Acknowledgment

We are indebted to Mrs. Lois R. Manning for her assistance in some of these studies.

References

Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66*, 375.
Greenstein, J. P., Birnbaum, S. M., and Otey, M. C. (1953), *J. Am. Chem. Soc. 75*, 1994.
Kagan, H. M., Manning, L. R., and Meister, A. (1965), *Biochemistry 4*, 1063.
Kagan, H. M., and Meister, A. (1966a), *Biochemistry 5*, 725.
Kagan, H. M., and Meister, A. (1966b), *Biochemistry 5*, 2423.
Khedouri, E., and Meister, A. (1965), *J. Biol. Chem. 240*, 3357.
Krishnaswamy, P. R., Pamiljans, V., and Meister, A. (1962), *J. Biol. Chem. 237*, 2932.
Levintow, L., and Meister, A. (1953), *J. Am. Chem. Soc. 75*, 3039.
Lineweaver, H., and Burk, D. (1934), *J. Am. Chem. Soc. 56*, 658.
Pamiljans, V., Krishnaswamy, P. R., Dumville, G., and Meister, A. (1962), *Biochemistry 1*, 153.
Wellner, V. P., and Meister, A. (1966), *Biochemistry 5*, 872.
Wellner, V. P., Zoukis, M., and Meister, A. (1966), Abstracts, 153rd National Meeting of the American Chemical Society, New York, N. Y., Sept.

Reaction Kinetics of the Ferrimyoglobin Azide System*

Donald Duffey,† Britton Chance, and Georg Czerlinski

ABSTRACT: Rate and equilibrium constants for azide binding by whale ferrimyoglobin have been determined and interpreted over essentially the entire pH range within which the myoglobin and its azide complex are stable. Kinetics of azide ferrimyoglobin (sperm whale) reactions measured over the range pH 4.5–7.0 gave the same rate constants at each pH whether determined by flow or relaxation techniques. Rates of formation and dissociation of the azide complex are highly pH dependent in this range. The dependence coincides with about a 300-fold preferential binding of hydrazoic acid ($k = \sim 10^6$ M^{-1} sec^{-1}) compared to azide ion.

In the range pH 7–10 rates determined by stopped flow showed much less sensitivity to pH. Binding is comparatively slower for freshly redissolved whale myoglobin crystals but faster for redissolved crystalline seal myoglobin.

Early studies on the binding of azide by ferrimyoglobin (Kiese and Kaeske, 1942) showed the system to follow a simple mass action law. It was not clear from this work, however, whether one form of the ligand was bound preferentially. Among other uncharged Bronsted acids and their conjugate bases that are known to act as ligands with hemoproteins, particularly ferrimyoglobin, fluoride is reported to be very much less reactive than hydrofluoric acid (George and Hanania, 1954) whereas cyanide (Coryell *et al.*, 1937; Chance, 1952) and phenolate ions (George *et al.*, 1961) are said to bind somewhat faster than their conjugate acids.

In some cases the conclusions concerning relative reactivities are based on equilibrium studies and in others on kinetic experiments. Reconciliation of results from both approaches has generally been difficult although Goldsack *et al.* (1965, 1966) have lately accomplished this to a limited extent with azide, cyanate, and hydrogen sulfide. To elucidate some details of ligand binding to ferrimyoglobin we have utilized equilibrium measurements as well as the intrinsically different kinetic techniques of stopped-flow and chemical relaxation in a study of the well-known azide ferrimyoglobin reaction.

Though the reaction of azide with crystalline metmyoglobin is much slower, and hence possibly quite different, than with dissolved metmyoglobin (Chance *et al.*, 1966), the over-all process in the solid state may be clearly pictured from X-ray diffraction results (Stryer *et al.*, 1964) to consist of a net isomorphous replacement of water by hydrazoic acid at the sixth coordination position of the heme iron(III) ion.

Experimental Section

Reagents. Solubilized sperm whale skeletal muscle

* From the Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania. *Received June 16, 1966.* This work was supported in part by Grants GM-10888-02 and U. S. Public Health Service-5T1-GM-277-05.

† Present address: Department of Chemistry, Mississippi State University, State College, Miss.

TABLE I: Ferrimyoglobin–Azide Equilibrium Dissociation Constants.

pH	4.5	5.0	5.5	6.0	6.5	7.0	7.6	9.0	10.0
$K_{(app)}$ (μM)	36 ± 4	26 ± 4	22 ± 4	24 ± 3	24 ± 4	27 ± 1	40 ± 5	132 ± 7	310 ± 40

myoglobin obtained from Seravac Laboratories was generally used without additional treatment after it was found that chromatography on Sephadex G-25 had no detectable effect on the rate or equilibrium constants. As expected, absorbance in the Soret band was slightly lower than the values obtained by Theorell and Åkeson (1955). At pH 7.6 the molar extinction coefficient was 13.6×10^4 M^{-1} cm^{-1} or only 85% of that of Breslow and Gurd (1962). Crystalline ferrimyoglobin was obtained out of 75% ammonium sulfate solution and washed with this solvent before redissolving in solutions of low ionic strength. Seal myoglobin crystals were kindly provided by Dr. N. Rumen of Johns Hopkins University. Practical grade sodium azide (Matheson Coleman and Bell) was generally used without further purification after it was found that recrystallization caused no appreciable alteration in the kinetic constants. Other chemicals used were of reagent grade. Citrate buffers were used over the pH range 4.5–6.0, phosphate buffers from pH 6.0 to 7.6, and glycine buffers at 9.0 and 10.0. Ionic strength adjustments were made with potassium sulfate.

Equilibrium Constants. The dissociation constants were determined by following titrations of the myoglobin by azide spectrophotometrically at 409 and 422 mμ, the strongest visible maxima of ferrimyoglobin and its azide compound, respectively. Myoglobin concentrations of about 10 μM were used most commonly, but a 20-fold variation in concentration at this level produced no change in the equilibrium constant. The ionic strength was adjusted to 0.2. Contributions of both species to the total absorbance at each wavelength were calculated from the molar extinction coefficients measured at zero and "infinite" azide concentrations at each pH. The equilibrium constants were then computed according to eq 1

$$K_{(app)} = \frac{(HN_3 + N_3^-)\Sigma(Mb_i)}{\Sigma(C_i)} \qquad (1)$$

where $\Sigma(Mb_i$ is the sum of the concentrations of all ferrimyoglobin species not containing azide and $\Sigma(C_i)$ is the sum of the concentrations of forms containing bound azide. Five to ten intermediate azide concentrations over about a 100-fold range provided the values and mean deviations for the equilibrium dissociation constants of whale ferrimyoglobin azide shown in Table I. The titrations were carried out on Beckman Model DB, Zeiss PMQII, and Cary Model 15 spectrophotometers.

Kinetic Methods. Apparent rate constants over the pH range 4.5–7.0 were obtained both by stopped-flow or, for faster processes, continuous-flow methods (Chance, 1964, 1965) and chemical relaxation following a small, rapid temperature increase (Czerlinski, 1962). Some of the dissociation rate constants were also measured by means of a regenerative stopped-flow apparatus (Chance and Legallais, 1954) using a 25-fold dilution ratio and concentrations such that the association reaction could be neglected. These reactions were carried out in the double-beam spectrophotometer using wavelengths of 630 and 600 mμ (Chance *et al.*, 1966). In all other kinetic studies reported here narrow pass interference filters with maximum transmission at 424 mμ (9-mμ half-width) were used. At pH 9.0 the dissociation rate constant was small enough to be determined from rapid mixing experiments in a cuvet where a 50-fold dilution was used.

In stopped-flow experiments to measure the apparent association rate constants the azide concentrations were made sufficiently large to give pseudo-first-order kinetics. A characteristic oscilloscope trace for this type of experiment is shown in Figure 1B, and typical experimental results are given in Table II.

TABLE II: Stopped-Flow Mixing Reaction of Myoglobin and Azide.[a]

Azide Concn after Mixing (μM)	$t_{1/2}$ (sec)
37	9.0 ± 1.4
75	7.5 ± 0.3
150	4.6 ± 0.3
310	2.6 ± 0.1
630	1.5 ± 0.1
1250	0.94 ± 0.07
2500	0.67 ± 0.05

[a] Whale ferrimyoglobin (7.5 μM) after mixing; pH 9.0, 0.03 M glycine, 0.2 μ.

The chemical relaxation studies were done by Joule's heating of 0.5–3.0° attained within about 50 μsec. In some experiments equal analytical concentrations of myoglobin and azide were used, and in these cases the relaxation time constants were related to the rate constants by eq 2. In other relaxation experiments a large excess of azide was used, and in these instances eq 3 was applicable.

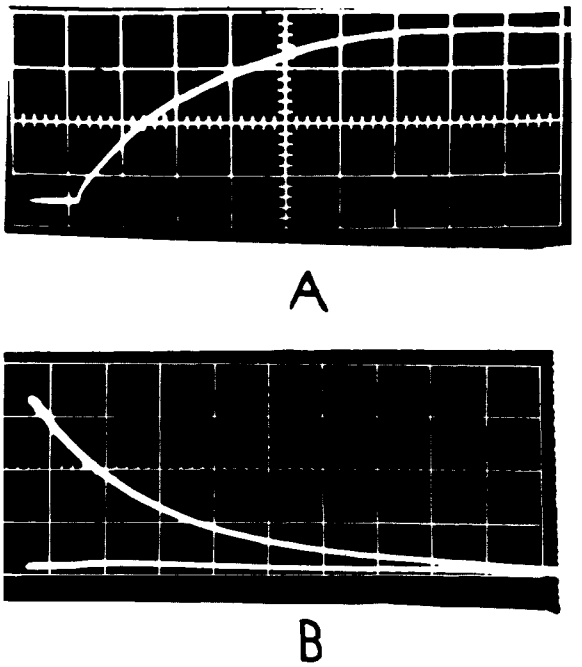


FIGURE 1: Relaxation trace (A) and stopped-flow trace (B). (A) 15 μM ferrimyoglobin; 15 μM azide; pH 5.5; 0.1 sec/horizontal division; 20 mv/vertical division. (B) 15 μM ferrimyoglobin mixed with 1.25 mM azide; pH 9.0; 1 sec/horizontal division, 0.5 mv/vertical division.

$$\tau^{-2} = 4k_2k_1c_A^0 + k_1^2 \quad (2)$$

$$\tau^{-1} = k_2c_A^0 + k_1 \quad (3)$$

In these equations τ is the time constant, k_2 and k_1 are the apparent association and dissociation rate constants, respectively, and c_A^0 is the analytical concentration of azide. The origin and limitations of these equations have been discussed by Czerlinski (1964). The relaxation time constants were evaluated analytically using at least three points at the early, middle, and late stages of the process. The time constants so obtained in a given experiment showed mean deviations of 10% or less. Equal consistency was obtained in the flow experiments.

An oscilloscope trace of a relaxation process is shown in Figure 1A. In this and all other chemical relaxation experiments the concentration of the complex was seen to decrease with increasing temperature. The base temperature for all equilibrium and kinetic measurements was the room temperature of 22 ± 2°. A characteristic set of results from the relaxation experiments is given in Table III. The error ranges indicated in this table and in Table II include reproducibility as well as the error estimate for a single experiment.

Both phosphate and citrate buffers were used at pH 6.0 and gave essentially the same rate constants in both media. Ionic strength effects were also found to be negligible in the range 0.01–0.20. At pH greater than 7.5 the reactions were too slow for measurement by the chemical relaxation apparatus before the onset of cooling and convection schlieren (about 20 sec).

TABLE III: Chemical Relaxation of Ferrimyoglobin Azide.[a]

Azide Concn (μM)	$\tau_{(msec)}$
40	2000 ± 300
80	1500 ± 200
120	1100 ± 200
200	790 ± 40
400	450 ± 20
800	230 ± 40

[a] Myoglobin (18 μM); pH 7.0, 0.03 M phosphate, 0.2 μ.

Results

The apparent second-order association rate constants over the pH range 4.5–10.0 for the whale ferrimyoglobin azide reaction are shown in Figure 2. The apparent first-order rate constants are presented in Figure 3. The line lengths in both figures represent the ranges of values observed in from three to ten experiment sets at each pH except pH 10.0 where only two sets of stopped-flow experiments were performed. Variation of the room temperature probably accounts for a significant but not major portion of the latitude in the results. In fact the correspondence of results from temperature-jump experiments where equal analytical concentrations and those where excess azide was used is within experimental error. Of greater importance, however, is the agreement between flow and relaxation results over the range pH 4.5–7.0. Experiments of all three types are included in this pH range (Figures 2 and 3). Below pH 4.5 all methods gave only erratic results, apparently attributable to denaturation (Hermans and Rialdi, 1965; Breslow and Koehler, 1965). Alkaline denaturation above pH 10 precluded experiments in quite basic solutions.

The effects of crystallization on the reaction of sperm whale ferrimyoglobin with azide was studied cursorily at pH 7.6 where the redissolved crystals after only a few minutes in solution reacted only about half as fast as lyophilized material that had been dissolved for several hours. Redissolved crystals of seal ferrimyoglobin on the other hand react considerably more rapidly with azide at pH 7.5 than does the whale myoglobin, though the dissociation constants are quite similar (*cf.* Table IV).

Discussion

In a given relaxation experiment the time constant

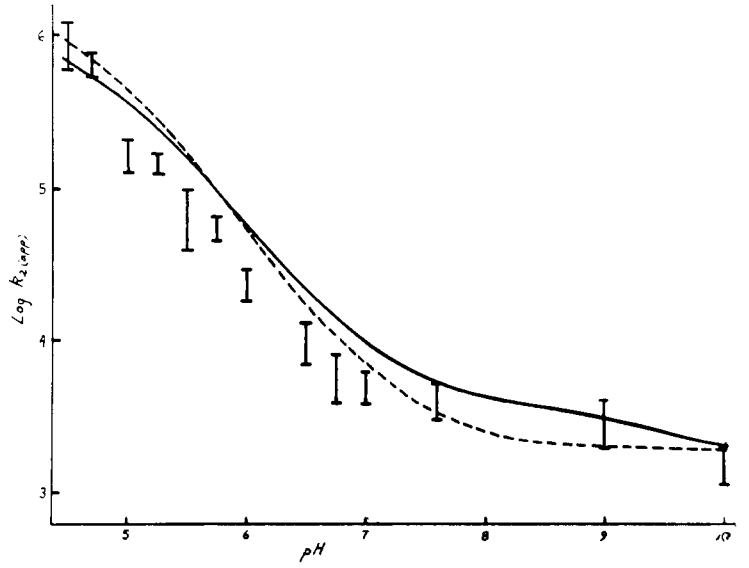


FIGURE 2: Dependence of $k_{2(app)}$ on pH. Solid-line curve calculated from set I parameters. Dashed line from set II, self-consistent parameters.

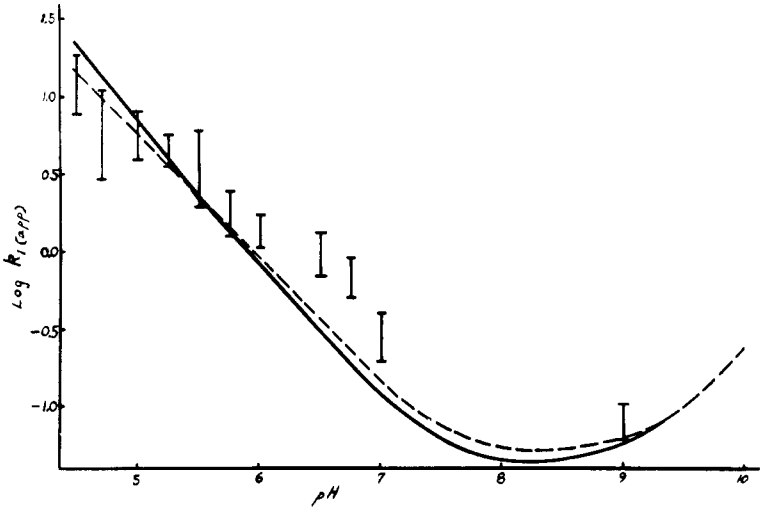


FIGURE 3: Dependence of $k_{1(app)}$ on pH. Solid-line curve from set I, dashed curve from set II, self-consistent parameters.

TABLE IV: Observed Constants for the Reaction of Seal Ferrimyoglobin with Azide.

pH	K (μM)	$10^{-4}k_2$ (M^{-1} sec^{-1})	k_1 (sec^{-1})
7.5	21 ± 2	1.0 ± 0.3	≥0.5
9.0	130 ± 15	0.24 ± 0.03	0.21 ± 0.02

when evaluated analytically at several points between about 10 and 75% reaction completion or better varied from the mean value by 10% or less. This constancy is good evidence that a single relaxation process was being observed (Hammes, 1966, and references therein). Since observations were made on the changes in the Soret band it seems probable that the process being followed is the attachment of azide to the iron of heme (Caughey *et al.*, 1965). From the close correspondence of apparent rate constants obtained by flow and relaxation methods, it is evident further that the spectroscopically observed reaction constitutes the rate-limiting step and that any other steps that affect the reaction with azide are considerably faster at least from pH 4.5 to 7.0.

In the relaxation experiments at high azide concentration such that virtually all of the ferrimyoglobin was in the form of its azide compound, a very fast relaxation (τ <100 μsec) was observable at 424 mμ

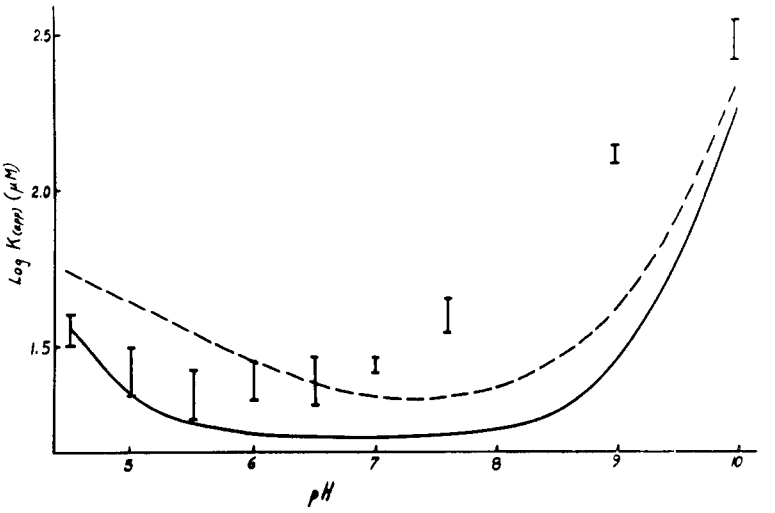


FIGURE 4: Dependence of $K_{(app)}$ on pH. Solid curve, set I. Dashed curve, set II, self-consistent.

but was not accurately separable from the heating time constant. This process was seemingly independent of pH and not detectable for ferrimyoglobin itself at this wavelength. A possible explanation of this fast step is the temperature-dependent equilibrium between different spin states of ferrimyoglobin azide (Beetlestone and George, 1964).

We have reported previously (Duffey *et al.*, 1965) that the variation of the association rate over the range pH 4.5–6.5 can be described by preferential binding of hydrazoic acid by ferrimyoglobin with a specific rate constant of 8 (± 4) $\times$ 10^5 M^{-1} sec^{-1}. Goldsack *et al.* (1965) have recently found a similar value from temperature-jump experiments in the range pH 5.1–7.5. At higher pH, however, reaction with azide ion appears to predominate. In addition, values of $k_{2(app)}$ at pH 9 and 10 reveal appreciable apparent reactivity of the hemic acid ionization product with azide ion. These general features of the azide ferrimyoglobin system are explicitly contained in the reactions of set I.

Set I

$$HN_3 \overset{K_A}{\rightleftharpoons} N_3^- + H^+$$

$$Mb_1 + HN_3 \underset{k_{11}}{\overset{k_{21}}{\rightleftharpoons}} C + H^+$$

$$Mb_1 + N_3^- \underset{k_{12}}{\overset{k_{22}}{\rightleftharpoons}} C$$

$$Mb_1 \overset{K_H}{\rightleftharpoons} Mb_2 + H^+$$

$$Mb_2 + N_3^- \underset{k_{13}}{\overset{k_{23}}{\rightleftharpoons}} C + OH^-$$

where K_A and K_H are the dissociation constants of hydrazoic and hemic acid. The experimentally accessible rate and equilibrium constants are related to the parameters of set I by eq 4–6 in which simple proton transfers are assumed to occur much faster than the ligand-binding steps.

$$k_{2(app)} = \frac{k_{21}(H)^2 + k_{22}K_A(H) + k_{23}K_AK_H}{[(H) + K_A][(H) + K_H]} \quad (4)$$

$$k_{1(app)} = k_{11}(H) + k_{12} + \frac{k_{13}K_W}{(H)} \quad (5)$$

$$K_{(app)} = \frac{k_{11}[(H) + K_A][(H) + K_H]}{k_{21}(H)} \quad (6)$$

Reaction of Mb_2 with hydrazoic acid is excluded from set I because an association rate constant larger than 10^8 M^{-1} sec^{-1} would be required for an appreciable contribution to $k_{2(app)}$ at pH 10, and such a large rate constant alone would give more than the observed binding rate at pH 9.

The parameter values in Table V for set I are sufficient for a good qualitative description of the ferrimyoglobin azide system as shown by the solid line curves of Figures 2–4, but it would seem likely that protonic processes on the protein may have some effect on the ligand-binding reactions even though they appear to affect the secondary structure of ferrimyoglobin very little (Breslow *et al.*, 1965; Samejima and Yang, 1964). Hence when two ionizations on the protein are included in the reaction scheme as in set II with values of the parameters such as shown in Table V under "Best Fit," it is not surprising that the calculated values of $k_{2(app)}$ and $k_{1(app)}$ fall within the experimental ranges at every pH (except pH 10).

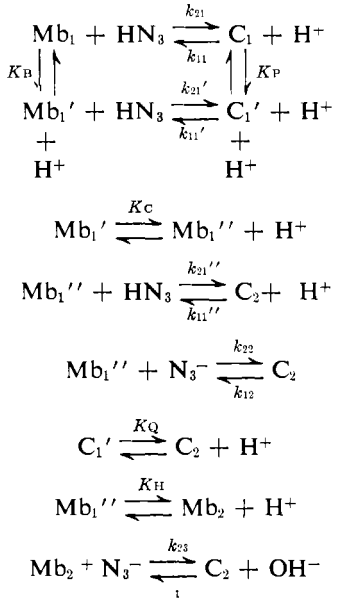
Goldsack *et al.* (1966) using our earlier data in the lower pH range as well as their own have in fact already shown graphically that protein ionizations affect the kinetics appreciably, thus excluding set I as a complete description of the azide ferrimyoglobin system. These

TABLE V: Kinetic Parameters for the Sperm Whale Ferrimyoglobin Azide System.

Constant	Set I	Set II	
		"Best Fit"	Self-Consistent
10^5K_A[a] (M)	2	4	2
10^5K_B (M)	—	1.8	2.7
10^6K_C (M)	—	0.66	2
10^9K_H[b] (M)	1	1	1
10^5K_P (M)	—	0.35	2
10^6K_Q (M)	—	0.25	3
$10^{-6}k_{21}$ (M^{-1} sec^{-1})	1.1	1.5	2
$10^{-6}k_{21}'$ (M^{-1} sec^{-1})	—	0.8	1.2
$10^{-6}k_{21}''$ (M^{-1} sec^{-1})	—	0.4	1
$10^{-3}k_{22}$ (M^{-1} sec^{-1})	4	5	2
$10^{-3}k_{23}$ (M^{-1} sec^{-1})	2	—	2
$10^{-5}k_{11}$ (M^{-1} sec^{-1})	7.5	3	3
$10^{-5}k_{11}'$ (M^{-1} sec^{-1})	—	7	6
$10^{-5}k_{11}''$ (M^{-1} sec^{-1})	—	20	10
k_{12} (sec^{-1})	0.04	0.07	0.04
$10^{-3}k_{13}$ (M^{-1} sec^{-1})	2	—	2

[a] International Critical Tables gives 1.8×10^{-5} at 20°. [b] George *et al.* (1961) gives $7.4\ 10^{-10}$ at 10°.

Set II



workers have, however, considered only the binding rates in their quantitative interpretation over only a portion of the pH range available. Nevertheless, they found it necessary to include two ionizations on the protein to obtain an accurate fit of their apparent association rate data. Our somewhat lower temperature and considerably higher ionic strength would appear to account for the minor differences in apparent rates.

On the other hand when the experimentally accessible equilibrium dissociation constants are compared with values of $K_{(app)}$ calculated from the "Best Fit" parameters, the agreement is little better than obtained with set I. A more serious objection to the "Best Fit" parameters is that they are not fully consistent with all conditions imposed by set II, *e.g.*, $k_{21}''/k_{11}'' = k_{22}K_A/k_{12} \sim 2$. The dissociation constant of hydrazoic acid is again termed K_A.

Adequate self-consistency is obtained with the parameters in Table V under "Self-Consistent," but in Figures 2–4 the dashed lines show this case to be little improvement over the results obtained using set I. It is significant that considerable variation in K_B and K_C or K_P and K_Q may be allowed without causing much change in the rate parameters or improving the agreement with experiment provided internal consistency is maintained.

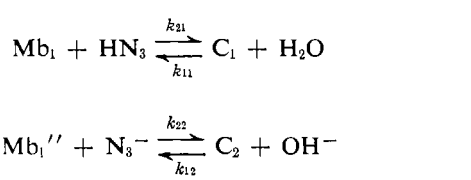
Equations 7–9 relate the observable quantities to the parameters of set II.

$$k_{2(app)} = \frac{(H)}{K_A + (H)}\left\{\frac{k_{21} + \frac{k_{21}'K_B}{(H)} + \frac{k_{21}''K_BK_C}{(H)^2}}{1 + \frac{K_B}{(H)} + \frac{K_BK_C}{(H)^2} + \frac{K_BK_CK_H}{(H)^3}}\right\} + \frac{k_{22}(H) + k_{23}K_H}{(H) + K_H} \quad (7)$$

$$k_{1(app)} = \left\{k_{11}(H) + k_{11}'K_P + \frac{k_{11}''K_PK_Q}{(H)} + \frac{k_{12}K_PK_Q}{(H)^2} + \frac{k_{13}K_PK_QK_W}{(H)^3}\right\}\left\{1 + \frac{K_P}{(H)} + \frac{K_PK_Q}{(H)^2}\right\}^{-1} \quad (8)$$

$$K_{(app)} = \frac{k_{11}''\{K_A + (H)\}\left\{\frac{1}{K_BK_C} + \frac{1}{K_C(H)} + \frac{1}{(H)^2} + \frac{K_H}{(H)^3}\right\}}{k_{21}''\left\{\frac{1}{K_PK_Q} + \frac{1}{K_Q(H)} + \frac{1}{(H)^2}\right\}} \quad (9)$$

Seemingly the only other likely alternate reaction scheme for the ferrimyoglobin azide system consists of modifying the right-hand sides of the ligand-binding reactions of set II as shown below.



These modifications would result, however, in no change in the calculated values of k_2(app) and would require

k_{12} to be greater than 10^{12} M^{-1} sec^{-1} or beyond the normal diffusion-controlled limit for solution reactions (Eigen, 1965).

Of the most likely reaction sequences, therefore, set I appears to offer a simple, fairly accurate description of the azide ferrimyoglobin system. Incorporation of as many as two protonic dissociations on the protein does not lead to significant change in the agreement between experimental and calculated values. Where discrepancies exist they are perhaps most easily attributed to dielectric changes stemming from protein ionizations that alter the effective value of K_A and the heme environment.

In conclusion it is interesting to note that though hydrazoic acid is the second most reactive ligand toward ferrimyolglobin yet studied, being exceeded only slightly by cyanic acid (Goldsack *et al.* (1965) report 1.8×10^6 M^{-1} sec^{-1}), its rate of combination is still considerably less than the rate with which oxygen combines with ferromyoglobin (Millikan, 1936; Gibson, 1959).

Acknowledgment

We wish to thank Mrs. Jutta Levin for her able technical assistance.

References

Beetlestone, J., and George, P. (1964), *Biochemistry 3*, 707.

Breslow, E., Beychok, S., Hardman, K. D., and Gurd, F. R. N. (1965), *J. Biol. Chem. 240*, 304.

Breslow, E., and Gurd, F. R. N. (1962), *J. Biol. Chem. 237*, 371.

Breslow, E., and Koehler, R. (1965), *J. Biol. Chem. 240*, 2266.

Caughey, W. S., Deal, R. M., Weiss, C., and Gouterman, M. (1965), *J. Mol. Spectry. 16*, 451.

Chance, B. (1952), *J. Biol. Chem. 194*, 483.

Chance, B. (1964), *in* Rapid Mixing and Sampling Techniques in Biochemistry, New York, N. Y., Academic, p 39.

Chance, B. (1965), *in* Discussions from Oxidases and Related Redox Systems, King, T. E., Mason, H. S., and Morrison, M., Ed. New York, N. Y., Wiley.

Chance, B., and Legallais, V. (1954), *Discussions Faraday Soc. 17*, 123.

Change, B., Ravilly, A., and Rumen, N. (1966), *J. Mol. Biol.* (in press).

Coryell, C. D., Stitt, F., and Pauling, L. (1937), *J. Am. Chem. Soc. 59*, 633.

Czerlinski, G. (1962), *Rev. Sci. Instr. 33*, 1184.

Czerlinski, G. (1964), *J. Theor. Biol. 7*, 435.

Duffey, D., Chance, B., and Czerlinski, G. (1965), *Biochem. Biophys. Res. Commun. 19*, 423.

Eigen, M. (1965), *Discussions Faraday Soc. 39*, 1.

George, P., and Hanania, G. I. H. (1954), *Biochem. J. 56*, xxxviii.

George P., Lyster, R. L. J., and Bettlestone, J. (1961), *J. Biol. Chem. 236*, 3246.

Gibson, Q. H. (1959), *Progr. Biophys. Biophys. Chem. 9*, 1.

Goldsack, D. E., Eberlein, W. S., and Alberty, R. A. (1965), *J. Biol. Chem. 240*, 4312.

Goldsack, D. E., Eberlein, W. S., and Alberty, R. A. (1966), *J. Biol. Chem. 241*, 2653.

Hammes, G. G. (1966), *Science 151*, 1507.

Hermans, J., and Rialdi, G. (1965), *Biochemistry 4*, 1277.

Kiese, M., and Kaeske, H. (1942), *Biochem. Z. 312*, 122.

Millikan, G. A. (1936), *Proc. Roy. Soc. (London) B 120*, 366.

Samejima, T., and Yang, J. T. (1964), *J. Mol. Biol. 8*, 863.

Stryer, L., Kendrew, J. C., and Watson, H. C. (1964), *J. Mol. Biol. 8*, 96.

Theorell, H., and Åkeson, A. (1955), *Acad. Sci. Fennicae, Ser. AII 60*, 303.