Allosteric Properties of Carbamylated Hemoglobins*

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Ted C. K. Lee and Quentin H. Gibson
From the Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

The spectra, ligand-binding properties, and conformational change of carbamylated human hemoglobin A, specifically modified at its α-NH₂ groups, have been compared with those of hemoglobin A. In this region, the spectra of all deoxyhemoglobins are identical. The Soret bands of carbonmonoxy α₂β₂ and α₂β₅ are shifted in opposite directions relative to hemoglobin A, α₂β₂ having its Soret band at the longer wavelength. Carbamylation of either chain slows oxygen dissociation from the α chain in the R-state, although the effect is larger for α₂β₂ and α₂β₅ than α₂β₂. Carbamylation of either chain also slows oxygen binding to the α chain while the rate for the β chain is not changed significantly, indicating that the chains influence each other in the R-state. The rates of oxygen binding after full and partial laser photolysis of oxygen-saturated α₂β₂ appear identical. The rate of oxygen rebinding for α₂β₅ after partial (10%) photolysis is the same as that for α₂β₂, but following full photolysis, biphasic rebinding was observed due to the appearance of T-state molecules. Carbon monoxide binding studies under photostationary conditions suggested an L value for α₂β₅ smaller than that for α₂β₂ and hemoglobin A. The R to T transition of the deoxy form of α₂β₅ is about 10 times slower than α₂β₂. Carbamylation of the β chain does not slow the transition, indicating that carbamylation of the α chain affects the allosteric equilibrium in deoxyhemoglobin. To a reasonable approximation with minimum assumptions, all of the observations made in this study may be rationalized using the two-state model of Monod, J., Wyman, J., and Changeux, J. P. ((1965) J. Mol. Biol. 12, 88-118).

Several investigations have shown that the hemoglobins carbamylated at the α-NH₂ groups are well defined materials with properties moderately different from those of native hemoglobin (1-4). The α chain carbamylated derivative, α₂β₂, has also recently been characterized by x-ray methods by O'Donnell et al. (5). The functional studies have dealt with the oxygen equilibrium and the Bohr effect (1, 2), and there have been few, if any, reports of the kinetics of the ligand reactions. The carbamylation of hemoglobins are especially interesting in relation to the two-state model (6-8) just because their properties do not depart widely from those of Hb A. Many mutant hemoglobins are either in the R-state under all conditions or reach it so easily that they show little evidence of allosteric behavior in the absence of powerful effectors, and sometimes tend to dissociate to dimers more than Hb A. The carbamylated hemoglobins, however, may be expected to show a full range of allosteric properties, analogous to, but different from, those of Hb A.

RESULTS AND DISCUSSION

The carbamylated hemoglobins were prepared substantially as described in Refs. 1-3 with a number of minor modifications set out in detail in Appendix 1. As a preliminary to the kinetic work, spectra were obtained for the deoxy and carbonmonoxy derivatives using a digital spectrophotometer (8). As described in detail in Appendix 2, the spectra of the deoxy derivatives were identical with those from hemoglobin A, but the carbamylated CO derivatives show shifts of the Soret band. The band is moved in opposite directions relative to Hb A for the two singly carbamylated derivatives. For α₂β₂ the shift is about 0.3 nm toward longer wavelengths, while for α₂β₅ it is about 2/3 as great and to shorter wavelengths. The α₂β₅ is almost identical with the α₂β₂. When the PMB derivatives were compared with PMB-treated Hb A, PMB-α was closely similar to PMB-α; the PMB-β gave a more complex spectrum suggesting sharpening of the Soret. There is a correlation between the position of the Soret band and affinity for ligand with the higher affinity derivatives having their Soret band at longer wavelengths.

Kinetics of Ligand Reactions—Data for the dissociation of oxygen from fully liganded hemoglobin were collected to represent a dissociation reaction from the R-state. The reactions are relatively slow, and as dimers and tetramers are alike, so far as is known, it is legitimate to use quite dilute solutions. The experiments also give estimates of the relative rates of oxygen and CO binding to the R-state without requiring the collection of additional data. The results, set out in detail in Appendix 3, may be summarized by saying that carbamylation of either chain slows oxygen dissociation from the α chain though the effect was larger for α₂β₂ than for α₂β₅. Doubly carbamylated hemoglobin resembled α₂β₂ quite closely, and the control experiments with Hb A agreed well with the data of Olson et al. (10), although the conditions were slightly different.

The ratio of the rate of O₂ binding to the rate of CO binding showed a similar pattern. Carbamylation of either chain slows the rate of oxygen binding to the α chain relative to that of CO, but carbamylation of the β chain has a larger effect. It is doubtful if the rate for the β chain is altered.

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1 The abbreviations used are: α₂β₂, α₂β₅, and α₂β₅, the hemoglobin specifically modified at the NH₂ termini of the α chains, β chains, and all four subunits, respectively; Hb, hemoglobin; PMB, p-mercuribenzoate.

2 Portions of this paper (including Appendices 1-7 and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014. Request Document No. 80 M-2274, cite authors, and include a check or money order for $16.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
These results were unexpected because there is little evidence that the chains influence each other in the R-state. There is, in fact, not much difference between dimers and tetramers in their ligand reactions, and only quite moderate differences between separated chains and chains present as tetramers. Olson et al. (10) found only small differences between α chains free and in combination, and thought that polymerization might be responsible for the larger differences seen with β chains. As already mentioned, dimers account for a considerable part of the heme in the dilute solutions used in these experiments, and it is possible that there are interactions between the chains in them. It is unlikely that there are large differences between dimers and tetramers, as this would have shown up as heterogeneity of the time course of replacement of oxygen by carbon monoxide.

**Oxygen Binding**—All of the experiments were performed by laser photolysis which, if applied to a solution with a low initial oxygen saturation may give information about binding and dissociation of T-state hemoglobin. The amplitude of the excursion after photolysis is a good approximate measure of the initial saturation, and if the experiment is carried out using a spectrometer the lower part of the dissociation curve may be obtained. If photolysis begins with a solution near saturation with oxygen, partial photolysis will usually give data on combination with the R-state, and comparison between full and partial photolysis may permit some conclusions about the rate of the R to T conformation change following photolysis.

Examination of α₂β₂ at low initial saturations gave a pattern of reaction rates which differs significantly from that of Hb A. As shown in Appendix 5, Fig. 1, A and B, there is a slow rate of about 350/s which accounts for about one-half of the observed reaction at high saturations, and much more at the lowest ones. The rate increases with the initial level of saturation of the hemoglobin before photolysis. The second component has a mean rate of about 1500/s and also increases with increase in initial saturation. Not only is the affinity for oxygen very high, with μ₀ of the order of 1 μM but the initial part of the equilibrium curve is much steeper still. As discussed in Appendix 5, this is likely to be due to a small degree of inequivalence in the amounts of the chain preparations used, and is not considered further. As a start toward interpretation of the results, an approximate calculation of the value of L required to give the high affinity observed (half-saturation at about 1 μM O₂) gives a value of only about 500, on the assumption that the properties of the R- and T-states are not changed. This is of the order of reciprocal c (the ratio of K₅ to K₆). In consequence, the dissociation velocity from T less quickly than α₂β₂. With α₂β₂ the rate and time course of oxygen binding after full and partial photolysis appear identical. With α₂β₂ the course of rebounding after partial (10%) photolysis is much the same as that for α₂β₂, but following full photolysis a notable biphasic reaction is seen. This may be explained as due to the appearance of T-state within the time of the experiment with α₂β₂ but not with α₂β₂. The approximate value of L is 1.5 × 10⁻⁵ under the standard conditions used.

The properties of the final derivative α₂β₂ may be dealt with quite briefly. It is intermediate between α₂β₂ and α₂β₂, perhaps favoring α₂β₂ over α₂β₂. Its affinity is high, and the kinetics does not suggest rapid R to T conversion of the deoxy form. The details of these experiments are set out in full in Appendix 5, together with a figure showing the equilibrium data.

The results so far presented in the various appendices allow the interpretation that the main effect of carbamylation is upon the allosteric equilibrium, but the argument is circular in that the conclusion is used in calculating the allosteric parameters. The actual numbers obtained for L also depend on the applicability of the model to the data of Ref. 11. An obvious extension of the experiments is to use other ligands. The results for the kinetics of the carbon monoxide reactions suggest it as a suitable candidate because there is no effect of carbamylation on the accessible rates. Unfortunately, its high affinity makes it difficult to study the equilibria with hemoglobins, and there is no easy method to measure the rate of CO dissociation from the T-state. These drawbacks can be largely overcome by measuring the equilibria in a strong light. The quantum yield is high and the dark dissociation reactions
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dow. It is easy, therefore, to decrease the apparent affinity greatly, so making measurement easier, and substituting a known rate of photo dissociation for the poorly defined dark reactions. The procedure, suggested by Brunori et al. (13), has been used by Torkelson and Gibson (14) with menhaden hemoglobin to simplify the application of the allosteric model. The residual difficulty remains that the partition between the R- and T-states depends upon L and c, and this is true in the light as well as in the dark. Equilibrium measurements made in a strong light must still depend, to some extent, on the values used for the dark dissociation reactions of carbon monoxide.

A complication in using human hemoglobin is its tendency to dissociate to dimers, and it is probably this difficulty which prevented Brunori et al. (13) from obtaining useful results with the dilute (2 to 4 μM in hemec) solutions they used. This was not a problem for menhaden Hb (14) because many fish hemoglobins dissociate much less than mammalian hemoglobins (15). The difficulty was met by including suitable terms in the equilibrium equation, and also by using flash photolysis as the method for determining the fractional saturation. If the concentration of carbon monoxide is low enough so that recombination after the flash is not competitive with the R to T transition, the course of carbon monoxide recombination in the R state will show two phases, a faster one due to binding to dimers, and a slower one due to binding to T-state tetramer. (Although true under most circumstances, there will be only one phase when the carbon monoxide is small and the concentration of T-state hemes is 30 times that of heme in dimers.) The amount of dimer may thus be measured and allowed for in interpreting the results. In principle, the appearance of dimers as a function of saturation of a hemoglobin solution is directly calculable from the model. In practice, it was found easy to represent the course of the appearance of dimer with increasing saturation (Appendix 6, Fig. 1), and the flash method does indeed seem to measure this quantity. Although interesting in themselves, the results failed to contribute toward the specific matter of the allosteric parameters for the carbamylated hemoglobins. It was found in numerical trials that the value of L required to reproduce a given affinity (measured as p50) was very sensitive to the light dissociation rate specified to the model. This is so great as to make comparisons between experiments performed on different days of somewhat doubtful value. With this restriction, the results given in Appendix 6 do seem to show, however, that α2β2 and α2β2 have smaller values of L than αβ2 and Hb A.

The next step in seeking to define differences in allosteric behavior between the carbamylated hemoglobins was to examine the kinetics of carbon monoxide binding over a wide range of ligand concentrations using the method of flash photolysis. The significant tetramer to dimer dissociation of the liganded derivatives gives an assurance that at least a substantial number of the tetramer molecules must be in the R-state. If ligand is removed from them quickly enough, the immediate product is deoxyhemoglobin in the R-state. These molecules may either combine at once with ligand, or they may change in conformation to the T-state, and, in doing so decrease their rate of combination with CO by some 30-fold. It is therefore easy to distinguish the molecules which bind in the R-state from those which bind in the T-state by observing the progress of ligand rebinding, and quantitative estimates of the rate of the R to T transition can be made by analyzing the progress curves.

Unlike the equilibrium experiments described earlier, the new results were unambiguous. Carbamylation of the α chain, alone or together with the β chain, gives a derivative for which the rate of the R to T transition of the deoxy form is some 10 times slower than the same change in Hb A. The rate of the R to T transition for α2β2 appears to be close to that of Hb A. These results show almost beyond doubt that carbamylation of the α chain affects the allosteric equilibrium in deoxyhemoglobin, and do so without requiring detailed calculations of values for L. Further, the behavior of the individual carbamylated hemoglobins agrees with the indications given by the combination of equilibrium and kinetic experiments described in earlier paragraphs. An additional method of observation of the R to T transition in deoxyhemoglobin has also been applied to experiments with carbon monoxide as ligand. This depends on the old observation that R-state deoxyhemoglobin has an absorption spectrum different from that of T-state deoxy. If, then, observations are made at the carboxy-deoxy isobestic point, the allosteric transition following flash photolysis may be observed directly rather than inferred from changes in ligand-binding rates. In hemoglobin A the change is so rapid that laser methods are required to follow it (16), but with the two α chain-carbamylated derivatives the change can readily be observed using apparatus based on conventional photographic flash tubes.

These kinetic experiments suffer from two weaknesses, first, only the rate of the transition from R to T is accessible. The reverse reaction is much too slow to influence the observed kinetics. Second, simply interpretable results are available for the R to T change only. Although partially liganded intermediates can be prepared, the absorbance changes associated with their conformation switch are usually too small to follow with the precision required for useful results. A detailed description of the procedures and results is given in Appendix 7 and its associated figures.

General Discussion—The main conclusion to emerge from this preliminary study of the kinetics of ligand binding to the carbamylated hemoglobins is that, although there is some effect on the kinetics of individual ligand reactions for derivatives which may reasonably be regarded as in similar allosteric states, these effects are small as compared with those mediated through changes in the allosteric parameter L. The changes in intrinsic rate constants follow a definite pattern with significant changes associated with a chain carbamylaion, and, where chain identification has been possible, it is the α chain which is affected (see Appendix 3, Table 1). It seems to be true, also, that oxygen reactions are more affected than reactions of carbon monoxide. In comparison, the effects of β chain carbamylaion are small, but with a tendency to be opposite in sign to those of a chain carbamylaion. In at least some of the properties studied, β chain carbamylaion tends to cancel the effect of a chain carbamylaion so that the doubly carbamylated compound is intermediate between the singly carbamylated forms.

The finding that the spectra of the CO compounds are shifted by carbamylaion is unexpected. In this case too, the effects of α and β chain carbamylaion are in opposite directions. It is difficult to relate the spectral changes to allosteric effects, since all of the CO forms must be in the R-state. They are associated with the tetramer, however, since the effects on the isolated α chains were small by comparison with those in the tetramer. There is a suggestion that the R-state in the carbamylated hemoglobins is not identical with that of hemoglobin A under similar conditions. All our work has been carried out in solutions buffered with phosphate, and it is, at present, only an attractive speculation that the spectral shifts are mediated by the inhibition of chloride binding reported by O'Donnell et al. (5) to follow a chain carbamylaion. It is a speculation which leads to experimentally verifiable predictions for Hb A as well as for the carbamylated hemoglobins.

Turning to the allosteric effect of carbamylaion, it is again
evident that the \( \alpha \) chain dominates, and that the allosteric equilibrium is indeed shifted in \( \alpha \beta_2 \) as compared with Hb A or with \( \alpha_2 \beta_2 \). As with the intrinsic rates, the effects of a chain carbamylation are partly reversed by \( \beta \) chain carbamylation. Although the functional effects are large, it has not been possible to obtain sound estimates of the actual size of \( L \). It was particularly disappointing that the studies of carbon monoxide binding in the photostationary state failed in this respect, especially when the experiments were arranged so that \( L \) was the only parameter to be determined. Although numbers are not available, an interpretation of the results in terms of the two-state model was easy and natural, and allowed rationalization of the data for oxygen binding at low saturations, although the data themselves differed widely between the derivatives. The actual oxygen affinities observed under our conditions are hard to compare with previous results although we have found a larger difference between \( \alpha_2 \beta_2 \) and Hb A than O'Donnell et al. (5). This may be explained in terms of their work on chloride and proton binding. Their experiments were performed in phosphate-free media chiefly at pH 7.6. We used 0.1 M KPi at pH 7. If, as seems likely, a chain carbamylation strongly favors the R-state, by preventing phosphate from binding at a site near to Val 1a, the apparent difference between \( \alpha_2 \beta_2 \) and Hb A will be greater the more the solution conditions favor the T-state for Hb A. The effect on \( p_50 \) in our experiment (Appendix 3, Fig. 5) is only 3 times greater than that reported in Fig. 2 of Ref. 5 and there is no necessary conflict between their result and ours.

The rate of the R to T transition is affected by carbamylation in the direction to be expected on the basis of the equilibrium experiments, and the effects are considerable, with a 20-fold span between \( \alpha_2 \beta_2 \) and \( \alpha_2 \beta_2 \). Comparison of the traces for \( \alpha_2 \beta_2 \) with published data of Sawicki and Gibson (16) for Hb A, suggests that \( \alpha_2 \beta_2 \) was little affected by carbamylation. Again, \( \alpha_2 \beta_2 \) is intermediate between \( \alpha_2 \beta_2 \) and \( \alpha_2 \beta_2 \).

In summary, the two-state model is able to rationalize a complex series of results to a reasonable approximation with a minimum of assumptions, and the results are entirely consistent with the recent structural work of O'Donnell et al. (5). Although it is axiomatic that kinetic data are incapable of proving the truth of a mechanistic hypothesis, the natural and easy description of the results so far obtained at least justify making further extension of observations to examine the predictions which may be made using the model together with the chemical proposals of O'Donnell et al. (5).

REFERENCES

1. Kilmartin, J. V., and Rossi-Bernardi, L. (1969) Nature (Lond.) 222, 1243-1246
2. Kilmartin, J. V., Fogg, J., Luzzana, M., and Rossi-Bernardi, L. (1973) J. Biol. Chem. 248, 7039-7043
3. Lee, C. K., and Manning, J. M. (1973) J. Biol. Chem. 248, 5861-5865
4. Nigen, A. M., Nijam, N., Lee, C. K., and Manning, J. M. (1974) J. Biol. Chem. 249, 6611-6616
5. O'Donnell, S., Mandaro, R., Schuster, T. M., and Arnone, A. (1979) J. Biol. Chem. 254, 12204-12208
6. Monod, J., Wyman, J., and Changeux, J. P. (1965) J. Mol. Biol. 12, 88-118
7. Edelstein, S. J. (1971) Nature (Lond.) 230, 224-227
8. Hopfield, J. J., Schulman, R. G., and Ogawa, S. (1971) J. Mol. Biol. 61, 425-443
9. Knowles, F. C., and Gibson, Q. H. (1976) Anal. Biochem. 76, 458-468
10. Olson, S. J., Andersen, M. E., and Gibson, Q. H. (1977) J. Biol. Chem. 252, 5919-5923
11. Sawicki, C. A., and Gibson, Q. H. (1977) J. Biol. Chem. 252, 7538-7547
12. Sawicki, C. A., and Gibson, Q. H. (1977) J. Biol. Chem. 252, 5783-5788
13. Brunori, M., Bonaventura, J., Bonaventura, C., Antonini, E., and Wyman, J. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 868-871
14. Torkelson, S. J., and Gibson, Q. H. (1978) J. Biol. Chem. 253, 7331-7335
15. Edelstein, S. J., McEwen, B., and Gibson, Q. H. (1976) J. Biol. Chem. 251, 6729-6737
16. Sawicki, C. A., and Gibson, Q. H. (1976) J. Biol. Chem. 251, 1533-1542

Additional references are found on pp. 4574-4577.
Allosteric Properties of Carbamylated Hemoglobin

Tom C. Lim and Hamilton E. Lipson

The Allosteric Properties of Carbamylated Hemoglobin

Carbamylated hemoglobin was prepared by the procedure described by Haldon and Lipson (1), with some modifications. The results of a previous study (2) on the effects of carbamylated hemoglobin on the oxygen affinity of human red blood cells have been reported. The oxygen affinity of carbamylated hemoglobin was measured using the method of Haldon and Lipson (1), with some modifications. The results showed that the oxygen affinity of carbamylated hemoglobin was significantly decreased compared to normal human red blood cells. The decrease in oxygen affinity was attributed to the carbamylated nature of the heme group, which is known to affect the oxygen affinity of hemoglobin.

References

1. Haldon, D. H. (1965) J. Biol. Chem. 239, 3190-3193.
2. Lipson, H. E. (1967) Blood 30, 113-117.

Figure 1: Fractionation of carbamylated hemoglobin. The volume (2.5 x 15 cm) was diluted to 50 ml per hour with a buffer containing 0.1 M Tris-HCl (pH 7.4), 0.05 M NaCl, and 0.001 M EDTA. After the buffer was added, the sample was centrifuged at 3000 rpm for 30 minutes. The supernatant was discarded, and the pellet was washed with the same buffer. The procedure was repeated three times, and the final pellet was resuspended in the buffer.

Figure 2: The distance of carbamylated hemoglobin from the native hemoglobin. The samples were obtained using a computerized solution of carbamylated hemoglobin at 0.01 M Tris-HCl pH 7.4. The distance was calculated by comparing the absorbance at 280 nm of the carbamylated hemoglobin to that of the native hemoglobin.

Figure 3: The difference in distance between the carbamylated and native hemoglobins in the native condition. The distance was determined by comparing the absorbance at 280 nm of the carbamylated hemoglobin to that of the native hemoglobin.

Figure 4: The Reaction of Carbamylated and Native Hemoglobins

Steady-state experiments were performed to measure the rate of reaction of the samples with 0.1 M Tris-HCl (pH 7.4) and 0.01 M EDTA. After the reaction was complete, the samples were analyzed by disc gel electrophoresis.

The results showed that the reaction rate of the carbamylated hemoglobin was significantly lower than that of the native hemoglobin. This was attributed to the formation of a more stable carbamylated derivative, which is resistant to the reaction conditions.

Figure 5: The difference in distance between the carbamylated and native hemoglobins in the oxygenated condition. The distance was determined by comparing the absorbance at 280 nm of the carbamylated hemoglobin to that of the native hemoglobin.

The results showed that the oxygen affinity of the carbamylated hemoglobin was significantly lower than that of the native hemoglobin. This was attributed to the formation of a more stable carbamylated derivative, which is resistant to the reaction conditions.

Figure 6: The difference in distance between the carbamylated and native hemoglobins in the deoxygenated condition. The distance was determined by comparing the absorbance at 280 nm of the carbamylated hemoglobin to that of the native hemoglobin.

The results showed that the oxygen affinity of the carbamylated hemoglobin was significantly lower than that of the native hemoglobin. This was attributed to the formation of a more stable carbamylated derivative, which is resistant to the reaction conditions.
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The data obtained by fitting the reaction curve with the appropriate model. If a value for the dissociation constant was not specified in the text, the data were collected for a single concentration of substrate, and the reaction was followed for 5 min. The data were then normalized to rate curves at different levels of substrate levels.

The appearance of the data in Fig. 1 demonstrates that the model fits the data well for all concentrations of substrate, with the exception of the lowest concentration where the fit is not as good. This is likely due to the lower signal-to-noise ratio for these data points.

The model used to fit the data was a modified Hill equation, where the Hill coefficient is 1.5. This is consistent with the results obtained by other investigators. The Hill coefficient is a measure of the cooperativity of the reaction, and a value of 1.5 indicates a weakly cooperative reaction.

The dissociation constant for the reaction is 1.5 M. This is also consistent with the results obtained by other investigators. The dissociation constant is a measure of the affinity of the reaction, and a value of 1.5 M indicates a moderate affinity for the substrate.

The activation energy for the reaction is 40 kJ/mol. This is consistent with the results obtained by other investigators. The activation energy is a measure of the energy required to overcome the activation barrier, and a value of 40 kJ/mol indicates a moderate energy barrier.

The model also predicts that the reaction rate is independent of the concentration of substrate. This is consistent with the results obtained by other investigators. The reaction rate is a measure of the rate of the reaction, and a value of 0 indicates that the reaction rate is not dependent on the concentration of substrate.

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Allosteric Properties of Carbamylated Hemoglobins

Figure 1. Combination of oxygen with hemoglobin following flash photolysis. A, 10% Hb in 0.05 M HEPES buffer, pH 7.0, 0.05 M KCl, 0.1 M MgCl₂, 0.1 M NaCl, 5.0 mM dithiothreitol (DTT), 5.0 mM NaCl, 0.1% Triton X-100. B, 0.1% Triton X-100. C, 0.1% Triton X-100. Other conditions as for A.

Figure 2. Titrations of Hb with oxygen. Conditions as for Fig. 1 with 50 mM NaCl. A, 0×C O₂; B, 2×C O₂; C, 5×C O₂. Other conditions as for A.

Figure 3. Titrations of Hb with oxygen. Conditions as for Fig. 1 with 50 mM NaCl. A, 0×C O₂; B, 2×C O₂; C, 5×C O₂. Other conditions as for A.

Figure 4. Titrations of Hb with oxygen. Conditions as for Fig. 1 with 50 mM NaCl. A, 0×C O₂; B, 2×C O₂; C, 5×C O₂. Other conditions as for A.

Figure 5. Equilibrium curves for oxygen binding to carbamylated hemoglobins. The data were obtained by ferrie photolysis as described in the text. The hemoglobin concentrations were 30 mg/ml in 0.1 M KCl. The buffer was 20 mM sodium phosphate, pH 7.0. Other conditions as for A.

Appendix A

Carboxyhemoglobin Formation under Physiological Conditions

The experiments were performed using the apparatus and methods described in reference to the text. The final concentration of formaldehyde was determined by titration with sodium hydroxide. The hemoglobin was precipitated by cold ethanol and re-dissolved in 0.1 M KCl, pH 7.0. Other conditions as for A.

Figure 6. Binding of CO to dimeric Hb in the presence of ethanol. The experiments were performed by photolysis as described in the text. The final concentration of formaldehyde was determined by titration with sodium hydroxide. The hemoglobin was precipitated by cold ethanol and re-dissolved in 0.1 M KCl, pH 7.0. Other conditions as for A.
Allosteric Properties of Carbamylated Hemoglobin

The results obtained with the derivatives Ψ_1 and Ψ_2 are shown in Fig. 3 and 4. In all of the data in this section, there are two widely separated rates of CO attachment, and, as expected in Fig. 1, their relative magnitudes are dependent on the concentration of CO. In fact, the second derivative with CO higher than 0.05 mM CO using Ψ_2 is essentially parallel to the rates for Ψ_2 with 0.05 mM CO. Thus the rate of the rapid (linearized) reaction is independent of the initial concentration of CO, in contrast to the slow reaction, which is dependent in a complex manner on the initial concentration of CO. This conclusion is supported by the data collected at 0.85 mM CO, a condition of which is shown in Fig. 3. There is a small but rapid change in Ψ_2 with a half-time of about 10 msec which is not significantly altered by a 10-fold change in CO concentration, though it is essentially constant with the lower CO. The results may be compared with the data of Table I, which is not significantly altered.

The values of Ψ_2 and Ψ_2 are different, but to obtain numerical estimates of the rate of conversion, more elaborate procedures are required. These have been described in [11]. The time course of the absorbance changes at 420 nm and 620 nm was represented by using the two components to give a set of differential equations. These were solved numerically, and the best fitting values of the rate constants were determined by computer simulation [12]. The results fit the data well, though the exact amount of CO is known to be fixed during the experiment and, as a result, the rate of the slow reaction is underestimated. The results obtained with CO concentrations up to 50 mM CO are shown in Fig. 5, which shows that Ψ_2 is increased by a factor of 2.3 when CO is increased from 0.05 mM CO to 50 mM CO. The observation of this effect is consistent with the observation that the rate of the fast reaction is increased by a factor of 2.3 when CO is increased from 0.05 mM CO to 50 mM CO. The observation of this effect is consistent with the observation that the rate of the fast reaction is increased by a factor of 2.3 when CO is increased from 0.05 mM CO to 50 mM CO.

References

1. Twitchell, J. A. and Gibson, G. B. (1967) J. Biol. Chem. 242, 1350-1360.
2. Kunkel, W. E. (1968) Biochemistry and Enzyme Analysis for the Physical Scientist, Chapter 11, Macmillan, New York.