proton introduces a repulsion. It is not clear from these experiments whether the second subunit undergoes a similar process after dissociation, but such a step could contribute to the irreversibility of dissociation.

Earlier results (Boeker and Snell, 1968) showed that the arginine decarboxylase dimer is at least much less active than the decamer and is probably inactive. In addition, the dimer of lysine decarboxylase, an enzyme with a very similar quaternary structure, is either inactive or absent under assay conditions (Sabo et al., 1974). Since arginine decarboxylase is produced by *E. coli* at acid pH (Melnykovich and Snell, 1958; Blethen et al., 1968), Gale (1946) has proposed that it serves to control the intracellular pH while Guirard and Snell (1964) have proposed that it controls the intracellular concentration of CO_2. Since either control would become less necessary as the pH increases, both suggestions are consistent with results presented here showing that the rate of dissociation to the apparently inactive dimer increases with pH. Since this dissociation can occur within a few minutes at pH values near neutrality, it may very well represent a physiological control mechanism for arginine decarboxylase activity.

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Mechanisms and Rate Equations for Dissociating Systems†

Elizabeth A. Boeker‡

ABSTRACT: The results presented in the previous paper (Boeker, E. A. (1978), *Biochemistry 17* (preceding paper in this issue)) indicate that the dissociation of the decamer of arginine decarboxylase of *Escherichia coli* B is enhanced by Na^+ and retarded by H^+. In this system, substances which increase the rate of dissociation can be treated kinetically either as substrates or activators, and substances which retard dissociation can be treated as products or inhibitors. In addition, the events needed for dissociation can occur in an ordered or a random sequence, and the dissociation itself, from a decamer to five dimers, can be a sequential or a concerted process. In order to provide a framework for the experimental results, mechanisms for the dissociation of arginine decarboxylase that take all of these factors into account are described. In addition, it is shown that the usual methods of steady-state kinetics can be applied to these systems when true initial rates are measured; rate equations are presented for each mechanism. The results can be used for any dissociating system of three or more subunits and will describe the dissociation of a dimer under certain conditions.

In the preceding paper (Boeker, 1978), the rate of dissociation of the inducible arginine decarboxylase of *Escherichia coli* B (Blethen et al., 1968; Boeker and Snell, 1968; Boeker et al., 1969, 1971; Boeker, 1977) was measured by allowing a sulfhydryl group, available on the dimer but not the decamer, to react with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB).[1] The results indicate that dissociation is enhanced by Na^+ and retarded by H^+ and can be used to deduce a dissociation mechanism for this enzyme. In order to verify this mechanism, it is necessary to derive rate equations for each of the theoretically possible mechanisms and to correlate them with the observed rate law. Although this type of analysis is sufficiently commonplace for enzyme reactions that mechanisms and rate equations have been extensively discussed (Cleland, 1963; Segel, 1975), similar discussions are not available for the dissociation of an enzyme into its subunits.

Two major differences between enzymic catalysis and dissociation must be considered. In dissociation, the final enzyme species is not identical with the original species; i.e., the enzyme does not behave like a catalyst. It is therefore not apparent that the ordinary methods of steady-state kinetics can be used to obtain equations for the rate of dissociation under various conditions. In addition, there is no way of knowing a priori whether a substance which promotes dissociation is doing so directly, as a substrate would in an enzyme catalyzed reaction, or indirectly, as an activator would; a substance which retards

† From the Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322. *Received August 15, 1977.* Supported in part by a grant from the Utah State University Research Development Fund.

‡ Established Investigator, American Heart Association.

[1] The abbreviations used are: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, thionitrobenzoate; E_{10}', arginine decarboxylase decamer (closed form); E_{10}, arginine decarboxylase decamer (open form); E_8, E_6, E_4, and E_2, arginine decarboxylase octamer, hexamer, tetramer, and dimer.

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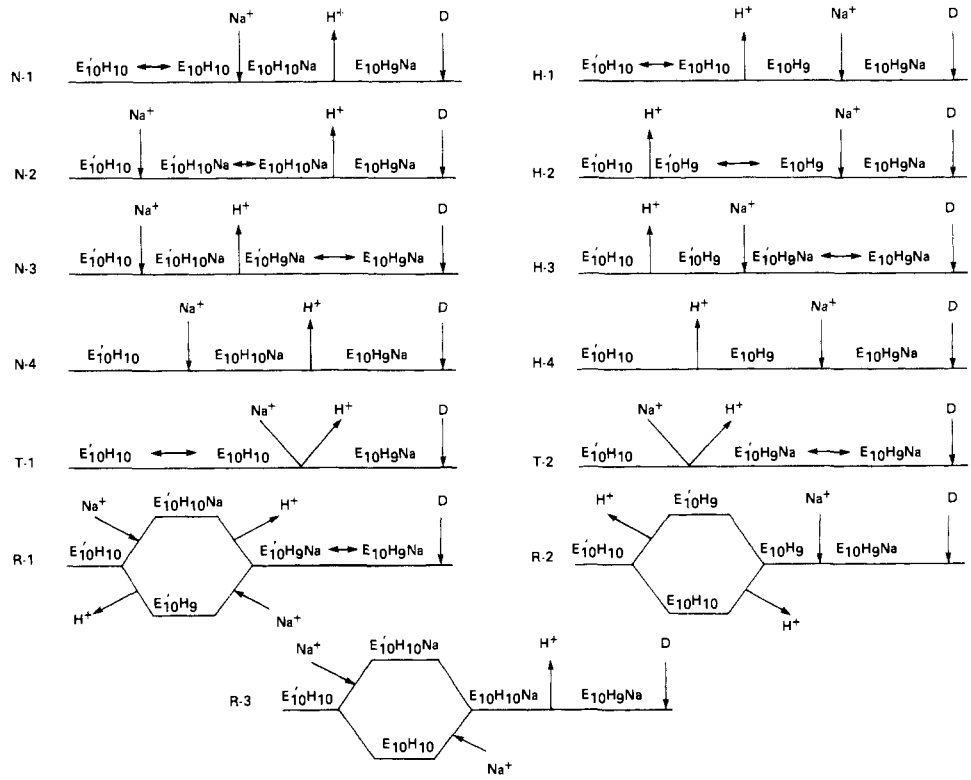

FIGURE 1: Mechanisms in which Na^+ behaves as a substrate and H^+ as a product. The diagrams apply specifically to the first step of a sequential process; they will apply to concerted processes if dimers replace the open form of the decamer. E_{10}' represents the closed decamer; E_{10} the open decamer; D, DTNB.

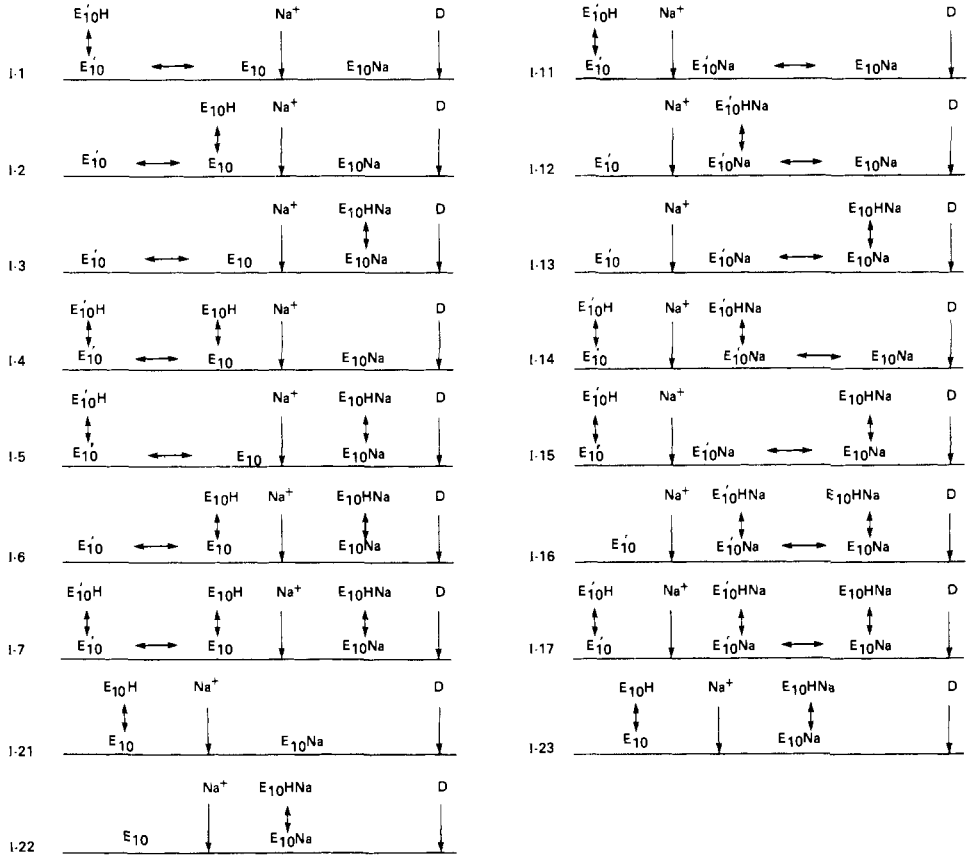

FIGURE 2: Mechanisms in which Na^+ behaves as a substrate and H^+ as an inhibitor. See the legend to Figure 1 for explanations.

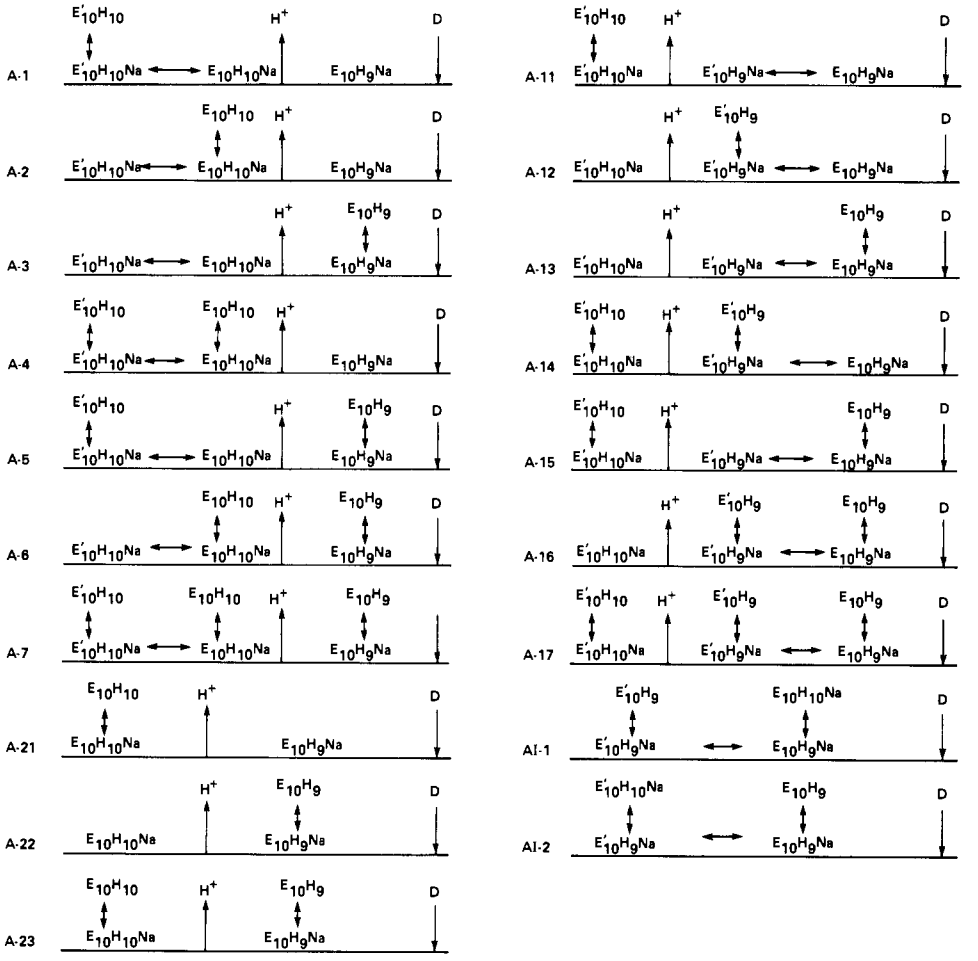

FIGURE 3: Mechanisms in which Na^+ behaves as an activator and H^+ as a product (A-1 to A-23) or an inhibitor (AI-1 and AI-2). See the legend to Figure 1 for explanations.

dissociation can also be acting either as a product or an inhibitor. The mechanisms considered must include both possibilities.

The experimental results (Boeker, 1978) show that the overall dissociation of arginine decarboxylase requires some combination of three events: Na^+ binding, H^+ ionization, and dissociation itself. The reaction of the newly formed dissociated species with DTNB must also be taken into account. In this paper, the possible combinations of these events that could lead to the experimental observations are set forth, and rate equations that can be tested against the observations are derived. It is assumed in all cases that the system behaves as if reaction with DTNB is the last step in the dissociation process. Although the dissociation of arginine decarboxylase is discussed specifically, the results are general and can be applied to most dissociating systems in the presence of an activator and/or an inhibitor.

Kinetic Considerations

A decamer can dissociate in one of two ways. In a concerted mechanism, the bonds between the dimers break simultaneously, while in a sequential mechanism, dissociation proceeds through a series of intermediate steps. The presence or absence of finite concentrations of intermediate species such as octamers, hexamers, etc., is the principal distinction between the two cases. Whether or not these intermediates are detectable of course depends on the technique for measuring dissociation.

Sequential Mechanisms. The sequential separation of a decamer into dimers can be thought of as requiring five steps. The first of these does not even produce a molecular weight change; the closed decamer is simply converted to an open form. The second step in such a process is indeterminate; either a dimer and an octamer or a hexamer and a tetramer could be produced. The experimental results (Boeker, 1978) show that, if the mechanism is sequential, each step must involve the binding of one Na^+ ion, the ionization of one proton, dissociation itself, and, for measurement purposes, reaction with DTNB. If, for example, Na^+ binding occurs first, H^+ ionization second, and dissociation third, the first step of the process can be written

$$E_{10}'H_{10} + Na^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_{10}'H_{10}Na \qquad (1)$$

$$E_{10}'H_{10}Na \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_{10}'H_9Na + H^+ \qquad (2)$$

$$E_{10}'H_9Na \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E_{10}H_9Na \qquad (3)$$

where E_{10}' represents the closed decamer and E_{10} the open decamer. In this step only, the reassociation reaction (k_6) is first order with respect to enzyme concentration; it will be second order in all of the other dissociation steps. In the presence of DTNB, the two newly available sulfhydryls (Boeker et al., 1969) on the dissociated species $E_{10}H_9Na$ can now react with DTNB. Thus, for one of them

$$E_{10}H_9Na + DTNB \xrightarrow{k_7} E_{10}H_9Na\cdot TNB + TNB \qquad (4)$$

TABLE I: Rate Equations Lacking a Term in $[H]/K_H$.[a]

Mechanism	Rate equation
N-1, N-2, N-4, A-1, A-2, A-4, R-3[b]	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D[H]}{[D]K_H} + \frac{K_N'K_D[H]}{[N][D]K_H}\right)$
H-1, H-3, H-4, I-2, I-11	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D'K_N}{[D][N]} + \frac{K_N[H]}{[N]K_H} + \frac{K_D'K_N[H]}{[D][N]K_H}\right)$
T-1	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_DK_N[H]}{[D][N]K_H}\right)$
T-2	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D'K_N}{[D][N]} + \frac{K_N[H]}{[N]K_H}\right)$
I-3, I-13, IA-1	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D'K_N}{[D][N]} + \frac{K_D[H]}{[D]K_H}\right)$
I-6	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D'K_N}{[D][N]} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D[H]}{[D]K_H} + \frac{K_D'K_N'[H]}{[D][N]K_H}\right)$
I-21, R-2	$R_m \Big/ \left(\frac{1}{[D]} + \frac{K_N}{[N]} + \frac{K_DK_N}{[D][N]} + \frac{K_N[H]}{[N]K_H} + \frac{K_DK_N[H]}{[D][N]K_H}\right)$
I-22	$R_m \Big/ \left(\frac{1}{[D]} + \frac{K_N}{[N]} + \frac{K_DK_N}{[D][N]} + \frac{[H]}{[D]K_H}\right)$
I-23	$R_m \Big/ \left(1 + \frac{[H]}{K_H}\right)\left(\frac{1}{[D]} + \frac{K_N}{[N]} + \frac{K_DK_N}{[D][N]}\right)$
A-3, A-22	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_D[H]}{[D]K_H} + \frac{K_DK_N}{[D][N]}\right)$
A-5, A-6, A-23[b]	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D[H]}{[D]K_H} + \frac{K_DK_N'}{[D][N]} + \frac{K_D'K_N'[H]}{[D][N]K_H}\right)$
A-7	$R_m \Big/ \left(1 + \frac{K_N}{[N]}\right)\left(1 + \frac{K_D}{[D]} + \frac{K_D[H]}{[D]K_H}\right)$
A-21	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D[H]}{[D]K_H} + \frac{K_N[H]}{[N]K_H}\right)$

[a] The compound constants K_D, K_N, K_H, K_D', and K_N' are defined in such a way that their number is minimized; R_m is the maximum attainable rate of dissociation. An example of the definitions is given in Table II. [b] In the equations for mechanism R-3 and A-23, $K_D' = K_D$ and $K_N' = K_N$.

As this sequence is written, Na^+ and H^+ do not behave symmetrically with respect to the pair of subunits undergoing dissociation; the Na^+ ion is bound and the proton is lost by only one member of the pair. Symmetrical behavior would require two each. Because the experimental results indicated that only one H^+ and one Na^+ are involved at each step, an asymmetric species such as $E_{10}H_9Na$ must be assumed to be reacting with DTNB. These species could subsequently bind an additional Na^+ and lose an additional H^+ to form intermediates with equivalent subunits. Taking this possibility into account, there are now a number of ways to write the second dissociation step; one possibility is

$$E_{10}H_8Na_2 + Na^+ \rightleftharpoons E_{10}H_8Na_3$$

$$E_{10}H_8Na_3 \rightleftharpoons E_{10}H_7Na_3 + H^+$$

$$E_{10}H_7Na_3 \rightleftharpoons E_8H_6Na_2 + E_2HNa$$

In this step, both $E_8H_6Na_2$ and E_2HNa have newly available sulfhydryls that can react with DTNB.

If each of the dissociation steps is written out as above, expressions for $d[TNB]/dt$ can be obtained; these expressions are so complex as to be uninterpretable. This problem can most easily be avoided by measuring only the first dissociation step; that is, by measuring initial rates. The entire system then reduces to eq 1 through 4, which can readily be solved for $d[TNB]/dt$.

In this analysis, the initial rate condition is rather different from, and considerably more stringent than, that for enzyme reaction kinetics. It is necessary not only that the concentrations of Na^+, H^+, and DTNB remain essentially unchanged, but also that no enzyme species beyond the first dissociated state be present in significant concentrations. In the example shown, there must not be significant amounts of intermediates beyond $E_{10}H_9Na$. This imposes very strict requirements on the

TABLE II: Rate Equations with a Term in $[H]/K_H$.[a]

Mechanism	Rate equation
I-7, I-17	$R_m \Big/ \left(1 + \frac{[H]}{K_H}\right)\left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D' K_N}{[D][N]}\right)$
I-12	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H}\right)$
I-15, I-16, A-12, A-16[b]	$R_m \Big/ \left(1 + \frac{K_N}{[N]} + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N' K_D}{[N][D]}\right)$
A-13, IA-2	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D K_N}{[D][N]} + \frac{K_D'[H]}{[D]K_H}\right)$
N-3, A-11	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
H-2, I-1, I-4	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D' K_N}{[D][N]} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
I-5	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D' K_N}{[D][N]} + \frac{K_D[H]}{[D]K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
I-14	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D' K_N'}{[D][N]} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
R-1	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D' K_N}{[D][N]} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
A-14	$R_m \Big/ \left(1 + \frac{K_N}{[N]} + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D K_N'}{[D][N]} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N[H]}{[N]K_H} + \frac{K_D' K_N[H]}{[D][N]K_H}\right)$
A-15	$R_m \Big/ \left(1 + \frac{K_N}{[N]} + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D K_N'}{[D][N]} + \frac{K_D'[H]}{[D]K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D' K_N'[H]}{[D][N]K_H}\right)$
A-17	$R_m \Big/ \left(\left(1 + \frac{K_N}{[N]}\right)\left(1 + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H}\right)\right)$

[a] The compound constants K_D, K_N, K_D', and K_N' are defined in such a way that their number is minimized; R_m is the maximum attainable rate of dissociation. In mechanism N-3, used as an example in Methods, $K_D = k_3(k_5 + k_7)/k_7(k_3 + k_5)$, $K_N = k_5(k_2 + k_3)/k_1(k_3 + k_5)$, $K_H = (k_3 + k_5)/k_4$, $K_D' = k_6/k_7$, $K_N' = k_2/k_1$, and $R_m = k_3k_5/(k_3 + k_5)$. [b] In mechanisms I-15 and I-16, $K_D' = K_D$.

experimental measurements. When these requirements are met and there are no intermediates present beyond the first dissociated state, the system behaves as if the last step in the sequence were producing the original enzyme species, just as it would in ordinary enzyme catalysis. Rate equations for each mechanism can therefore be derived by the ordinary methods of steady-state kinetics, including the method of King and Altman (1956). The equations shown in this paper have been obtained in this way.

Concerted Mechanisms. Rate equations for concerted mechanisms can be obtained in the same way as those for sequential mechanisms if it is assumed that reassociation, which depends on the fifth power of the dimer concentration, does not occur. The sequential mechanism shown above becomes a concerted mechanism if eq 3 is written:

$$E_{10}'H_9Na \rightarrow 4E_2H_2 + E_2HNa$$

In this example, four of the dissociation events differ from the fifth. This is unlike the sequential mechanisms, where the dissociation process is the same for each pair of subunits undergoing dissociation, but the events which occur on each member of the pair are different. Mechanisms in which there are two types of dissociation are extremely unlikely. In the example above, the dissociation processes are identical only if 5 (or 10) Na^+ ions and protons are involved in the initial steps. For example

$$E_{10}'H_{10} + 5Na^+ \rightleftharpoons E_{10}'H_{10}Na_5$$

$$E_{10}'H_{10}Na_5 \rightleftharpoons E_{10}'H_5Na_5 + 5H^+$$

$$E_{10}'H_5Na_5 \rightarrow 5E_2HNa$$

Mechanisms such as this produce fifth (or tenth) order dependences on Na^+ and H^+, a result which is eliminated experimentally.

Concerted mechanisms can give first-order dependences on Na^+ and H^+, and still have five identical dissociation steps, only if dissociation occurs before Na^+ binding and H^+ ion-

TABLE III: Rate Equations for Mechanisms with Irreversible Steps.[a]

Mechanism	Rate equation
A-11, $k_2 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]}\right)$
A-14, A-15, A-17,[b] $k_2 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{K_D K_N'}{[D][N]}\right)$
R-1, k_2, $k_8 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H}\right)$
R-1, $k_{10} = 0$	$R_m \Big/ \left(1 + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_N'[H]}{[N]K_H}\right)$
N-3, $k_2 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D'[H]}{[D]K_H}\right)$
H-2, $k_4 = 0$; I-1, $k_2 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D'K_N}{[D][N]}\right)$
I-5, $k_2 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_D'K_N}{[D][N]} + \frac{K_D[H]}{[D]K_H}\right)$
I-5,[b] I-14,[b] $k_4 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D'[H]}{[D]K_H}\right)$
A-15, A-17,[b] $k_4 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_N'[H]}{[N]K_H} + \frac{K_D K_N'}{[D][N]}\right)$
N-3, H-2, I-4, $k_6 = 0$; I-1, A-11, A-14,[b] $k_4 = 0$	$R_m \Big/ \left(1 + \frac{K_D}{[D]} + \frac{K_N}{[N]} + \frac{[H]}{K_H} + \frac{K_N'[H]}{[N]K_H}\right)$

[a] An example of the definition of the constants K_D, K_N, K_H, K_D', and K_N' is given in Table II. [b] In Mechanisms A-14, A-17, and I-14, $K_N' = K_N$, and in I-5, $K_D' = K_D$.

ization. An example of this type of mechanism is

$$E_{10}'H_{10} \rightarrow 5E_2H_2$$

$$E_2H_2 + Na^+ \rightleftharpoons E_2H_2Na$$

$$E_2H_2Na \rightleftharpoons E_2HNa + H^+$$

$$E_2HNa + DTNB \rightarrow E_2HNa{\cdot}TNB + TNB$$

In all of the mechanisms where the dependences on Na^+ and H^+ can be first order, the irreversible dissociation step occurs first; kinetically, this means that the rate equations obtained are identical with those for the corresponding sequential cases. The two cases are therefore not treated separately in this paper.

Mechanisms and Rate Equations

There are four factors which must be taken into account in defining the possible ways in which arginine decarboxylase dissociation can occur: It can be sequential or concerted, the reactions with Na^+, H^+, and DTNB can be ordered or random, the Na^+ ion can be treated kinetically as a substrate or as an activator, and the proton as a product or an inhibitor. The possible combinations of Na^+ binding, H^+ ionization, and dissociation are shown in Figures 1–3. In each case, the dissociation process is shown only as far as the first reaction with DTNB; i.e., the only steps shown are those which are kinetically visible when initial rates are measured.

If the dissociation process is ordered and if Na^+ behaves as a substrate and the proton as a product, there are six possible combinations of binding, ionization and dissociation. These are mechanisms N-1 to N-3 and H-1 to H-3 in Figure 1. Kinetically, there are four additional cases in which one of the intermediate enzyme species is invisible under experimental conditions. These are equivalent to Theorell–Chance mechanisms and are N-4, H-4, T-1, and T-2 in Figure 1. Although Theorell–Chance mechanisms are really only simplifications of other cases, they are considered separately here because the empirical rate law apparently represents such a simplification (Boeker, 1978).

If the dissociation process is ordered and if Na^+ behaves as a substrate, but the proton is assumed to be a dead end inhibitor rather than a product, there are two possible orders of binding and ionization, each with seven ways in which the proton can form complexes. These are mechanisms I-1 to I-7 and I-11 to I-17 in Figure 2. There are also three Theorell–Chance mechanisms, I-21 to I-23.

Possible sequences for ordered dissociation with Na^+ behaving as an activator and H^+ as a product are shown in Figure 3. In these mechanisms, enzyme species which lack a Na^+ ion act as dead end complexes. There are two possible orders, shown in A-1 to A-7 and A-11 to A-17, and three Theorell–Chance mechanisms, A-21 to A-23. There are also two mechanisms in which Na^+ behaves as an activator and H^+ as an inhibitor; these are AI-1 and AI-2 in Figure 3.

The experimental observations impose several constraints on the possible types of mechanisms in which Na^+ binding, H^+ ionization, and dissociation can occur in random order. Only rapid equilibrium random mechanisms will give kinetics which are first order with respect to Na^+, H^+, and DTNB. The mechanisms must also contain at least one slow, nonequilibrium step; if all of the steps were in rapid equilibrium, the dimer and the decamer would not be separable in the ultracentrifuge (Boeker and Snell, 1968). In an ordinary enzymatic reaction, the nonequilibrium step could occur anywhere in the sequence; i.e., either before or after the two random steps. However, in this system, it can only be last. As discussed above, the enzyme species formed after the addition of DTNB is not the same as the first enzyme species in these mechanisms. This means that an initial slow step will not produce a steady state involving the first and last reactions, but will simply produce a preliminary slow equilibrium. Kinetically, such mechanisms do not result in the observed saturation by DTNB and have therefore been eliminated. The remaining rapid equilibrium random cases, in which the random steps are followed by a slow step, are mechanism R-1 to R-3 in Figure 1.

In each of the mechanisms just described, dissociation can be either sequential or concerted. However, as discussed above, concerted mechanisms will give kinetics that are fifth order or higher in all cases except those where both Na^+ binding and H^+ ionization occur after dissociation. The only possible concerted mechanisms, then, are N-1, N-4, T-1, H-1, H-4, I-2, I-3, I-6, A-2, A-3, and A-6.

The rate equations for the mechanisms in Figures 1–3 are shown in Tables I and II. Many of these equations lack a denominator term in $[H^+]$ alone, depending on it only in combination with other variables. Since these mechanisms are readily eliminated experimentally, they have been grouped together in Table I. The first five equations in Table II can also be eliminated experimentally, one because a term in $1 + [H^+]/K_H$ factors in the denominator, and the others because they lack a term in $[H^+]/[Na^+]$.

The last eight equations in Table II have all of the experimentally required terms, but they also contain a denominator term in $[H^+]/[DTNB]$ which the results (Boeker, 1978) show must be negligible. Since this appears to be a fundamental property of the dissociation mechanism, rather than a result of the concentrations chosen, the actual mechanism must have a simplifying property such as an irreversible step. The rate equations obtained when each of the possible mechanisms is assumed to have one irreversible step are shown in Table III. Most of these equations lack one or more of the experimentally required terms. However, two of them, the last two in Table III, fulfill all of the experimental requirements, containing denominator terms in $[H^+]$, $1/[Na^+]$, $1/[DTNB]$, and $[H^+]/[Na^+]$, and lacking a term in $[H^+]/[DTNB]$. Altogether, these equations represent eight possible mechanisms. However, five of them (N-3, A-11, A-14, A-15, and A-17) represent the same sequence of events, differing only in the number of complexes which lack Na^+ (see Figures 1 and 3). In addition, mechanisms H-2 and I-4 differ only in their kinetic details, not in the actual events (see Figures 1 and 2). The rate equations which are experimentally possible, then, represent only three underlying sequences through which dissociation can occur.

Mechanisms and rate equations have been presented here in very specific terms: dissociation of the arginine decarboxylase decamer in the presence of an activator and an inhibitor. These results can easily be generalized to other dissociating systems. The method for obtaining rate equations is independent of the type of dissociation; it requires only that true initial rates be measured. The equations themselves can be applied directly to systems which lack an inhibitor or an activator simply by setting the appropriate concentrations equal to zero. Mechanisms where both of the dissociating subunits interact with the activator or inhibitor will simply depend on the square of the appropriate concentration.

The mechanisms and equations discussed here are applicable, without modification, to dissociation in any system containing three or more subunits; e.g., conversion of a closed trimer to an open form, etc. Dissociation of a dimer to monomers is more complex, however, since the reassociation reaction is second order with respect to the enzyme concentration. If, as a first approximation, the dissociation reaction is assumed to be irreversible, these systems are then equivalent to the concerted cases discussed here. Rate equations for a number of these cases have been presented; the remainder can easily be obtained.

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