The Reaction of Hemoglobin Zürich with Oxygen and Carbon Monoxide* 

Giorgio M. Giacometti, Maurizio Brunori, and Eraldo Antonini
From the Istituti di Chimica e Chimica Biologica, Facolta di Medicina, Universita di Roma and Centro di Biologia Molecolare del Consiglio Nazionale delle Ricerche, Roma, Italy

Ernesto E. Di Iorio and Kaspar H. Winterhalter
From the Laboratorium fur Biochimie, Biochemie I, Universitatsstrasse 16, Eidgenossische Technische Hochschule, 8092 Zurich, Switzerland

The present paper reports a spectroscopic and kinetic study of the reaction of oxygen and carbon monoxide on (a) the abnormal hemoglobin Zurich (p63, E7 His → Arg, E7 His → Arg, j3) its isolated abnormal chains (pZH), and (a) a reconstituted hybrid containing cobalt instead of iron on the normal α chains (Coα)-(Fe β34). The abnormal β34 chains, isolated from hemoglobin Zurich (HbZH) tetramer, display very peculiar spectral properties in the Soret region which were determined, for the oxy and deoxy derivatives, by kinetic difference spectra.

The spectral properties of abnormal chains are maintained in the native and reconstituted hybrid tetramer. The rate constants for CO and O₂ binding to isolated β34 chains were compared with those of the normal α and β chains. It is confirmed that the CO combination rate constant to β34 is much higher than that of normal chains, whereas for O₂ is similar for the normal and abnormal chains.

The CO binding rate constant for Fe β34 chains in the hybrid tetramer is the same as in the native HbZH molecule, i.e. much higher than that of normal chains.

The new data are fully consistent with the sequential mechanism for CO binding previously proposed by us; on the other hand, on the basis of the spectral and kinetic results, and contrary to what has been suggested by other authors, a sequential binding of the ligand is excluded in the case of O₂.

Understanding of the relationship between structure and function of hemoglobin has been based largely on the identification of the amino acid residues which play a crucial role in determining the functional characteristics of the molecule. Investigation of the functional properties of mutant hemoglobins, carrying substitutions at crucial positions, has been valuable in this task. Hemoglobin Zurich (HbZH, p63 E7 His → Arg) is a suitable example to investigate the role of the distal histidine. For this reason, since its discovery, a great deal of interest has been directed toward the elucidation of the structural and functional properties of this molecule (1–3).

In a previous paper (4), we have proposed a kinetic model for the reaction of HbZH with CO. The model implies that

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The abbreviations used are: HbZH, hemoglobin Zurich; HbA, the abnormal β34 chains in a tetramer with a deoxy-T configuration have (i) higher affinity for CO than the α chains and (ii) higher combination rate constant (1.3 x 10⁴ M⁻¹ s⁻¹) than the normal β chains in T (10³ M⁻¹ s⁻¹). Thus, in the case of HbZH, the reaction with CO can be regarded, to a first approximation, as a sequential one involving: (i) the binding of the ligand to the abnormal β34 chains in T; (ii) the switchover of the quaternary conformation from T to R; (iii) the binding of the ligand to the normal α chains in R.

Recently, the tridimensional structure of carboxy-HbZ has been determined (5). The data available allowed us to provide a stereochemical interpretation of some of the abnormal functional properties of HbZH. Moreover, by analogy with the sequential mechanism proposed for CO (4), the reaction with O₂ of this abnormal Hb has also been tentatively interpreted (5).

It has often been assumed, and it is certainly true, that the main features of the binding properties of HbA for CO and O₂ may essentially be fitted by the same scheme (6). However, in view of the differences in the electronic and geometrical contributions to the binding of O₂ and CO to the iron, different interactions of these two ligands with the imidazole ring of the distal histidine have been proposed (7). Thus, it is possible that the replacement of this residue in HbZH may produce different effects on the binding of the two ligands.

The purpose of this work is to extend the investigation of the spectral and kinetic properties of HbZH and its isolated chains to the reaction with oxygen and to test whether or not the same model which accounts for the reaction with carbon monoxide is also valid for the physiological ligand. This paper shows that the two ligands follow different mechanisms since binding of O₂ to deoxy-HbZH is not sequential.

MATERIALS AND METHODS

Hemoglobin Zurich was purified, from the blood of a patient heterozygote for this condition, by following the procedure previously described (1). Chain separation was achieved by an adaptation of the original method of Bucci and Pronicelli (8). Sulphydryl groups were regenerated by passing 10-ml aliquots of the isolated chains (in the PMB form) through a Sephadex G-25 column (20 x 2 cm) equilibrated with 0.1 M Tris-HCl buffer at pH 7.4 containing 5 x 10⁻⁴ M EDTA and 0.1 M KCl. On the same column, a 10-ml aliquot of buffer

adult human hemoglobin; β34, abnormal β chains from HbZH; PMB, p-chloromercuribenzoate; β, normal β chains from HbA; α, normal α chains from HbA or HbZH; β34, abnormal β chains from the sulfhydryl groups blocked by PMB; Coα, α chains containing cobaltous protoporphyrin; (Coα)-(Fe β34), hybrid tetramer in which the two α subunits contain Co and the β34 abnormal subunits contain Fe.
containing 10 mM β-mercaptoethanol had been applied just before the chains. Cobalt-containing α chains (Coα) were obtained starting from cobalt-containing Hb A, prepared according to Yonetani et al. (9).

The (Coα) 2(Fe β 3β H 4) 4 hybrid tetramer was prepared by mixing, immediately before use, stoichiometric amounts of Coα-oxy and Fe β 3β-carboxy chains. Addition of an excess of Coα chains did not modify the kinetic properties of the reconstituted hybrid (see below). The absence of free β 3β chains in the reconstitution product was controlled by electrophoresis.

Photodissociation experiments were performed by a conventional flash photolysis apparatus described elsewhere (6). "O2 pulse" experiments were performed with a Durrum stopped flow apparatus equipped with a 2-cm observation cell. Static spectra were recorded on a Cary 219 spectrophotometer.

Oxygen titration of HbZH was performed using the thin film oxygenation apparatus developed by Gill and collaborators (10).

**RESULTS AND DISCUSSION**

Properties of the Isolated Chains—Because of the intrinsic instability of all derivatives of the isolated abnormal β 3β chains except the CO derivative, the spectral properties of the oxygen and deoxy derivatives have been determined by an indirect (kinetic) method. In Fig. 1, three kinetic difference spectra are reported, i.e. (i) the difference spectrum deoxy-carboxy, which was obtained by flash experiments in the presence of dithionite; (ii) the difference spectrum oxy-carboxy, obtained by flashing a solution of β 3β in the presence of suitable concentrations of both carbon monoxide and oxygen. Under these conditions the following reactions occur:

\[
\begin{align*}
& \text{β}^{3β}\text{CO} \xrightarrow{hv} \text{β}^{3β} + \text{O}_2 \\
& \text{β}^{3β} + \text{CO} \xrightarrow{hv} \text{β}^{3β}\text{CO} \\
& \text{β}^{3β}\text{O}_2 \xrightarrow{} \text{β}^{3β}
\end{align*}
\]

and the difference spectrum given in Fig. 1, corresponds to the reaction (III → I); (iii) the difference spectrum deoxy-oxy as obtained by subtracting Spectra i and ii.

These experiments were carried out using the chains with free or blocked (by PMB) sulfhydryl groups. The difference spectra are essentially the same for β 3β and β 3β, only minor differences being observable, both in maxima and isosbestic points. Thus, the deoxy-oxy difference spectrum has a Δω of ~42 at λ = 435 nm for the β 3β and ~32 at the same wavelength for the β 3β. The isosbestic point at 424 nm for the β 3β is displaced of ~3 nm toward the blue in the β 3β. These differences can be largely attributed to the oxy-CO difference spectrum at wavelengths above ~430, while the difference spectrum deoxy-CO are very similar in both cases. Therefore, the effect of PMB seems to be limited to the spectroscopic properties of the oxy derivative.

The transformation of absorbance changes into absolute extinction changes was carried out using the extinction coefficient for the β 3β CO derivative determined by the pyridine hemochromogen method (4). Using the kinetic difference spectra of Fig. 1 and the absolute spectrum of the CO derivative, the absolute spectra of the oxy and deoxy derivatives were calculated. They are reported in Fig. 2, where they are compared with those of the normal β 4 chains. Within experimental errors the absolute spectra are independent from the presence or absence of PMB on the —SH groups.

The peculiar spectral properties of the CO derivative of the isolated β 3β chains was found to be, as reported previously (4), an essential element in the interpretation of the kinetics. We have now shown that the deoxy and oxy derivatives of β 3β also display very different spectral properties as compared to the normal isolated β 4 chains. For the oxy complex, the absorption maximum is red-shifted by about 6 nm, whereas the deoxynated form has an absorption maximum shifted toward the blue by about the same amount; in the latter case, a shoulder is observable at about 435 nm. The extinction coefficients are given in Table I.

The very unusual spectral properties of deoxy and oxy β 3β chain given in Fig. 1, demand some comment. First of all, it is known that the isolated β 3β chains are unstable and were thus prepared under pure CO. This instability may cast some doubts on the quality of the material. However, the combination with O2, occurring immediately after the flash, can be reproduced numerous times without changes in rates and spectral properties. Thus, the data certainly refer to a native hemoprotein reversibly binding O2. Moreover, it was shown that the β 3β CO chains can reassociate with normal chains, either containing iron or cobalt protoporphyrin (see below), to give a functional tetramer.

The kinetics of recombination of the isolated β 3β chains with CO was investigated for both the —SH and PMB subunits. At various wavelengths, the second order rate constants are identical for the two, within experimental errors, and in very good agreement with the value previously reported for the PMB form (Table II and ref. 4).

The kinetics of combination with O2 was investigated by the flash experiments outlined above, which were performed at different relative concentrations of the two ligands (CO and O2). For a simple displacement reaction, the rate of displacement (R) of O2 by CO is given by

\[
\frac{1}{R} \cdot \frac{1}{k + k'} (O_2)
\]

provided that \(k' (O_2) \ll k' (CO)\), where \(k, k' \) and \(l, l'\) are the "off" and "on" rate constants for O2 and CO binding, respectively. Replacement of O2 by CO was found to be always first order. For both chain derivatives, a very good linear dependence in a plot of \(1/R \) versus \(O_2/CO\) was observed (Fig. 3), indicating that the above assumption is valid. Thus, using the independently determined value of \(l' (2.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1})\), both \(k\) and \(k'\) can be determined from this set of data. The value for the oxygen combination rate constant is found to be \(k' = 6.5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}\). From a parallel set of experiments on the PMB derivative, a value of \(k' = 8.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}\) was determined. Direct observations of the oxygen combination immediately after CO photodissociation yield a value of \(k'\) of approximately \(10^{10} \text{ M}^{-1} \text{ s}^{-1}\). Taking into account the errors due to the high velocity of the process, the agreement between the values reported above may be considered acceptable. The dissociation rate constants for O2 have been determined from
the intercept, as reported above (see Fig. 3). As shown in Table II, the values differ by one order of magnitude for the \( \beta^{\text{N}} \) (33.8 s\(^{-1}\)) and the \( \beta^{\text{PM}} \) (470 s\(^{-1}\)). This finding shows: (a) that the \( \beta \) dissociation rate constants for the abnormal \( \beta^{\text{PM}} \) chains are significantly higher than the corresponding values of normal \( \beta \) chains and (b) that the effect of PMB, which is known to speed up the process in the normal chains (6), is observed also for the \( \beta^{\text{PM}} \) chains.

**Properties of Fe-Co Hybrids**—In order to follow the kinetic and spectral properties of the abnormal \( \beta^{\text{PM}} \) chains in an assembled molecule without interferences from the normal \( \alpha \) chains, we have prepared a reconstituted hybrid containing cobalt instead of iron on the \( \alpha \) chains (Coo,\( \alpha(\text{Fe}^{\text{PM}}) \)). Since the cobalt-containing porphyrins do not react with carbon monoxide (11), it is possible to follow the reaction of this ligand exclusively with the \( \beta^{\text{PM}} \) chains in the tetramer.

**TABLE I**

Millimolar extinction coefficients and absorption maxima (in parentheses) of normal and abnormal isolated \( \beta \) chains at pH 7.4, 0.2 M \( p \), 20°C and normal cy chains, CM

<table>
<thead>
<tr>
<th>( \lambda (\text{nm}) )</th>
<th>( \beta^{\text{N}} )</th>
<th>( \beta^{\text{PM}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxy</td>
<td>113 (430)</td>
<td>130 (424)</td>
</tr>
<tr>
<td>Oxy</td>
<td>125 (415)</td>
<td>130 (421)</td>
</tr>
<tr>
<td>Carbonmonoxy</td>
<td>193 (419)</td>
<td>176 (422.5)</td>
</tr>
</tbody>
</table>

\( ^a \) Values extracted from Ref. 6.

\( ^b \) Value extracted from Ref. 4.

**TABLE II**

Rate constants for association and dissociation of \( \text{O}_2 \) and \( \text{CO} \) to normal and abnormal isolated chains in the 

Data relative to normal chains are taken from Ref. 6.

<table>
<thead>
<tr>
<th>( \text{O}_2 )</th>
<th>( \text{CO} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k' )</td>
<td>( k )</td>
</tr>
<tr>
<td>( \text{Normal} )</td>
<td>( \text{PMB} )</td>
</tr>
<tr>
<td>( \text{O}_2 \text{H} )</td>
<td>( 5 \times 10^2 )</td>
</tr>
<tr>
<td>( \text{O}_2 \text{SH} )</td>
<td>( 5.5 \times 10^2 )</td>
</tr>
<tr>
<td>( \beta^{\text{PM}} )</td>
<td>( (6.5 \pm 2) \times 10^2 )</td>
</tr>
<tr>
<td>( \beta^{\text{PM}} \text{H} )</td>
<td>( (8.2 \pm 2) \times 10^2 )</td>
</tr>
</tbody>
</table>

Possible complications arising from the presence of dimers was reduced by the use of high protein concentrations (70 \( \mu \text{M} \) in terms of sites). At this protein concentration, dissociation into dimers should not exceed \( -30\% \), assuming that the dissociation constant measured for HbZH-CO (12) applies to the hybrid. In the presence of dithionite, the time course for CO recombination to the Fe\( ^{\text{III}} \) sites in the hybrid molecule conforms to a single process, with a second order rate constant of \( 1.7 \times 10^7 \text{ M}^{-1} \text{s}^{-1} \). This is in very good agreement with the value previously reported for the \( \beta^{\text{PM}} \) chains in a tetrameric molecule in the T quaternary state (4). This finding has two implications in so far as 1) it confirms the mechanism of sequential binding of CO which we have previously proposed for HbZH, whose central feature is represented by a very high reactivity of the abnormal \( \beta^{\text{PM}} \) chains even in a T state molecule, and 2) it suggests that the tertiary state of the iron-containing mutant \( \beta^{\text{PM}} \) chains in the hybrid molecule is similar to that of the same subunits in the HbZH tetramer in T, excluding any dramatic changes due to the presence of cobaltoporphyrin on the partner \( \alpha \) chains.

Fig. 4 reports the kinetic difference spectrum obtained by
flashing a solution of the Fe-Co hybrid saturated with CO in the presence of dithionite. The positions of the maxima and of the isosbestic points are identical with those of the isolated $\beta^{AB}$ chains (Fig. 1) although $\sim70\%$ of the molecules are in the tetrameric form under these conditions. This finding confirms that the same difference spectrum deoxy-CO is associated with the abnormal chains, whether isolated or in the tetramer (4).

The difference spectrum oxy-carboxy Fe $\beta^{AB}$ in the hybrid molecule has been studied by flashing a solution of the protein saturated with CO in the presence of suitable concentrations of oxygen. The experiment may be schematized as follows:

\[
\text{Co-O}_2 \xrightarrow{h\nu} \text{CoO}_2 \xrightarrow{O_2} \text{Co-O}_2 \xrightarrow{CO} \text{CoO}_2 \xrightarrow{O_2} \text{CoO}_2
\]

(1) (II) (III) (I)

Since the reaction between (II) $\rightarrow$ (III) is too fast at the O$_2$ concentrations used (from 1360 to 25 $\mu$M), only the replacement indicated by the step (III) $\rightarrow$ (I) could be followed directly. The half-time for the replacement of O$_2$ by CO on the Fe $\beta^{AB}$ chains becomes larger as the O$_2$ concentration increases. At the lowest concentrations of oxygen (stoichiometric with the Fe$^{2+}$ sites), only the Fe $\beta^{AB}$ sites in species (III) were occupied by O$_2$, the Coa subunits being ligand-free due to their much lower affinity; on the other hand, at the highest concentration, all the sites were largely saturated by O$_2$ before the displacement. We have therefore compared the kinetic difference spectrum for the exchange of O$_2$ by CO on the Fe $\beta^{AB}$ chains under conditions where the Coa chains were either ligated or unligated. As shown in Fig. 4, the two difference spectra are equal within the experimental errors. This finding indicates that the difference spectrum oxy-carboxy for the $\beta^{AB}$ chains is insensitive to the state of ligation of the partner chains in the tetramer. Moreover, the overall shape of this kinetic difference spectrum and the position of the isosbestic points are equal to those obtained for the isolated abnormal $\beta^{AB}$ chains for the same process (Fig. 1).

Therefore, from the data in Fig. 4, it is easy to calculate the deoxy-oxy difference spectrum for the Fe $\beta^{AB}$ chains in the hybrid molecule. This spectrum, also shown in Fig. 4, is identical with the same spectrum reported in Fig. 1 for the isolated $\beta^{AB}$ chains.

**Native HbZH Tetramer**—The kinetic difference spectrum deoxy-oxy for native tetrameric HbZH was determined at two saturations (100% and 25%) by the "O$_2$ pulse method." This approach, described by Gibson (13), is based on mixing a solution of the protein containing dithionite with a buffer containing oxygen. If the concentrations of dithionite and oxygen are properly adjusted, the initial reaction is represented by binding of O$_2$ to the protein; the ligand thereafter dissociates since its free concentration is kept to zero by the excess dithionite. This method allows the investigation of spectral and kinetic properties of intermediates, as shown by Gibson for HbA (13). In addition, since the starting material is deoxy-Hb, dissociation into dimers can be neglected at every protein concentration.

Under appropriate conditions, O$_2$ dissociation can be measured from a solution being fully or partially saturated with this ligand. The data in Fig. 5 show that the same kinetic difference spectrum may be calculated from dissociation experiments with fully and partially ($Y = 0.25$) saturated HbZH.

From the data in Fig. 5, the difference spectrum of deoxy-oxy $\beta^{2H}$ in the HbZH tetramer may be computed. The contribution of the a chains to the difference spectrum was estimated from a corresponding set of data obtained with HbA (curve A, Fig. 5) on the assumption that both types of chains contribute equally to the difference spectrum (which is obviously valid only to a first approximation). The overall shape of the deoxy-oxy difference spectrum calculated for the $\beta^{2H}$ chains (curve B, Fig. 5) is similar in shape to those characteristic of the isolated $\beta^{AB}$ chains (Fig. 1) and of the $\beta^{2H}$ chains in the hybrid (Fig. 4). Thus, we feel that, given the assumptions involved in the calculation, the spectral contribution of the abnormal $\beta^{2H}$ chains in the HbZH tetramer should not be significantly different from that of the isolated chains.

In a static experiment, we have measured, over the whole saturation range, the spectra of HbZH using a thin film apparatus according to Gill and co-workers (10). This experiment, which allows us to follow achievement of equilibrium at any O$_2$ partial pressure from pure O$_2$ downwards, was performed at high protein concentration ($\sim70 \mu$M in terms of sites). Moreover, since the experiment was carried out in the presence of inositol hexaphosphate (0.4 mM), the bottom asymptote in the binding isotherm extends up to $Y \sim 0.3$ (14), i.e. over a saturation range which is easily accessible with the thin film apparatus (10). The isosbestic points identified in going from oxy- to deoxy-HbZH, which are located in the Soret region at $\lambda = 447.5$, 321.5 and 388 nm, did not indicate...
an obvious shift over the whole saturation range. If, similarly to what was reported for CO (4), the binding of O₂ to HbZH was strictly sequential among the β⁡H chains and the α chains, we would have expected a shift in isosbestic points along the binding curve of about 6 nm around 422 nm (see Fig. 4).

As elucidated by the paper of Tucker et al. (5) on the crystallography of carboxy-HbZH, Arg E7 in the β⁡H chains binds to the proximate of the heme ring, leaving a large gap inside the heme pocket and a more open entrance for the ligand to approach the binding site. Moreover, the porphyrin is distorted, and its orientation becomes more similar to that characteristic of the R state.

The spectral properties of the β⁡H chains are dramatically modified compared to those of β⁡A and α (see Fig. 2 and Table I). This shows that the presence of a “distal” arginine and the small rotation of the heme around its perpendicular axis reported for HbZH-CO (5) not only modify the intrinsic reactivity of the heme iron, but also have a major effect on the absorption bands of the porphyrin ring. The differences both in absorption maxima and extinction coefficients (shown in Fig. 2 and Table I) are such that a possible spectral change in going from isolated chains to the αβ⁡H tetramer is probably negligible at this level of resolution. Moreover, it is now proven that, to a first approximation, the abnormal spectral properties of the β⁡H chains are maintained in the assembled molecule, whether the partner chains contain Fe- or Co-porphyrin.

The crystallographic data fit very well the ideas beneath the kinetic model previously reported for the binding of carbon monoxide (4), from which it was concluded that the main effect of the distal substitution in HbZH was experienced by the T state of the β subunits.

Inspection of the results reported in Table II shows that, in the isolated chains, the rate constant for the binding of CO to β⁡H is increased by about 1 order of magnitude compared to normal chains, whereas for O₂, the rate is similar for β⁡A and β⁡H. Since for the isolated chains all the derivatives (deoxy, oxy, carboxy) are in a structural state analogous to the R structure (6), this result shows that the steric advantage of a more open hydrophobic pocket is greater for CO than for O₂. On the other hand, the fact that the O₂ dissociation rate constant is increased by approximately a factor of 2 shows that the alteration of the binding site has a definite effect also on this ligand, and thus suggests that the lack of an effect on the “on” constant may be due at least partially, to diffusion control.

In a laser-photolysis study at low temperature on the O₂-CO replacement reaction in myoglobin, Beece et al. (15) observed a different behavior for the two ligands which may be accounted for by a multibarrier model (16). In this model, the access of CO to the binding site is governed by four barriers, whereas for O₂ only three barriers separate the binding site from the solvent.

We think that abnormal β⁡H chains would be a particularly interesting system with respect to this approach as far as it could give information on the physical nature of the energy barriers.

A strong evidence that the modulating effect of the protein structure on the reactivity of the ferrous heme is still present in the abnormal isolated chains is given by the effect of PMB on the “off” constant for O₂. Despite the difference in the value of k between normal and abnormal β chains, in both cases k is increased by approximately 1 order of magnitude in the PMB derivatives (see Table II).

Speculating on the physical basis of the abnormal spectral properties of β⁡H chains, it may be noticed that the blue shift of ~6 nm for the absorption maximum of the deoxy form and the associated increase in the CO binding rate recall the behavior of model compounds (17) and that of sperm whale myoglobin in acid conditions (18), for which a tetracordinate deoxy intermediate, correlated to the protonation of the proximal imidazole, has been suggested. However, the deoxy spectral properties may be consistent also with an hemochrome type spectrum; moreover, the red shift of the liganded derivatives is of difficult interpretation. Therefore, at this stage, we refrain from providing a definite structural interpretation of the spectra.

The new spectral and kinetic experiments on the Co-Fe hybrids are in total support of the mechanism for binding of CO to HbZH which we proposed previously on the basis of more limited data (4). It is confirmed unequivocally that the abnormal β⁡H chains in a tetramer having a T quaternary structure display a reactivity toward CO very much greater than that characteristic of either normal chains in HbA. This is the property which determines the complete sequentiality of CO binding to tetrameric HbZH.

The recently published binding curves for O₂ have shown that, in the presence of phosphates, the initial binding in HbZH corresponds to an affinity higher than that for HbA, the value of K₄ (first Adair constant) being 7.5 times higher in HbZH. Ikeda-Saito et al. (14) have proposed an interpretation of the binding data, based also on parallel results on the Co-Fe compounds, which envisages weaker subunit interactions in the low affinity state of HbZH. This interpretation is also based on the finding that different allosteric effectors change the position of the lower asymptote.

The same set of data, however, has been interpreted by Tucker et al. (5) along different lines. In particular, by analogy with the sequential binding proposed for CO (4), these authors have suggested that the lower asymptote of the binding curve represents preferential binding of O₂ to the abnormal β⁡H chains in HbZH, and therefore the higher asymptote corresponds largely to saturation of the normal α chains.

However, the results reported above exclude that, in the case of O₂, binding of the ligand is sequential as between the two types of chains. In particular, the presence of unique isosbestic points along the O₂ titration of HbZH and the finding that the spectrum of the β⁡H chains in the tetramer is different from that of the α chains support this conclusion. Thus, the presence of arginine on the distal side has different effects on O₂ and CO, and a priori assumptions of identical mechanisms for the binding of these two ligands may be misleading. The observation reported by Tucker et al. (5) that the CO/O₂ partition coefficient is strongly saturation-dependent for HbZH is obviously consistent with the difference for the two ligands reported in this paper.

The finding (14) that deoxy-HbZH in a T quaternary state has an intrinsic affinity higher than that of HbA in T must, of course, be dependent on the structural alteration on the distal side. The result that the isolated β⁡H chains are characterized by an O₂ affinity lower than that of normal β chains is not in contradiction with the increased affinity of HbZH in T since the properties of the individual chains, when embedded in a deoxy tetramer, are strongly dependent on the subunit interactions. On the contrary, the low O₂ affinity of isolated β⁡H chains may partially account for the lower value of the fourth Adair’s constant (K₄) reported by Ikeda-Saito et al. (14) for HbZH.

A more detailed mechanistic interpretation in terms of rate constants for the individual chains in HbZH is not possible at present. Any superficial extrapolation is dangerous in view of the demonstration that, even in HbA, the kinetic behavior of the α and β chains in a T state molecule is different, since laser photolysis experiments (19) have shown two kinetic components with different “on” and “off” constants. This type of experiment may provide direct information on the dynamics...
of $O_2$ binding to HbZH necessary to extend the interpretation.

We may point out, however, that the increased reactivity of 
HbZH toward $O_2$ and CO cannot be associated to a zero spin 
configuration of the deoxygenated $\beta^{2+}$ chains since, as shown 
in the accompanying paper, all four chains in deoxy HbZH are high spin (20).

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