Properties of the Product of Partial Photodissociation of Carbon Monoxide Hemoglobin

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Eraldo Antonini, Naomi Margaret Anderson, and Maurizio Brunori

From the C.N.R. Center for Molecular Biology and Institute of Biochemistry, University of Rome and Regina Elena Institute, Rome, Italy

SUMMARY

Some properties of the quickly reacting hemoglobin generated by partial photodissociation of human CO hemoglobin have been studied using flash photolysis methods. The absorption spectrum of the rapidly reacting photoproduct is different from that of slowly reacting "normal" hemoglobin and, similarly to other quickly reacting forms, corresponds to that of isolated deoxyhemoglobin chains. The fraction of the fast reacting material increases linearly with the fraction of nonphotodissociated hemoglobin.

On the basis of these results and of other information on hemoglobin kinetics it is suggested that this fast reacting species represents partially saturated intermediate which appears transiently when the ligand is suddenly removed from ligand-bound hemoglobin.

The kinetic behavior of hemoglobin after flash photolysis of the ligand-bound derivatives is complex. Under many conditions the fraction of the unliganded product of photodissociation reacts with ligand at a rate much higher than that observed in rapid mixing experiments (1-3). This material was first observed by Gibeon (4) who termed it "quickly reacting hemoglobin."

It has now become clear that quickly reacting hemoglobin may originate under different sets of conditions which presumably involve different structural states. Three conditions produce large amounts of quickly reacting hemoglobin on rapid photodissociation: (a) photodissociation of CO hemoglobin at high concentrations (>10^{-5} M) and at pH 9 (4); (b) partial photodissociation of concentrated CO hemoglobin solutions at pH 7 (5); (c) photodissociation of CO hemoglobin in dilute solutions (<10^{-4} M) at pH 7 (6).

At pH 7 little quickly reacting material is obtained on complete photodissociation of CO hemoglobin at protein concentrations greater than 10^{-5} M. The present paper deals with the properties of the quickly reacting hemoglobin obtained when concentrated solutions of human hemoglobin at pH 7 are only partially photodissociated.

MATERIALS AND METHODS

Human hemoglobin was prepared by the procedure used routinely in this laboratory (7). The final solution was deionized by passage through a mixed bed ion exchange resin.

Flash photolysis experiments were performed with the apparatus described previously (8). In order to obtain photodissociation of only a fraction of the CO hemoglobin the amount of photolytic light was varied by screening the flash lamps with aluminum foil. The constancy of the light output under each screening condition was established by the reproducibility (5%) of the amount of CO hemoglobin photodissociated on repetitive flashes.

RESULTS

Spectral Properties of Quickly Reacting Hemoglobin Obtained on Partial Photodissociation—When the intensity of the photolytic light is reduced so that only a fraction of CO hemoglobin is photodissociated, recombination with CO in the dark is diphasic. The kinetic curves can be analyzed in terms of two simultaneous second order reactions which represent binding of CO to the quickly and slowly reacting materials. The second order rate constants (k') calculated from the dependence on CO concentration of the slow and fast rates are: 2 x 10^5 M^{-1} sec^{-1} for the slow and 4 x 10^4 M^{-1} sec^{-1} for the fast component, at pH 7 in phosphate buffer and 20°C.

Experiments were devised to see whether the quickly reacting material obtained on partial photodissociation has the same spectral properties as the other types of rapidly reacting hemoglobin (1, 2, 4) which, in turn, correspond to those of the isolated alpha and beta chains (9). The results in Fig. 1 show that, at a constant level of photodissociation, the fraction of optical density change due to the fast component varies with wave length. The continuous line was calculated on the assumption that, in the reaction with CO, the spectra of the fast and slow phases observed after partial photodissociation correspond to those of the isolated hemoglobin chains and of hemoglobin, respectively. The agreement between the calculated line and the experimental data is good, indicating that the spectral properties of the quickly reacting component obtained on partial photodissociation are indeed similar to those of the isolated chains. The relative amounts of the fast and slow components were computed from observations at 437 nm, the isosbestic wave length for deoxyhemoglobin and deoxy chains (9). On this basis, the percentage of photodissociation in the experiment shown in Fig. 1 was 55%. The wave length dependence of the fraction of fast and slow optical density change was in agreement with that reported in Fig. 1 also for other levels of photodissociation, although no systematic analysis of the data was carried out.

Distribution of Fast and Slow Components—The distribution of fast and slow components as a function of the degree of photodissociation has been investigated at different hemoglobin and CO concentrations. The range, however, was rather narrow due to overlap with other forms of quickly reacting hemoglobin. A linear relation was found between the fraction of quickly reacting hemoglobin and the fraction of nonphotodissociated CO hemoglobin (Fig. 2). This trend was observed under all conditions examined, including low protein concentrations in which a rela-
Partial Photodissociation of Carbon Monoxide Hemoglobin

Vol. 247, No. 1

FIG. 1. Dependence on wave length of the percentage of fast optical density change obtained on partial photodissociation. The points represent the experimentally determined fraction of fast optical density change from observations performed at different wave lengths; the photodissociating light intensity was maintained constant. The continuous line was calculated on the assumption that the spectrum of the deoxygenated derivative corresponds for the slow component to the spectrum of normal hemoglobin, and for the fast component to that of the isolated \(\alpha\) and \(\beta\) chains. The isosbestic wave length for the two types of deoxy spectra is at 437 nm where \(s = 60.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}\). Conditions: pH 7, 0.2 M phosphate buffer, 20°; total hemoglobin concentration = 5 \times 10^{-5} \text{ M} in heme; total CO concentration = 1 \times 10^{-5} \text{ M}.

FIG. 2. Relationship between the percentage of the quickly reacting component and the percentage of photodissociation. Conditions: pH 7, 0.2 M phosphate buffer, 20°; total hemoglobin concentration = 5 \times 10^{-5} \text{ M} in heme. Observation at \(\lambda = 437 \text{ nm}\). Different symbols indicate different experiments in which hemoglobin was either completely (O) or only partially (\(\bullet\,\Delta\)) saturated with CO.

relatively large fraction of hemoglobin reacted quickly with CO even on complete photodissociation.

Several experiments were performed with equilibrium mixtures of deoxyhemoglobin and CO hemoglobin, the total hemoglobin concentration being held constant. When the distribution of the fast and slow components was referred to the degree of photodissociation of the CO hemoglobin initially present, the relationship was the same as it is in fully saturated CO hemoglobin. Thus, in partial photodissociation experiments, the presence of unliganded hemoglobin does not alter the behavior of that fraction of hemoglobin which is initially present as CO hemoglobin.

In addition, when the equilibrium mixtures of deoxyhemoglobin and CO hemoglobin were allowed to react with CO in rapid mixing experiments, the combination with the ligand was found to be similar to that of fully unliganded hemoglobin and, in agreement with previous results by Legge and Roughton (10), no quickly reacting material was ever detected.

In contrast to the behavior of equilibrium mixtures, Gibson and Parkhurst (11) in flow-flash experiments found that the rapidly reacting hemoglobin appears, on partial photodissociation, with a marked lag in relation to the combination of hemoglobin with CO. Thus, at the same degree of saturation, the liganded species of hemoglobin present in the kinetic experiments and in the equilibrium mixtures behave differently with respect to photodissociation.

CONCLUSIONS

Measurements of the rate of reaction with ligands after photodissociation of a small fraction of a ligand-bound hemoglobin derivative provided the basis, in the framework of a simple four-stage Adair scheme, for the evaluation of \(k^4\), the velocity constant for the combination of the fourth heme with the ligand (1).

From these data and from results of rapid mixing experiments, the conclusion was drawn that in the combination of tetrameric hemoglobin with ligands, the first three sites reacted with velocity constants close to their statistical values and, at this stage, a large increase in the combination velocity constant—corresponding to binding of the fourth ligand—occurred (1). However, this view now appears oversimplified and the results reported here reinforce the conclusion that the fast rate associated with the ligand reaction of partially photodissociated material does not have the unequivocal meaning previously suggested.

The main arguments for this conclusion are the following.

1. The distribution of the quickly reacting material does not correspond to that expected for the form Hb(dCO)4, as calculated on the assumption that the quantum yield for photodissociation is the same for all of the sites.

2. Rapid mixing experiments on the combination with CO of artificial intermediates (12, 13), of "aged" mixtures of liganded and unliganded hemoglobin, and of ferric and unliganded hemoglobin (14) fail to show the presence of steps with reaction rates corresponding to the quickly reacting material. On the other hand, the artificial intermediates give predominantly fast rates with CO when studied by flash photolysis.

This suggests that the rapidly reacting species represents partially saturated intermediates which appear transiently when the ligand is suddenly removed from fully ligand-bound hemoglobin. According to this view the results of the partial photodissociation

1 E. Antonini, M. Brunori, and K. H. Winterhalter, unpublished observations.
experiments might be interpreted in the framework of reaction schemes of the type:

\[
X + \text{Hb}^oX = \text{Hb}^*X + X
\]

1

\[
\text{Hb}^oX = \text{Hb}^*X
\]

where the forms with superscripts * and o differ either in conformation, or in the binding of a third component, or in any other ligand-linked property. Rates of ligand-linked processes have generally been found to be high in comparison with those of uptake or removal of ligand (2, 3); however, if it is assumed that in some of the intermediates the transition from the * to the form occurs slowly in comparison with the rates of ligand binding, situations like that discussed here can be easily reproduced.

Effects of the type just discussed emphasize the difficulty of treating hemoglobin kinetics with a small number of kinetic constants and suggest that only phenomenological descriptions and adoption of approximate schemes are feasible at present.

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