Studies on the Relations between Molecular and Functional Properties of Hemoglobin

VII. KINETIC EFFECTS OF THE REVERSIBLE DISSOCIATION OF HEMOGLOBIN INTO SINGLE CHAIN MOLECULES*

(Received for publication, October 2, 1967)

ERALDO ANTONINI, MAURIZIO BRUNORI, AND SONIA ANDERSON

From the Institute of Biochemistry, University of Rome, The Consiglio Nazionale delle Ricerche Center for Molecular Biology, Rome, and the "Regina Elena" Institute for Cancer Research, Rome, Italy

SUMMARY

The kinetics of the reactions of human hemoglobin with carbon monoxide and oxygen has been studied in photochemical and rapid mixing experiments over a large range of hemoglobin concentration.

When the reaction is initiated by rapid removal of the ligand from ligand-bound hemoglobin, the kinetics of the combination of hemoglobin with CO shows a marked concentration dependence in both the photochemical and the rapid mixing experiments. In dilute hemoglobin solutions (below $10^{-5}$ M in heme), dissociation of the ligand from oxyhemoglobin or carbonmonoxyhemoglobin is followed by slow changes (half-time of the order of seconds) in the properties of the system.

These results lead to the following picture, which is also consistent with other as yet unexplained aspects of hemoglobin kinetics. (a) Ligand-bound hemoglobin dissociates reversibly into single chain molecules at concentrations below $10^{-5}$ M. (b) Deoxygenated hemoglobin has a much lower tendency to dissociate into single chain molecules, and there is no appreciable dissociation even at concentrations of the order of $10^{-4}$ to $10^{-3}$ M. (c) The association of deoxygenated $\alpha$ and $\beta$ chains is a relatively slow process. Therefore, after sudden dissociation of the ligand from dilute hemoglobin solutions, the properties of the system, for a brief time, are those of a mixture of deoxygenated hemoglobin and deoxygenated $\alpha$ and $\beta$ chains. (d) The properties of the single chain molecules obtained by dilution of ligand-bound hemoglobin are the same as those of isolated $\alpha$ and $\beta$ hemoglobin chains as obtained by preparative procedures.

Although the kinetics of hemoglobin reactions has been under active investigation for more than 40 years, in detail it still represents largely an unsolved problem (1-3).

In an attempt to correlate the functional behavior of hemoglobin with the subunit structure of the protein, studies of the kinetics of the reaction with ligands under conditions that promote dissociation into subunits have been recently undertaken (4, 5).

In the previous paper of this series (5), observations on the kinetic behavior of hemoglobin in concentrated salt solutions have been reported. Under these conditions, which promote dissociation of the $\alpha_2\beta_2$ tetramer into $\alpha\beta$ dimers, there is no change in the kinetics associated with the splitting of the molecule; thus it had been concluded that in hemoglobin the $\alpha\beta$ dimer is the relevant unit in the kinetics of the reaction with ligands.

The kinetic studies have also been extended to conditions that presumably favor dissociation into single chain molecules. Previous observations on the kinetic behavior of dilute hemoglobin solutions after flash photolysis (4) were shown to be fully consistent with the assumption of dissociation of ligand-bound hemoglobin but not of deoxyhemoglobin into single chain molecules. Investigations on the kinetic behavior of dilute hemoglobin solutions both in rapid mixing and in photochemical experiments have now been extended and are reported in the present paper. The results provide further evidence that ligand-bound hemoglobin dissociates into single chain molecules. It is shown that the different tendency of ligand-bound hemoglobin and deoxyhemoglobin to dissociate into single chain molecules may explain a number of peculiar effects seen in hemoglobin kinetics and in turn leads to predictions of the behavior of the system that have been verified by experiment.

MATERIALS AND METHODS

Human hemoglobin was prepared from freshly drawn blood according to the toluene procedure (6). The final oxyhemoglobin solutions (3 to 7% concentration) were stored in the cold and used within 10 days.
The hemoglobin α and β chains were prepared according to Bucci and Fronticelli (7) and Bucci et al. (8). Bound hydroxymercuribenzoate was removed with dodecanethiol by the procedure of De Renzo et al. (9). Hemoglobin and chains concentrations are given in heme equivalent.

Other reagents were commercial products of analytical grade. Gases were obtained from S.I.O. (Rome).

Spectrophotometric measurements were carried out with a Cary model 14 recording spectrophotometer. Stopped flow experiments were made with a Gibson or a Gibson-Durrum apparatus.

Flash photolysis experiments were performed with the apparatus described before (5); the same basic apparatus was also used for experiments in which the sample was kept photodissociated for different lengths of time. In this case the flash lamps were turned off, and a 250-watt tungsten lamp (operated with alternating current) was placed on the top of the magnesium oxide-coated chamber containing the cell. With the relatively small light intensities supplied by this lamp, very small CO concentrations were needed to obtain almost complete photodissociation of the carboxyhemoglobin. Under these conditions even the monitoring light of the flash photolysis apparatus produced some photodissociation; this was made negligible by proper filtering of the monitoring light before it reached the cell. Temperature control in the photochemical experiments was achieved by immersion of the cell until immediately before the experiments in a constant temperature bath.

All of the other procedures and the preparations of the reagent solutions were the same as described in previous papers (4, 5, 11, 12).

RESULTS

Photochemical Experiments

As mentioned in the introduction, the kinetic behavior on flash photolysis of dilute HbCO solutions may be explained on the basis that the ligand-bound derivatives of hemoglobin undergo a reversible dissociation into single chain molecules of the type:

\[
\alpha X + \beta X \xrightarrow{k_1 \text{ to } k_2} \text{(αβ)2}
\]  

(1)

The values of the constants involved were estimated to be, at pH 7 and room temperature, \(k_1 = 2.2 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}\); \(k_2 = 0.49 \text{ sec}^{-1}\) (4).

On the other hand, kinetic evidence indicates that in deoxyhemoglobin the equilibrium is much more in favor of the dimer, so that no appreciable dissociation into single chains occurs with this derivative within the accessible concentration range (4, 5).

Thus, with dilute hemoglobin solutions (<10^{-5} M), the kinetics of recombination of CO after flash photolysis of the CO derivative is biphasic, unlike the kinetics of combination with CO in rapid mixing experiments. The biphasic recombination is explained, on the basis of the equilibrium shown in Scheme 1, by the following reactions, which take place on flash photolysis.

Accordingly, the “slow” rate corresponds to that observed in rapid mixing experiments on the reaction of deoxyhemoglobin with CO (\(l' \sim 10^5 \text{ sec}^{-1}\)) (1); the “fast” rate corresponds to that measured directly with isolated hemoglobin chains (\(l' \sim 4 \times 10^5 \text{ sec}^{-1}\)) (11, 12). The amount of fast reacting material increases with dilution of the protein.

The kinetic behavior of hemoglobin on flash photolysis, as a function of hemoglobin concentration, is illustrated by the experiment reported in Fig. 1. The reader is referred to other papers (1, 4, 5, 13) for a discussion of the situation at high hemoglobin concentration, particularly with respect to the effect of pH, the presence of concentrated salts, and the profile of the photolytic flash. The main purpose of the experiment shown in Fig. 1, in connection with the present paper, is to show that the “dilution effect” on the behavior of hemoglobin is similar at pH 7 and 9, in the presence and absence of concentrated salts, and with the two types of photolytic flashes.

In all of the work described above, the photodissociation of the hemoglobin is obtained by flashes lasting less than 1 msec. If photodissociation of a dilute CO hemoglobin solution is not carried out by a brief photolytic flash but is maintained for different lengths of time by prolonged illumination, the results shown in figure 2 are obtained. The most striking effect which now appears is that the kinetics of the recombination with CO in the dark varies with the time of illumination of the sample. These kinetic curves can be analyzed in terms of two components, one reacting rapidly, the other slowly, with CO, if the fraction of optical density change taking place rapidly is plotted against the time of illumination, the results shown in Fig. 3 are obtained. At a hemoglobin concentration of 2.9 \(\times 10^{-4}\) M the half-time for the change shown in the figure is about 2 sec.

These results can be explained by assuming that the following reactions take place during illumination:

\[
\text{(αβ)2} \xrightarrow{h\nu} \text{(αβ)} + 2X
\]

\[
\alpha X \xrightarrow{h\nu} \alpha + X
\]

\[
\beta X \xrightarrow{h\nu} \beta + X
\]

\[
\alpha + \beta \xrightarrow{k_3} \text{(αβ)}
\]

In other words, the explanation is that in the light the single chain molecules associate to form slowly reacting αβ dimers; the time course of the change in kinetics shown in Fig. 3 allows an estimate of \(k_3\).
Fig. 1. Effect of hemoglobin concentration, pH, ionic strength, and flash profile on the kinetics of CO combination after flash photolysis. The ordinates give the optical density change in arbitrary units. ◆, data obtained with "brief" flash (100 joules; duration to 10% peak ~100 μsec); ○, data obtained with "long" flash (300 joules; duration to 10% peak ~300 μsec); □, the same data have been obtained with both flashes. The CO concentration is 1 × 10^{-4} M; λ is 435 μm; the temperature is 20°.

Fig. 2. Tracing of oscilloscope records showing changes occurring on illumination of dilute HbCO solutions. Arrows indicate beginning and end of illumination (see the text). The record in the top panel is obtained with a brief photolytic flash (300 joules; duration to 10% peak ~300 μsec). Hb concentration, 2.95 × 10^{-7} M; CO concentration, 6.2 × 10^{-3} M; 0.1 M phosphate buffer, pH 7.0; temperature, 21°. Degree of saturation of hemoglobin with CO in the dark, 0.92; light path, 10 cm.

Also, other features of the curves shown in Fig. 2 are consistent with the presence of single chain molecules in dilute CO hemoglobin solutions. Thus, on shining the light, the kinetics of the approach to the steady state is biphasic, part of the optical density change occurring rapidly, part slowly. This may be accounted for mainly by the fact that the rates of approach to the steady state are different for hemoglobin and the isolated hemoglobin chains.

Other sets of experiments similar to that shown in Fig. 2, which were carried out under different conditions, notably different hemoglobin and CO concentrations, gave results consistent with the general picture outlined above. Thus at a lower hemoglobin concentration (5.6 × 10^{-4} M) than in the experiment

2 The kinetics of approach to the steady state in the light and in particular the heterogeneity varied, at constant hemoglobin concentration, with the CO concentration. This too is not inconsistent with the schemes proposed. The biphasic approach to the steady state in the light was present also when the reaction was followed at different wave lengths in the Soret zone, notably at a wave length where deoxyhemoglobin and the isolated deoxygenated chains are isosbestic. Thus the small increase in optical density associated with the combination of α and β chains at 430 μm (14) can be only in small part responsible for it.
of Fig. 2 and at a similar CO concentration \((6.8 \times 10^{-7} \text{ M})\),
the fraction of rapidly reacting material initially present was
greater (>90%) and the half-time for the change in the kinetics
of recombination on prolonged illumination was longer, about
5 sec.

Control experiments for the results described above were
carried out with myoglobin; in this case the kinetics of photo-
dissociation and recombination corresponded to simple
approaches to equilibrium, and there was no effect on varying the
length of illumination of the sample.

**Rapid Mixing Experiments**

**Kinetics of CO Combination with Freshly Reduced Hemoglobin**—If oxyhemoglobin is partially dissociated into single
chain molecules, the following reactions should take place when
the solution is rapidly deoxygenated by mixing with sodium
dithionite.

\[
(\alpha\beta)(O_2) \rightarrow \alpha + \beta + 2O_2
\]

\[
\alpha O_2 \rightarrow \alpha + O_2
\]

\[
\beta O_2 \rightarrow \beta + O_2
\]

\[
\alpha + \beta \rightarrow (\alpha\beta)
\]

Deoxygenation of hemoglobin and hemoglobin chains in the
presence of dithionite is a rapid process (owing to the high
values, \(-10\) to \(50\) sec\(^{-1}\), of the \(O_2\) dissociation velocity constants)
in comparison with the association of \(\alpha\) and \(\beta\) chains (12, 14).

Thus, just after deoxygenation of a dilute \(HbO_2\) solution, a
fraction of the material should be present as deoxygenated \(\alpha\)
and \(\beta\) chain molecules. If the system is now allowed to react
with CO, it should show the presence of species reacting with
CO at a rate much higher than that for hemoglobin and similar
to that for the reaction with CO of isolated hemoglobin chains.
The situation has obvious analogies with the rapid photodis-
sociation of dilute HbCO solutions.

It was already noticed a long time ago by Roughton (15) that
"freshly reduced hemoglobin" shows a biphasic kinetics on
reaction with CO, with an initial phase more rapid than the
ordinary rate found for "aged" hemoglobin. The effect has
been confirmed in the experiments described below, the results
of which correspond to the expectations outlined in Scheme 4.
The experiments are carried out as follows. An oxyhemoglobin
solution in 0.1 M phosphate buffer at neutral pH is mixed in
the stopped flow apparatus with a solution containing dithionite
(\(\sim 0.2\%\)) and carbon monoxide, the concentration of CO being
kept as low as possible. The experiment is followed at wave
lengths between 450 and 440 nm, where oxyhemoglobin and
CO hemoglobin have a lower extinction than deoxyhemoglobin.

On mixing the two solutions, deoxygenated hemoglobin forms
rapidly, and then combines with CO. The two processes
naturally overlap to some extent, but the conditions may be
adjusted so that they occur largely in sequence. The results of a
typical experiment of this kind are shown in Fig. 4. If \(HbO_2\)
is mixed with the same dithionite solution used in the experiments
just described sometime (several seconds) before allowing it to
react with CO, the rapid phase in the reaction with CO disap-
pears. Fig. 4 also shows the behavior on flash photolysis of the
\(HbCO\) solution obtained at the end of the rapid mixing experiments.

It is clear that both the relative amount of fast and
slow components and the rates of reaction with CO are similar
in the flash photolysis and in the rapid mixing experiment start-
ing with \(HbO_2\). Fig. 5 gives the results of other experiments
performed at different hemoglobin concentrations, other con-
ditions being the same: it is clear that the fraction of the rapid
phase in the combination with CO increases with decrease in pro-
tein concentration, just as should be expected for a phenomenon
involving dissociation. It should also be pointed out that the
I should expect that the process $\alpha + \beta \rightarrow k_3 (\alpha \beta)$ in Scheme 4 would show up as a slow increase in optical density at 430 nm following the rapid deoxygenation of the system. Fig. 6 shows the slow optical density change, lasting a few seconds, occurring after mixing a dilute HbO$_2$ solution with dithionite.

The nature of this change may be further identified by following it at different wavelengths. As is evident from Fig. 7, the kinetic difference spectrum so obtained corresponds to the static or kinetic difference spectrum obtained on mixing the isolated hemoglobin $\alpha$ and $\beta$ chains. In addition, the fraction of optical density change taking place slowly increases with a decrease in protein concentrations (Table I); its size corresponds to the expectations from the degree of dissociation into single chains estimated from other experiments and the differences in extinction between deoxyhemoglobin and the hemoglobin chains.

In view of the reported side effects of dithionite (1, 16, 17),

---

**Fig. 5.** Percentage of "rapidly reacting hemoglobin" when oxyhemoglobin is mixed with a solution of dithionite containing CO (see Fig. 4) as a function of protein concentration. The continuous line is a theoretical curve calculated for the equilibrium $a\beta \leftrightarrow a + \beta$ with $K = 1 \times 10^{-4} M$ on the assumption that the dimers react slowly and the monomers rapidly with CO. The abscissae give hemoglobin concentration before mixing since the approach to the equilibrium for the association-dissociation process in ligand-bound hemoglobin is a relatively slow process (4). CO concentration, $1 \times 10^{-4} M$ (before mixing), 0.1 M phosphate buffer, pH 6.5; $\lambda$, 437 nm; temperature, 19°.

**Fig. 6.** Oscilloscope trace showing a slow increase in optical density at 430 nm following the rapid deoxygenation of dilute oxyhemoglobin solution in the presence of dithionite. The trace corresponds to Experiment 19 reported in Table I.

Relative amounts of fast and slow optical density changes in these experiments vary slightly with wavelength, in agreement with the difference in extinction in the Soret zone between deoxy- genated hemoglobin and hemoglobin chains (11, 14).

**Slow Changes in the Soret Region Following Deoxygenation of HbO$_2$**—On the basis of the reactions described in Scheme 4, another effect can be predicted to occur on rapid deoxygenation of a dilute HbO$_2$ solution. The isolated hemoglobin chains have, in the deoxy form, spectral properties in the Soret region significantly different from those of deoxygenated $a\beta$ dimers (11, 14); in particular, the extinction coefficient at 430 nm is about 15% lower for the isolated chains than for hemoglobin. Thus one

---

**Table I**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Hb concentration (before mixing)</th>
<th>$\Delta$O.D. total$^a$</th>
<th>$\Delta$O.D. slow$^b$</th>
<th>$\Delta$O.D. total ( \times 300 )</th>
<th>$% \times 100$</th>
<th>$t^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>$2.6 \times 10^{-4} M$</td>
<td>0.200</td>
<td>0.012</td>
<td>6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>$1.3 \times 10^{-4} M$</td>
<td>0.104</td>
<td>0.009</td>
<td>8.5</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>32-34</td>
<td>$0.65 \times 10^{-4} M$</td>
<td>0.047</td>
<td>0.006</td>
<td>12</td>
<td>3.7</td>
<td></td>
</tr>
</tbody>
</table>

*a* Total optical density change between time 0 and ~1 min.

*b* Optical density change taking place "slowly" (see Fig. 6).

*c* Half-time for the "slow" optical density change.
which might cast doubt on the meaning of the experiments just reported, several controls were carried out with isolated \( \alpha \) and \( \beta \) chains. When the oxygenated derivatives of the isolated chains were mixed with dithionite under the same conditions used for hemoglobin, there was no sign of slow optical density changes in the Soret zone, following the main reaction, in the time range up to several seconds.

The rates for the slow change in optical density following deoxygenation of dilute \( \text{HbO}_2 \) at different hemoglobin concentrations are given in Table I. They are similar to those measured directly by mixing isolated deoxy \( \alpha \) and \( \beta \) chains, and therefore give further support to the proposed interpretation of the effect.

**Kinetics of Dissociation of Oxygen from Oxyhemoglobin**—The dissociation of oxyhemoglobin into single chain molecules at low protein concentration should also be reflected in a change in the kinetics of oxygen dissociation with protein concentration. This between pH 6 and 7 the rate of dissociation of oxygen from oxyhemoglobin at high protein concentration is higher (2- to 3-fold) than that for the isolated hemoglobin chains (1, 12).

Fig. 8 shows the time course of oxygen dissociation measured by mixing oxyhemoglobin with dithionite, at a protein concentration from \( 5 \times 10^{-4} \) M to \( 1.3 \times 10^{-6} \) M. The two curves differ in the expected direction: at low protein concentration there is clear evidence of kinetic heterogeneity, the reaction tending to slow down as it proceeds. Although this effect is less straightforward than the others reported in the previous sections, it is nevertheless entirely consistent with the proposed picture.

Also in this case, controls were carried out with isolated hemoglobin chains; with these, no significant change in the kinetics of oxygen dissociation was observed with protein concentration.

**DISCUSSION**

This paper presents a number of old and new observations on hemoglobin kinetics which may all be reconciled and explained on the basis of association-dissociation processes, involving single chain molecules, which occur in dilute hemoglobin solutions. The structural origin of the effects in terms of a dissociation process is clearly shown by their dependence on protein concentration: they become more and more evident below \( 10^{-5} \) M hemoglobin, a protein concentration range in which, for technical reasons, experiments on hemoglobin kinetics are often done. This conclusion is in agreement with recent ultracentrifuge experiments (18) and with other reports (19, 20), which indicate that hemoglobin may dissociate into monomers in very dilute solutions.

The behavior of hemoglobin in all the kinetic experiments is consistent with the following picture.

1. **Ligand-bound** hemoglobin tends to dissociate into single chain molecules because of reversible equilibria of the type

\[ (\alpha \beta) X \rightleftharpoons \alpha X + \beta X \]

Dissociation starts to be large at concentrations below \( 10^{-5} \) M in heme. Dissociation of ligand-bound hemoglobin is independent of the nature of the ligand, carbon monoxide and oxyhemoglobin being the same in this respect.

2. Deoxygenated hemoglobin has a much lower tendency than ligand-bound hemoglobin to dissociate into single chain molecules, and there is no appreciable dissociation even at concentrations of the order of \( 10^{-6} \) to \( 10^{-7} \) M in heme. When ligand-bound hemoglobin loses its ligand (by photodissociation of the CO compound or by mixing of the oxygenated form with dithionite), the single chain molecules associate to form \((\alpha \beta)\) dimers.

3. The association of deoxygenated \( \alpha \) and \( \beta \) chains is a relatively slow process, so that after sudden dissociation of the ligand from dilute hemoglobin solutions, the properties of the system, for a brief time, are those of a mixture of deoxygenated hemoglobin and deoxygenated \( \alpha \) and \( \beta \) chains.

4. The properties of the single chain molecules obtained by dilution of ligand-bound hemoglobin are the same as those of isolated \( \alpha \) and \( \beta \) chains obtained by the hydroxymercuribenzoate procedure (7). This concerns the rates of reaction with ligands (11, 12), the spectral properties (11), and the speed of association to form hemoglobin (14).

It is tempting to try to calculate from the kinetic experi-

\(^3\) Description of the dissociation equilibrium in the simple terms \((\alpha \beta) \rightleftharpoons \alpha + \beta \) is probably an oversimplification since the isolated chains themselves, notably the \( \beta \) chains, have a strong tendency to associate (8). For this reason it is desirable in the text to speak of single chain molecules instead of monomeric single chains. The situation is further complicated by the presence of the other dissociation-association equilibrium \((\alpha \beta) \rightleftharpoons 2(\alpha \beta)\), which has been discussed elsewhere (5). Finally, the combination of deoxy \( \alpha \) and \( \beta \) chains is kinetically complex and cannot be described as a simple bimolecular process (14).
ments equilibrium and kinetic constants corresponding to the association-dissociation processes into single chains in ligand-bound hemoglobin and deoxyhemoglobin. Although in principle this might be done, actually, since most of the information is admittedly semiquantitative and it is not clear whether the association-dissociation can be described in the simple terms of a single chain molecule, only a rough estimate of the size of the constants is justified. Another difficulty in this respect arises from the unexplained variability that is often observed in the "dilution effect" in flash photolysis experiments with different hemoglobin samples. This has been reported before (5, 13) and might appear also in comparing the amount of rapid phase in what lower, value was obtained in studies of the reaction of the isolated hemoglobin chains (14).

The rate of association of the deoxygenated chains, which can be deduced by the experiments shown in Figs. 2 and 6, corresponds to a velocity constant, assuming a simple bimolecular reaction, of the order of $10^4$ m$^{-1}$ sec$^{-1}$. A similar, although somewhat lower, value was obtained in studies of the reaction of the isolated $\alpha$ and $\beta$ chains (14).

The specific aspects of the single experiments will now be discussed briefly. The results reported in Fig. 1 emphasize the distinction already discussed elsewhere (4, 5) between the "quickly reacting forms" on flash photolysis at high and low hemoglobin concentration. In particular, it may be noticed that at high hemoglobin concentration ($>10^{-5}$ M) the amount of rapid phase depends on the length of the photolytic flash and is larger at pH 9 than at pH 7; these differences tend to vanish on dilution. This pattern is similar in dilute buffer solutions and with added 2 M NaCl, confirming that the kinetic behavior of the $\alpha_2\beta_2$ tetramer and of the $\alpha\beta$ dimer is the same (5).

The experiments just discussed serve as an introduction to those in which photodissociation is produced by light of constant intensity but varying duration. It has already been pointed out that even in this more complex case the behavior of the system is entirely consistent with the proposed picture involving association-dissociation into single chains. It may also be noted that these experiments give further evidence that the peculiar kinetic behavior of dilute hemoglobin after flash photolysis is not due to photochemical side effects and that the only noticeable effect of the light is to remove the bound ligand since after prolonged illumination the recombination with CO is similar to that observed in rapid mixing experiments.

A brief comment is required on the use of dithionite in the rapid mixing experiments, in connection with possible side effects due to this reagent. Dalziel and O'Brien (16, 17) have made a careful study of the spectral changes occurring on treating oxyhemoglobin with dithionite after the rapid deoxygenation reaction, and the problem has also been discussed in connection with kinetic experiments involving the use of this reagent (1). It has been concluded that dithionite must be used with great care in hemoglobin studies, although clear side effects are evident only under special conditions. The present experiments, on the other hand, provide evidence that the spectral changes occurring within a few seconds after mixing oxyhemoglobin with dithionite are real kinetic features of the system, independent of chemical side effects of the reagent. Evidence for this is given by the absence of the slow optical density changes in the controls carried out with isolated hemoglobin chains and by the consistency of the size and velocity of the slow changes with the model proposed.

In conclusion, the results reported in the present paper show a clear correlation of the kinetic behavior of hemoglobin with association-dissociation into single chain molecules. In cases like this, where the degree of dissociation of the protein depends on the presence of the ligand, a number of peculiar kinetic effects will appear; the extent to which they will be evident, however, will depend on the way the experiments are carried out and on the relative size of the rates for the association-dissociation processes and for the reactions with ligands. In this respect the case of hemoglobin might be taken as an example of a situation that is likely to occur in other proteins and enzymes.

Acknowledgments—We wish to thank Professor Q. H. Gibson and our colleagues in Rome for helpful discussions. We also thank Mr. Mario Sanchioni and Mr. Saverio Condó for skillful technical help.

REFERENCES
Studies on the Relations between Molecular and Functional Properties of Hemoglobin: VII. KINETIC EFFECTS OF THE REVERSIBLE DISSOCIATION OF HEMOGLOBIN INTO SINGLE CHAIN MOLECULES
Eraldo Antonini, Maurizio Brunori and Sonia Anderson


Access the most updated version of this article at [http://www.jbc.org/content/243/8/1816](http://www.jbc.org/content/243/8/1816)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/243/8/1816.full.html#ref-list-1](http://www.jbc.org/content/243/8/1816.full.html#ref-list-1)