KINETICS OF THE FERRIMYOGLOBIN-AZIDE-SYSTEM

Donald Duffey, Britton Chance, Georg Czerlinski*
Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pennsylvania 19104

Received March 17, 1965

Crystalline sperm whale myoglobin (from Seravac Labs, Cape Town, South Africa) has been shown to be isomorphous with its crystalline azide (from Matheson, Coleman and Bell, Cincinnati) complex (Stryer, et al., 1964). The net reaction corresponds to a simple replacement of a water molecule from the coordination sphere of the ferric ion by the complexing agent. We were interested in studying this complexing process by both mixing and relaxation techniques. The approach to equilibrium from the side of the two reactants was observed with a stopped- and continuous-flow apparatus (Chance, 1964, 1965). Chemical relaxation was observed by perturbing the equilibrium in a temperature-jum apparatus (Czerlinski, 1962).

Equilibrium constants between pH 4.5 and 6.5 were determined by spectrophotometric titrations (Beckman Model DB and Cary Model 15) at 409 mμ and at 420 mμ for the reaction

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA$$

with E = active site of sperm whale ferrimyoglobin, A = (total) azide, and k_1, k_2 = the rate constants. The experiments were mainly conducted in citrate buffer. The equilibrium and kinetic results are summarized in Table I.

In the rapid-flow experiments and in the relaxation experiments, the ferrimyoglobin concentrations range from 2 to more than 200 μM. The azide concentrations vary from 1 μM to 100 mM. In spite of these

* To whom requests for reprints should be addressed

TABLE I

Summary of Equilibrium and Kinetic Results

pH	6.5	6.0	5.5	5.0	4.5
K (μM)	24 $\pm$ 4	24 $\pm$ 4	23 $\pm$ 4	26 $\pm$ 4	36 $\pm$ 2
$10^{-4}k_1(M^{-1}sec^{-1})^a$	1.4 $\pm$ 0.3	2.7 $\pm$ 0.4	---	14 $\pm$ 5	---
$k_2(sec^{-1})^{a,b}$	0.3 $\pm$ 0.1	0.8 $\pm$ 0.2	---	3 $\pm$ 2	---
$10^{-4}k_1(M^{-1}sec^{-1})^c$	0.9 $\pm$ 0.2	2.0 $\pm$ 0.5	6 $\pm$ 2	18 $\pm$ 4	70 $\pm$ 20
$k_2(sec^{-1})^c$	1.0 $\pm$ 0.2	1.4 $\pm$ 0.3	5 $\pm$ 1	5 $\pm$ 2	13 $\pm$ 4
$k_2/k_1(\mu M)^c$	110	70	83	28	19

a = Flow method

b = k_2 was obtained in the rapid-flow experiments by using the spectrophotometrically obtained dissociation constant K.

c = Relaxation method

large variations in concentrations, the rate constants never deviated significantly from those given in the Table. At pH 6, phosphate buffer was also used in place of citrate buffer, leading to values in the rate constants which are within the error limit given in Table I. The dependence of the rate constants upon ionic strength ($0.03 \leq \mu \leq 0.2$) seemed to be negligible. Table I also shows a reasonably good agreement between the kinetic parameters from the two different techniques.

The pH-dependence of k_1 can largely be attributed to a preferential association of ferrimyoglobin with protonated azide (Duffey and Czerlinski, 1965) similar to that observed by Chance (1952) with catalase. In fact a value for k_1 of $(8 \pm 4) \times 10^5 M^{-1}sec^{-1}$ may be calculated on the basis of complexing between the enzyme and hydrazoic acid only, over the pH range 4.5---6.5 where the dissociation constant of hydrazoic acid is taken as 2×10^{-5} M (from International Critical Tables). The pH-dependence of k_2, on the other hand, can be explained

most readily by assuming a protonic equilibrium on the protein (Duffey and Czerlinski, 1965).

It is interesting to find that the association rate constant for the binding of azide lies between the association rate constant for imidazole (around $10^2 M^{-1} sec^{-1}$ [Alberty, 1964]) and cyanide (around $10^3 M^{-1} sec^{-1}$ [George and Hanania, 1955] and [Chance, 1952]) to the ferri-form and that for O_2 to the ferro-form (Millikan, 1936). At low pH the on-rate for azide to the ferri-form is quite similar to the on-rate for CO to the ferro-form ($4 \times 10^5 M^{-1} sec^{-1}$ [Millikan, 1936] and [Gibson, 1959]). Differences in the rapid monomolecular interconversions between the diffusion-limited step and the step associated with the spectral change may account for these differences in the association rate constants (Duffey and Czerlinski, 1965). As a matter of fact, the temperature-jump experiments showed a very fast change at 424 mμ (still under investigation), which is quite indicative of at least one rapid (monomolecular) step.

While the titrimetrically determined dissociation constant (K in Table I) remains rather constant with pH, the dissociation constant derived from relaxation experiments (k_2/k_1 in Table I) shows a strong trend with pH. This disparity may result from the inclusion in K of all pertinent prior equilibria in addition to the process at 424 mμ, whereas k_2/k_1 refers primarily to the "slow" step. The difference between these association constants serves as an additional indication of one or more rapid monomolecular processes.

This work was supported in part by grants from the National Institutes of Health (GM-10888-02 and USPHS-5T1-GM-277-05).

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