecules for a better understanding of this mechanism and the overall hemoglobin redox reactions that keep the oxygen carrier active in its ferrous form. We used simple methodologies to discriminate the bi-directional transfers α → β and β → α and intra versus inter-tetramer. Our results are discussed in light of Hb oxidation and reduction differences between the α and β subunit.

MATERIALS AND METHODS

Hb A was purified from the blood of healthy non-smoking donors, stripped of organophosphates, and stored under liquid nitrogen until use as described previously (3). Hb S from homozygous sickle cell patients was purified on a DEA‑Sephadex column equilibrated with 50 mM Tris-HCl buffer, pH 8.3, after running a pH gradient 8.3 to 8.0. Natural variant Hb Chesapeake α92 Arg → Leu was found in a poly‑globulic patient (4) and characterized by electrophoresis and molecular biology studies (DNA sequence analysis). The separation was accomplished by preparative isoelectric focalization as described below.

MetHb was generated by autooxidation of ferrous Hb at 37 °C overnight at pH 6.5 in 50 mM bis-Tris buffer with 100 mM NaCl. The remaining ferrous fraction was oxidized by a small excess of potassium ferricyanide at room temperature. The residual ferricyanide and its reduced form were removed by chromatography on an analytical grade ion exchange resin 501-X5 (Bio-Rad). Human α and β chains were split from Hb A upon reaction with p-mercubenzozate after the procedure of Winterhalter and Colosimo (5) with some modifications. Then para-mercubenzozate chains were separated by preparative isoelectric focusing at 5 °C using granulated gel (Ultrodox) and ampholines (with a pH gradient from 6 to 8; No. 80-1125-93) purchased from Amersham Biosciences, Inc. The SH groups were regenerated by incubation with dithiothreitol according to the method developed by Parkhurst and Parkhurst (6). Because the UV spectra are a sensitive probe of the presence of para-mercubenzozate bound to the cysteines, the αSH and βSH spectra were recorded to assess the absence of residual para-mercubenzozate. Analytical isoelectric focusing in polyacrylamide...
PAGE along a gradient from pH 6.5 to 8.5 was run to confirm the homogeneity of each preparation and their correct pI, which are different from those of para-mercuribenzoate-bound chains (this control was also performed for all the other protein preparations). Finally, visible spectra of the carboxylated chains did not show a contamination of oxyhemoglobin.

Cross-linked DCL Hb was generously provided by Baxter Healthcare Corporation. High performance liquid gel filtration chromatography on Superose 12 PC (Amersham Biosciences, Inc.) showed an elution profile for a homogenous population of tetramer under conditions (micromolar concentration) for which oxy-Hb A shows a significant amount of dimers and trimers.

Fe$^{3+}$/$Fe^{2+}$ Hybrid Preparation; Species ($\alpha'-\beta'\alpha\beta$)$_2$ and ($\alpha\beta'\alpha'\beta$)$_2$.—The symmetric hybrids ($\alpha'\beta'\alpha\beta$)$_2$ and ($\alpha\beta'\alpha'\beta$)$_2$ were prepared according to the method of Cassoly (7). The respective deoxy ferrous subunits were oxidized for several minutes in a 1 M glycine buffer, pH 8.4, by a slight excess of ferricyanide at 4 °C and immediately loaded onto a G25 column to strip the oxidant products. Then a 10% excess of the carboxylated heterologous subunits was added to the stripped oxidized subunits. The hybrid solution was cleared of small contamination of fully meth and carboxylated Hb A by preparative isoelectric focusing at 5 °C. The spectrum of each hybrid was analyzed by simulating the spectrum as a linear combination of the fully CO and aquo-metHb spectra. As expected, near equal amounts of both heme contributions were observed in each case.

Two alternative methods were used that do not require separation of the chains, as reported by Tomoda and Yoneyama (8). Hybrid ($\alpha'\beta'\alpha\beta$)$_2$ was obtained from metHb based on the preferential reduction of the $\beta$ subunit by sodium ascorbate under anaerobic conditions. The metHb solution equilibrated with 10 mM potassium phosphate, pH 6.8, was thoroughly deoxygenated by replacing air with nitrogen gas. Then 20 mM sodium ascorbate was added, and the reduction reaction was monitored by spectrophotometry. Once 45% of the reduction was achieved, the sample was immediately loaded onto a G25 column to remove the ascorbate, thus stopping the reaction. After elution, preparative isoelectric focusing at 5 °C was performed overnight to remove the remaining metHb and also a small amount of fully reduced tetramer. Finally, the intermediate isoelectric band enriched in hybrid ($\alpha'\beta'\alpha\beta$)$_2$ was further purified by fast protein liquid chromatography. Stationary phase was monitored during the 24-h incubation under anaerobic conditions, because remaining traces of oxygen or addition during sampling via the Hamilton syringe might provoke the oxidation side reaction. Samples were monitored during the 24-h incubation under anaerobic conditions, and the spectrum of each aliquot was analyzed. In the presence of the O$_2$-scavenging system, deoxy Hb samples did not show the formation of metHb after 24 h of incubation. For the hybrid species, methemoglobin formation did not exceed 5% after 24 h and did not introduce a systematic error in the electron transfer measurement because the autoxidation rates for $\alpha$ and $\beta$ chains are equivalent.

Absorption Spectra for Monitoring the Kinetics of Methemoglobin Reduction.—The kinetics of metHb reduction were recorded with the rapid-scanning diode array spectrophotometer HP8453. We measured the reduction of metHb ($\alpha\beta$)$_2$, ($\alpha'\beta'\alpha\beta$)$_2$, and ($\alpha\beta'\alpha'\beta$)$_2$ hybrid species by the system NADPH/ferrodoxin-NADP/ferrodoxin as described by Hayashi et al. (11). Enzymes and chemicals were from Sigma. We also measured the chemical reduction of this species by 20 mM sodium ascorbate.

Absorption Spectra for Monitoring the Kinetics of Electron Transfer.—Absorption differences between heterologous subunits have been reported in the ferrous deoxy and high spin ferric states (12). We used the same procedure to determine the differences in the electron transfer transfer between Hb subunits. These inequalities of optical properties between Hb chains arise essentially from their aquo-metHb forms. Such differences have been observed for high spin ferric heme-ligand complexes but not for low spin ligands such as azide or cyanide for instance (12). This could be related to the difference between the chains in their $pK_a$ for the transition between the high spin aquo-metHb and the low spin hydroxy-metHb forms.

Azide Association Kinetics for Monitoring the Ferric Fractions of the $\alpha$ and $\beta$ Chains.—Biphasic kinetics of azide association to aquo-metHb were observed, with rates of 600 and 140 M$^{-1}$ s$^{-1}$ at 25 °C, 50 mM potassium phosphate, pH 7. From the work of Gibson et al. (13), the faster rate was attributed to the $\beta$ subunits; these rate assignments were confirmed by kinetic measurements of the hybrid species, fully $\alpha$- or $\beta$-oxidized (Fig. 1A). In the presence of IHP, the ratio of the rates is 30 +/− 5 for metHb A, as previously observed by Edeleanu and Gibson (14); the ratio was 40 +/− 5 for met-DCL Hb with IHP. The larger ratio in the presence of IHP was due essentially to the $\beta$ chain contribution (Fig. 1B). Indeed the slow rate of azide binding to the $\alpha$ subunits is similar for metHb A, met-DCL Hb, and ($\alpha'\beta'\alpha\beta$)$_2$ hybrid species with those observed without deoxy cyanide.

We used this large difference in rates to monitor the relative changes between $\alpha$ and $\beta$ ferric fractions during the electron transfer reactions (insert Fig. 1A). Kinetics were recorded at 403 nm, a wavelength for which the maximum change of absorbance occurred between aquo-metHb and azide-metHb forms. The estimation of the $\alpha$ and $\beta$ ferric fractions depended on the accurate measurements of the total signal...
amplitude and the proportion of the two binding phases. As shown in the inset of Fig. 1B, the expected amplitude from the static spectrum is in agreement with the measured amplitude by stopped-flow after mixing a metHb solution with a final concentration of 15 mM azide in the presence or absence of IHP. Each stopped-flow kinetic curve was an average of at least five stopped-flow mixings.

Note that for metHb A, the amplitudes of the two phases are not identical, due to spectral differences of the chains (inset of Fig. 1A). At the different wavelengths tested, the percentage of fast phase is shown between 40 and 55%; in particular, at 403 nm, the fast phase is equal to 42 ±/−2% of the signal but lower after the addition of 0.2 mM IHP (Fig. 1B). This was taken into account to relate the proportions of the two binding phases to the ferric α and β subunits, which conformed to the static variation of absorbance (solid line, azido-metHb minus aquo-metHb). This control confirms that in our experimental conditions the entire kinetics were recorded after the stopped-flow mixing.

The curve for metHb shows a biphasic pattern, reflecting the difference in reaction rate between the chains; azide binding is four times faster for β relative to α subunits (A). The inset shows the variation of absorbance in the Soret region upon azide binding to the valency hybrids (αCOβ+1)2 (solid line) and (α′β′CO)2 (dashed line). After the addition of IHP, which stabilizes the deoxy structure, the rate for azide binding is 30 times faster for the β chains (B). The inset shows the amplitude of the azide binding signal in the presence of IHP (solid line) compared with the static variation of absorbance (dashed line, azido-metHb minus aquo-metHb). This control confirms that in our experimental conditions the entire kinetics were recorded after the stopped-flow mixing.

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FIG. 1. Stopped flow kinetics of azide binding to metHb A and valency hybrids, monitored at 403 nm. The curve for metHb shows a biphasic pattern, reflecting the difference in reaction rate between the chains; azide binding is four times faster for β relative to α subunits (A). The inset shows the variation of absorbance in the Soret region upon azide binding to the valency hybrids (αCOβ+1)2 (solid line) and (α′β′CO)2 (dashed line). After the addition of IHP, which stabilizes the deoxy structure, the rate for azide binding is 30 times faster for the β chains (B). The inset shows the amplitude of the azide binding signal in the presence of IHP (solid line) compared with the static variation of absorbance (dashed line, azido-metHb minus aquo-metHb). This control confirms that in our experimental conditions the entire kinetics were recorded after the stopped-flow mixing.

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Electron Transfer between Hb A and Hb S—We measured the electron transfer in hemoglobin by incubation of metHb A or met-DCL Hb with deoxy-Hb S; these species were easily separated by biochemical methods, since their pI values differ by 0.4 pH units. Use of the cross-linked DCL Hb ensured that dimer exchange will not occur. Dimer exchange was initially minimal when one of the species was the deoxy tetramer, which required hours to dissociate as opposed to a few seconds for liganded Hb. Thus, for the mixing of metHb-A and deoxy-Hb S, as the electron transfer reaction reached equilibrium, the partially oxidized Hb S may dissociate into dimers and hybridize with the Hb A dimers. There was little difference in the electron transfer kinetics for Hb A versus DCL Hb for experiments at the same hemoglobin concentrations.

Each aliquot collected during the incubation under anaerobic conditions of the Hb mixtures was diluted in 20 mM potassium phosphate, pH 6.8, containing 500 μM KCN and equilibrated under 1 atm of CO gas to prevent the autoxidation of the ferrous heme, further electron transfer reaction, and to neutralize the difference of charge between ferric and ferrous hemes before separation of the Hb species by a fast protein liquid chromatography column. The separation was carried out by a specific elution step in a 1-mm optical cuvettes. A typical kinetic curve with a detection wavelength at 436 nm was the average of at least 10 measurements. This technique was used to compare the allosteric properties for ligand binding between Hb Ch apeake and Hb A at pH 7.2. Sodium dithionite solution was added up to a final concentration of 0.5 mM in the experiments to prevent an O2 contamination and to keep the Hb samples fully reduced.

RESULTS

Kinetics of electron transfer between the Hb subunits are shown in Figs. 2 A and B. When a symmetric hybrid (α or β oxidized) is incubated under anaerobic conditions, the fraction of oxidized form begins to become more evenly distributed, with a slightly larger amount of α chain in the oxidized form at equilibrium. At pH 7.0, 25 °C, and 100 μM heme concentration, the observed rates are 0.2 h⁻¹ for the reaction starting with (α−deoxy)+2 or (α−deoxy)+2; however, the latter species showed about a 20% slightly faster phase. At equilibrium the ferrous heme fraction was slightly more populated in β subunits, which indicates that the microscopic rate of electron transfer is faster in the direction α → β than β → α, with the observed rate the sum of the microscopic rates. The ratio between β and α ferrous fractions at equilibrium, equivalent to the ratio between the microscopic rates, is 1.7 and 1.3 with or without the effector IHP.

Each point in the curve (Figs. 2) is the fraction of α- and β-oxidized subunits, determined from the azide binding kinetics. As described under “Materials and Methods,” oxidized α and β subunits react with azide (13) at distinct rates, with β much faster than α chains. Fig. 3 illustrates the variation of azide binding kinetics due to electron transfer under anaerobic conditions after incubation of the symmetric valency hybrid (α−deoxy)+2. The fraction of the fast recombination phase increases as the β chains transfer their electron to the α-oxidized subunits.

Likewise, for experimental conditions closer to those in vivo, namely pH 7.4, 37 °C with 100 μM phosphate to mimic the 2,3-diphosphoglycerate effect, the same pattern was observed for the electron transfer reaction in hemoglobin (0.1 mM total heme) with a ratio α/β at equilibrium equal to 1.3 and observed rates of 0.4 h⁻¹ for the 2 hybrid species. Again the (α-deoxy-β)+2 hybrid species showed an initial phase (−20%) with a faster rate; the kinetics could be fitted with two exponential terms, with 80% having the slower rate, similar to the other valency hybrid. This might suggest a different pathway

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FIG. 2. Electron transfer kinetics monitored by the absorbance changes in the Soret region for a solution initially (α'β-deoxy)$_2$ (□) or (α-deoxyβ')$_2$ (B, □, O) hybrids. The difference of static spectra between Hb symmetric valency hybrids met/deoxy is shown in the inset. The initial and final fractions of oxidized subunits were confirmed by azide stopped-flow kinetics. The experimental conditions were 100 mM phosphate, 0.1 mM EDTA, pH 7.4, 37 °C for 20 μM total heme concentration of Hb hybrid solutions. Solid lines are simulations using a mono-exponential decay.

or the possibility of more than two distinct species with regard to their electron transfer reactivity arising from the 10 different Hb ligation micro-states.

We also used the small differences of the absorption spectra of the hybrids to monitor the electron transfer kinetics at 37 °C, pH 7.4 (Fig. 4). Using this method we obtained transition rates for β-oxidized and α-oxidized hybrids equal to 0.5 and 0.4 h$^{-1}$, respectively, at 20 μM protein on a heme basis. The final distribution of the oxidized sites was confirmed by the relative amplitudes of fast and slow phases in azide binding kinetics. Note that with this method of detection we did not observe the biphasic kinetics for the (α-deoxyβ')$_2$ hybrid. However, in these experiments part of a rapid phase could be missed because the time required to deoxygenate the solution in the optical cuvette with the enzymatic system is about 5 min; in the other method (with azide kinetics on aliquots), the hybrid solutions were readily deoxygenated by adding mechanical stirring to the enzyme system action.

Control 1; Dependence on the Type of Ligand—We did not observe electron transfer between Hb subunits under 1 atm of O$_2$ or CO at 25 °C after a 48-h incubation (data not shown), confirming a very low rate for liganded ferrous subunits. Similarly, the addition of CN$^-$ as the ligand for the oxidized subunits greatly decreased the transfer rate (15, 16). In a similar manner, at alkaline pH the electron transfer decreases when the oxidized subunits exhibit a transition of ligand from water molecules to hydroxyl anions (Fig. 5). Consequently the electron transfer occurs mainly between deoxy and aquo-met subunits for which the heme configuration is in the high spin state.

Control 2; Heme Exchange—Initially heme exchange was proposed to explain the valency exchange between Hb subunits. Hemin loss from high spin aquo-metHb is possible since hemin dissociation from metHb takes only a few minutes (17, 18), but ferrous heme dissociation is much slower, and valency exchange involving heme is unlikely to occur in vivo. No heme exchange reaction was demonstrated between ferrous and ferric Hb because of the high affinity of globin for the heme (19); such an exchange would involve a concomitant heme dissociation from both types of subunit. Although this might occur under conditions of low protein stability, our experiments were always within the range for which Hb unfolding is not probable. Because free heme is immediately oxidized by O$_2$, any hemes that were exchanged would be oxidized; this would imply an increase in the overall percentage of metHb, which was not observed.

To check for a possible heme exchange reaction under anaer-
The concentration of both species was 90 °C at 37 °C. The rate between cross-linked DCL metHb and deoxy Hb S at 37 °C was determined using fast protein liquid chromatography (see the inset in Fig. 7). The pK values of acid-alkaline transition (Fe²⁺-H₂O ≈ Fe³⁺-OH⁻) range from 7 to 10 in ferric hemoproteins (29).

At pH 8.0, 25 °C, which reflects the slow dissociation rate of the ferrous heme from the globin.

Allosteric State—A dependence on the allosteric state of Hb was tested by use of IHP, a potent effector that favors the deoxy conformation, and a mutant Hb, which shifts the allosteric equilibrium in the opposite direction. In general Hb with two ligands display a mixture of the two allosteric states and is therefore sensitive to shifts in the allosteric equilibrium. For example, the difference spectra for both valency hybrids measured by Raman spectroscopy showed half of the difference between deoxy and fully liganded Hb (20). We did not observe a change in the rate of electron transfer for samples with IHP; only a slight increase on the inequivalence between bi-directional electron transfer pathways was observed. This result suggests that the Hb quaternary conformation does not significantly influence the electron transfer.

Electron transfer kinetics with symmetric hybrids of Hb Chesapeake support this hypothesis. Indeed this natural variant has been described as a high oxygen affinity mutant due to a pronounced destabilization of the deoxy conformation (21, 22). The kinetics of CO rebinding measured by flash photolysis for 50% ligand dissociation show much slow phase, characteristic of the deoxy species, compared with Hb A (inset in Fig. 6). This confirms that the Hb Chesapeake tetramers are strongly shifted toward the liganded conformation. Electron transfer kinetics with Hb Chesapeake valency hybrids exhibit rates similar to those of Hb A (0.15 h⁻¹) at pH 7.0, 25 °C, with the same preferential pathway toward the β subunits (Fig. 6).

Inter-tetramer Electron Transfer—To test for transfer between tetramers, we mixed a near equal amount of deoxy-Hb S with met-DCL Hb or metHb A. The cross-linked DCL Hb was used to prevent dimers exchange. After incubation, the collected aliquots were loaded onto a strong cation exchange column to separate each component for further analysis of their relative ferrous and ferric content by spectroscopy. Fig. 7 shows the spectral variation upon electron transfer from deoxy Hb A toward the metHb S after 24 h. After separation by chromatography (see the inset in Fig. 7) the Hb spectrum was simulated as a linear combination of fully cyano-metHb and Hb CO spectra, two stable forms preventing any further electron transfer.

The dependence of the electron transfer rate on heme concentration is shown in the inset of Fig. 8; electron transfer is faster at higher hemoglobin concentrations, with a value of 1.6 h⁻¹ at 1.2 mM (total heme). This is expected if the electron transfer occurs by collision between tetramers. A deviation from linearity at low protein concentration could indicate that a parallel mechanism is present.

**DISCUSSION**

Studies of partially liganded Hb are difficult because of their transient nature, especially with oxygen, which dissociates rapidly. There are numerous *in vitro* studies on hemoglobin that are based on valency hybrids or metal-substituted Hb. These Hb hybrids molecules have been extensively produced as stable analogues for a better understanding of the Hb oxygenation mechanism. However, electron transfer may play a significant role in the redistribution of the valency state between α and β subunits after a prolonged incubation under conditions...
for which hemes are not fully liganded. In vivo the electron transfer between hemoglobin molecules within the erythrocyte will occur in parallel with the enzymatic reduction and are capable of reducing metHb on the order of a few minutes (23).

Electron transfer is a well known reaction for both the cytochromes and Hb. In fact Hb is probably reduced more than 50 times during the 120-day lifetime of the cell, since the rate of autoxidation is about 2 days. Thus electron transfer due to collisions between heme proteins is a common reaction, and some transfer between Hb molecules, although at a slower rate, is to be expected.

In the present study, an electron transfer reaction on the time scale of a few hours was observed. For the symmetric hybrids, this reaction will tend to more evenly distribute the fraction of each type of subunit that is oxidized. A slight preference for the α-oxidized form was observed, indicating a higher rate for electron transfer from α to β chains. The slight tendency of the β chains to gain an electron from the α chains could be explained by its higher oxidation-reduction potential, 0.11 V compared with 0.05 V (24). However we did not observe an inequivalence in the reduction of the oxidized subunits by the ferrodoxin-NADP system (data not shown), as has been reported for the methemoglobin reductase system in the absence of IHP (25). A factor of 2 at pH 7.4 was found in favor of the β chains with ascorbate (data not shown) as previously described (26).

From these results, one cannot discriminate whether the transfer occurs intra-Hb tetramer from the symmetric Hb hybrids mixture rather by collision via inter-tetramer exchange or from these two different mechanisms. Inter-molecular electron transfer is obviously a mechanism by which numerous oxidation or reduction reactions are supplied such as the reduction of metHb by the cytochrome b5. From the results of mixing deoxy tetramers with oxidized cross-linked tetramers, the mechanism of transfer via tetramer collisions was confirmed. Based on the concentration dependence study (Fig. 8) we were able to extrapolate the bimolecular rate of transfer under physiological conditions of the erythrocyte, where the Hb concentration is very high (6 mM in tetramers). Taking a venous hemoglobin oxygen saturation (SaO2) about 75% the electron transfer rate between deoxy and ferric subunits would be 6 h⁻¹. The electron transfer rate decreases when the oxygen saturation increases; in an arterial hemoglobin oxygen saturation (SaO2), about 97% the electron transfer rate will then approach a value of 0.7 h⁻¹. Consequently in the venous compartment, electron transfer is likely to redistribute the oxidized fraction between the pool of Hb tetramers concomitantly with the reduction process by the natural enzymatic system.

Heme loss depends on the heme oxidation state, with a very high affinity of the order of picomolar for the ferrous state (27) but greatly decreased for the ferric heme (18). From ⁵⁹Fe-labeled heme studies, heme loss or exchange for the ferrous deoxy or liganded Hb or cyano-met Hb has been shown to require more than 5 days (28), whereas hemin may dissociate within a few minutes. The affinity of the heme for the apoglobin is essentially driven by its dissociation rate; association is rapid, and no difference was observed between heme and heme-CO binding rates to apoglobin (19). Hemin dissociation increases 10-fold for dimers compared with tetramers (17), attributed mainly to a significant increase of the rate of dissociation of heme from the β chains (19, 28). Therefore an increase in hemoglobin oxidation, especially for the β chains, or an increase in hemoglobin dimerization will invariably accelerate the hemoglobin degradation in vivo, ending with the precipitation of the apoglobin.

For a normal red cell the reduction system is sufficient to ensure a very low level of methemoglobin. However, if the methemoglobin fraction increases due to a decrease of the activity of the NADH-cytochrome b5 reductase or to a decrease of the substrate concentrations including the cytochrome b5 such as in case of red cell senescence or methemoglobinemia, the role of the electron transfer between hemoglobin molecules with a higher rate for transfer from α to β could help prevent an accumulation of the unstable oxidized β chains. The same mechanism could be involved in the erythrocyte disorders observed for thalassemia and hemolytic anemia due, respectively, to unpaired chains or unstable hemoglobin variants. The presence of a rapidly oxidizing form would drain electrons from the normal fraction. In these diseases unstable species are highly susceptible to autoxidation, forming aggregates and inclusion bodies. This autocatalytic degradation after oxidation and removal of these forms is necessary to avoid further electron transfer with the remaining fraction. Electron transfer in hemoglobin will play a role in vivo during the transit of the red cells, since it will effect the distribution of oxidized subunits.

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