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Arginine Decarboxylase from *Escherichia coli* B: Mechanism of Dissociation from the Decamer to the Dimer[†]

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ABSTRACT: The mechanism by which arginine decarboxylase dissociates from a decamer to a dimer has been examined by allowing a sulfhydryl group, available in the dimer but not the decamer, to react with 5,5′-dithiobis(2-nitrobenzoic acid). Initial rates of dissociation were obtained by following the resulting increase in absorbance at 412 nm in a stopped-flow spectrophotometer. The rate of dissociation increases linearly with the protein concentration and reaches a maximum as a function of the concentrations of 5,5′-dithiobis(2-nitrobenzoic acid), Na^+, and $1/[H^+]$. Experiments in which the rate of dissociation was measured while one reagent was varied at fixed levels of a second indicate that dissociation requires three events: binding of one Na^+ ion, dissociation of one proton, and the irreversible dissociation of subunits, in that order. The results also show that the decamer dissociates in stages rather than all at once. The activation energy for the overall process is 16 kcal/mol.

Interactions between the subunits of enzymes have been investigated almost exclusively from a thermodynamic point of view, since instruments such as the analytical ultracentrifuge provide information only about the equilibria that exist between associated and dissociated states. An entirely different type of information, the underlying mechanism of the dissociation process, would be obtained if kinetic techniques could be adapted to the study of dissociating systems. This is not ordinarily possible, since enzymes which dissociate undergo only a molecular weight change, not a chemical change; there is usually no way to assay the newly formed species. In cases where dissociation makes available a previously unreactive group, however, it should be possible to develop such assays and to study dissociation kinetically.

The inducible arginine decarboxylase of *Escherichia coli* B appears to be particularly favorable for such a study; dissociation from the decamer to the dimer unmasks one sulfhydryl group per subunit (Boeker et al., 1969). Since there are no other available sulfhydryls, it is possible to assay for dissociation by titrating this group with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)[1] (Boeker, 1977).

As determined in the analytical ultracentrifuge (Boeker and Snell, 1968), arginine decarboxylase occurs as a decamer (mol wt 820 000) at pH values below 6.5 or salt concentrations above 0.08 M and dissociates to dimers (mol wt 160 000) as the pH increases and the salt concentration decreases. Dissociation appears to be complete above pH 7 and below 0.04 M salt. The ultimate subunits (mol wt 82 000) are identical by several criteria (Boeker et al., 1969). Electron micrographs show that the decamer consists of five dimers arranged in a pentamer. Unless there is more than one type of interdimer bond in this structure, these bonds must be heterologous, or head to tail (Boeker et al., 1969). The intradimer bonds, on the other hand, must be isologous, or head to head, since the decamers themselves show no tendency to associate. The subunit structure of *E. coli* lysine decarboxylase is very similar (Sabo et al., 1974).

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[1] The abbreviation used is: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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Since high concentrations of either protons or Na^+ ions are required for decamer formation, while low concentrations of both favor the dimer, it seems likely that arginine decarboxylase loses both Na^+ ions and protons when it dissociates. However, the results do not indicate how many are lost, nor do they give any clues to their roles. Since the intermediate dissociation states represented by the tetramer, hexamer, and octamer have been observed in the ultracentrifuge only during reassociation and only when a substrate analogue was present, it is not clear whether dissociation occurs by a sequential or a concerted mechanism. A kinetic technique for answering these questions is developed in this paper. Under the proper conditions, the kinetics of dissociation are analogous to enzyme reaction kinetics: H^+, Na^+, and DTNB behave as if they were substrates and products or activators and inhibitors. Their mode of action can therefore be defined by measuring the rate of dissociation when one is varied at fixed levels of a second, as is done for ordinary enzyme reactions (Cleland, 1963).

A preliminary investigation of the dissociation mechanism has been carried out by measuring rates of dissociation under a variety of conditions in an ordinary spectrophotometer (Boeker, 1977). However, interpretable rate equations can be derived only if the rates measured are true initial rates; this condition is more restrictive than the usual initial rate condition for enzyme kinetics (Boeker, 1978). In order to be certain that true initial rates were obtained, the experiments presented here were carried out in a stopped-flow spectrophotometer.

Methods

Arginine decarboxylase was purified by the procedure of Boeker et al. (1969) and was assayed with the technique of Morris and Pardee (1965) in 0.2 M sodium acetate buffer, pH 5.2, containing 0.15 M L-[U-^{14}C]arginine and 0.05 mM pyridoxal-P. Protein concentrations were determined in a Beckman DB spectrophotometer using an extinction coefficient, $E_{280}{}^{1\%}$, of 15.7 (Blethen et al., 1968).

DTNB was obtained from Sigma; solutions were prepared by titrating aqueous mixtures of the free acid to pH 4 with NaOH and were filtered. DTNB concentrations were determined by diluting the stock solution with 10 mM cysteine in 0.1 M sodium phosphate buffer, pH 7.2, and reading the absorbance at 412 nm. An extinction coefficient of 13.6 mM^{-1} for the thionitrobenzoate anion (Ellman, 1959) was used for calculating both the concentration of DTNB and the rate of dissociation of arginine decarboxylase.

In all cases, the pH values reported were measured on a Corning Model 10 pH meter. When necessary, phosphate or Na^+ concentrations were calculated using a pK_a of 6.8 for $H_2PO_4^-$ (Alberty et al., 1951; Bernhard, 1955).

Measurements of the rate of arginine decarboxylase dissociation were carried out in an Aminco-Morrow stopped-flow apparatus equipped with a Digital Electronics PDP-8 computer for data acquisition. Solutions of arginine decarboxylase and DTNB, as specified below, were allowed to stand for 15 min in the mixing syringes in order to ensure thermal equilibrium, and dissociation was begun by mixing 100 μL of each. Measurements were made from the first 5% of the total reaction in order to be certain that true initial rates were obtained.

Arginine decarboxylase solutions were prepared by dialyzing the enzyme exhaustively against 1 or 2 mM NaH_2PO_4, which had been titrated to pH 5.2 with NaOH, and diluting to the appropriate concentration with dialysis buffer. Arginine decarboxylase solutions contained at least 95% decamer initially, as determined in a Beckman Model E analytical ultracentrifuge.

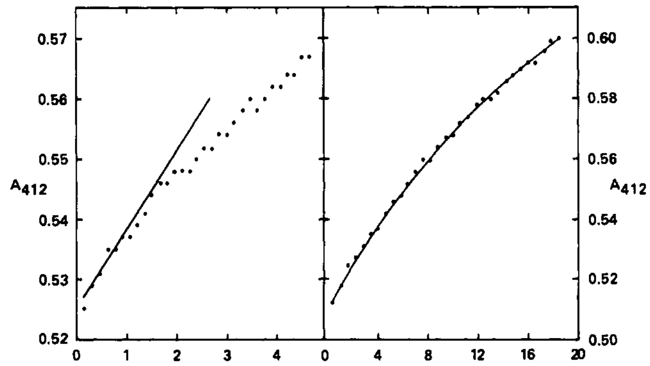

FIGURE 1: Dissociation of arginine decarboxylase, measured in the stopped-flow apparatus. The data shown are part of the protein concentration study described in the text. Arginine decarboxylase was 3 mg/mL. The initial rate is shown on the left, where the total absorbance change shown is 8% of that which would occur if all of the arginine decarboxylase present were to dissociate. The absorbance change shown on the right is 18% of the total possible.

For the protein concentration and temperature dependence studies, dissociation was begun with a solution containing 9.6 mM NaH_2PO_4 and 10 mM DTNB, titrated to pH 7.6 with NaOH. The Na^+ concentration of this solution was 38 mM, as calculated from the amount needed to neutralize the DTNB and $H_2PO_4^-$. These conditions were also used for the DTNB dependence experiment at pH 7.25 except that the DTNB concentration was varied appropriately and the Na^+ concentration was maintained at 38 mM by adding NaCl as needed. When DTNB was varied at pH 6.54, dissociation was begun with a solution which had been titrated to pH 6.57 with H_3PO_4 and which contained 4.5 mM Na_2HPO_4 and the appropriate concentrations of DTNB and NaCl. For the pH dependence study, solutions containing 25 mM Na_2HPO_4, 28 mM NaCl, and 1 mM DTNB were titrated to the appropriate pH with H_3PO_4 and used to initiate dissociation. Similarly, the solutions used for the Na^+ dependence studies contained, with one exception, 4.5 mM Na_2HPO_4, 2.0 mM DTNB, and NaCl as needed, and were titrated either to pH 7.60 or 6.48 with H_3PO_4. The solution used for the lowest Na^+ concentration at pH 6.32 contained 2.5 mM Na_2HPO_4 and 2.0 mM DTNB and was titrated to pH 6.55.

Results

Figure 1 shows the type of results obtained when arginine decarboxylase dissociates in the presence of DTNB. All of the results presented here were obtained in a similar way: dissociation was begun by increasing the pH in the presence of fixed concentrations of Na^+, DTNB, etc., and followed by the increase in absorbance at 412 nm. In general, the observed rate of dissociation was divided by the extinction coefficient of thionitrobenzoate (Ellman, 1959) and the protein concentration in order to obtain an apparent rate constant. The kinetic data are presented in plots similar to Eadie plots.

The rate of dissociation of arginine decarboxylase was first measured as a function of the protein concentration, between 0.5 and 3.0 mg/mL. In this experiment, dissociation was begun by mixing equal parts of arginine decarboxylase (in a pH 5.2 P_i buffer) and DTNB (in a pH 7.6 P_i buffer). The final conditions were: Na^+, 20 mM; P_i, 5.8 mM; DTNB, 5 mM; pH, 7.25; μ, 39 mM; T, 37.2 °C. The results of this experiment showed clearly that dissociation is first order with respect to protein concentration. Although this is the expected result regardless of the dissociation mechanism, it is extremely im-

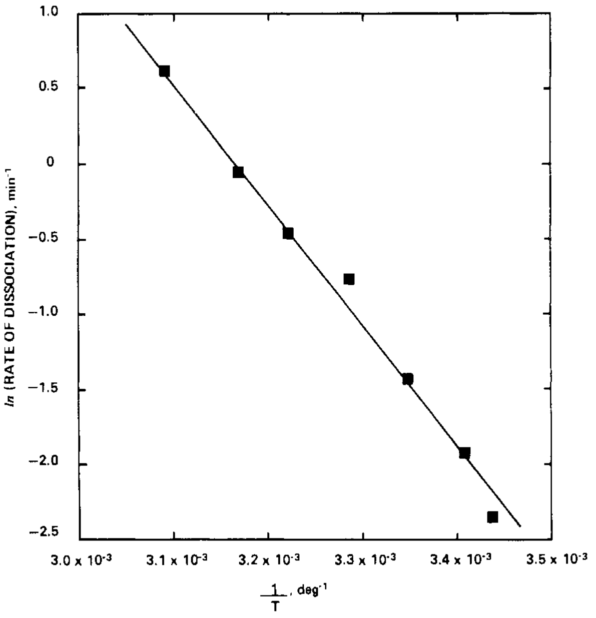

FIGURE 2: Dissociation of arginine decarboxylase as a function of temperature. Dissociation was begun by mixing equal parts of arginine decarboxylase (in a pH 5.2 P_i buffer) and DTNB (in a pH 7.6 P_i buffer). Final conditions: arginine decarboxylase, 2 mg/mL; Na^+, 20 mM; P_i, 5.8 mM; DTNB, 5 mM, pH 7.25; μ, 39 mM. The measured rates have been divided by the concentration of arginine decarboxylase, using a subunit mol wt of 82 000.

portant; it means that results obtained at the protein concentrations used here, which are around 2 mg/mL, are applicable to much lower concentrations.

The change in the rate of dissociation as a function of temperature between 17.7 and 50.5 °C is shown in Figure 2. The activation energy calculated from this plot is 16 kcal/mol. The temperature limitations of this measurement were dictated by the temperature regulation on the stopped-flow apparatus rather than by arginine decarboxylase, which is a very heat stable enzyme (Blethen et al., 1969).

The dependence of the rate of dissociation on the concentration of DTNB is shown in Figure 3. Although DTNB is not involved in the actual dissociation, it is required for the dissociation assay and therefore behaves kinetically as if it were the last step in the process. Since initial rates are being measured, there is no reason to expect that the DTNB reaction will be anything other than first order; i.e., the kinetically visible reaction will be that with the first sulfhydryl group to become available. Figure 3 shows that this is indeed the case. Unlike the previous results where rapid mixing was not used (Boeker, 1977), Figure 3 also shows that the reaction is saturable with DTNB. The amount of kinetically available information will, therefore, not be reduced by a rate-limiting assay reaction. The DTNB concentration producing half the maximum rate of dissociation is 2.5 mM at pH 7.25 and 0.5 mM at pH 6.54; both experiments were performed at a nonsaturating Na^+ concentration of 20 mM.

The previous results (Boeker, 1977) indicated that the rate of dissociation was second order with respect to both $[H^+]$ and $[Na^+]$. When rapid mixing is used, as in Figures 4 and 5, the dependence is clearly first order. It is also clear from these experiments that, since the rate of dissociation increases with increasing $[Na^+]$ and decreasing $[H^+]$, Na^+ is behaving as a substrate or an activator would in an enzymatic reaction while H^+ is behaving as a product or an inhibitor would. Because the behavior with respect to Na^+ and H^+ is first order, it appears that the binding of only a single Na^+ ion and the loss of only a single proton are required to initiate dissociation. The Na^+ concentration giving half the maximum rate of dissociation is 9.1 mM at pH 7.02 and 7.0 mM at pH 6.32; both numbers were obtained at a nonsaturating DTNB concentration of 1.0 mM. The pH value corresponding to the half-maximal rate is 7.7 at 40 mM Na^+ and 0.5 mM DTNB.

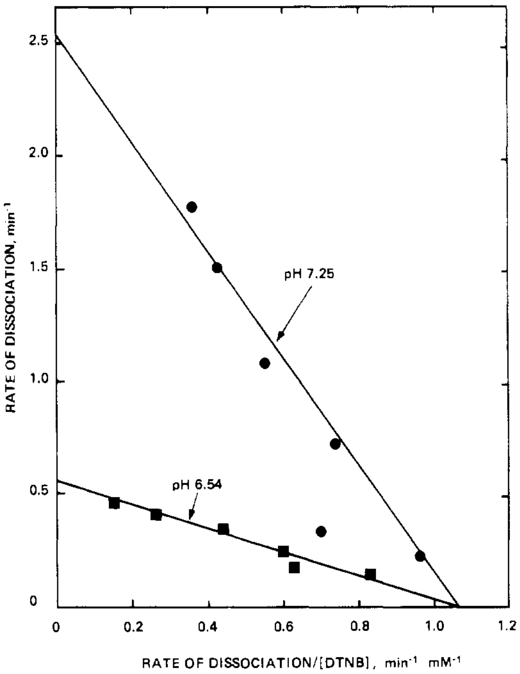

FIGURE 3: Dissociation of arginine decarboxylase as a function of DTNB concentration. Dissociation was begun by mixing equal parts of arginine decarboxylase (in a pH 5.2 P_i buffer) and DTNB (in a pH 6.57 or 7.60 P_i buffer). Final conditions: arginine decarboxylase, 2 mg/mL; Na^+, 20 mM; P_i, 4.1 mM at pH 6.54 and 5.8 mM at pH 7.25; Cl^-, 0 to 15 mM; μ, 22 to 25 mM at pH 6.54 and 25 to 29 mM at pH 7.25; T, 37.2 °C. The measured rates have been divided by the concentration of arginine decarboxylase, using a subunit mol wt of 82 000.

If the empirical rate law is written in the form $r = R_m$/denominator, where r is the observed, and R_m the maximum rate of dissociation, the results in Figures 3–5 show that the denominator must contain terms in $1/[Na^+]$, $1/[DTNB]$, and $[H^+]$, whether alone or in combination with some other variable. In order to define the rate law more closely, the experiments shown in Figures 3 and 4 were conducted at two pH values. In the plots shown, the intercept at $r = 0$ gives the value of R_m/K_{app} for the variable substrate. Since this intercept changes in Figure 4, where Na^+ is varied at fixed H^+, the denominator of the rate law must have a term in $[H^+]/[Na^+]$. Similarly, since this intercept is constant in Figure 3, where DTNB is varied at fixed pH, the denominator must lack terms in $[H^+]/[DTNB]$, at least under the conditions used.

In both Figures 3 and 4, the apparent maximum rate varies with the pH. The denominator of the rate law, therefore, must either have a term in $[H^+]$ alone or separate terms in both $[H^+]/[Na^+]$ and $[H^+]/[DTNB]$. Since the second possibility has already been eliminated, the minimum rate law that will satisfy the experimental observations must have a denominator term in $[H^+]$ alone, terms in $1/[Na^+]$, $1/[DTNB]$, and $[H^+]/[Na^+]$ either alone or in combination, and must be simplifiable so that it will lack terms in $[H^+]/[DTNB]$.

In the following paper (Boeker, 1978), I have defined 49 possible sequences of events that could lead to the observed rate law and have derived equations for them. The cases considered include sequential and concerted dissociation steps, protons as either products or inhibitors, Na^+ ions as either substrates

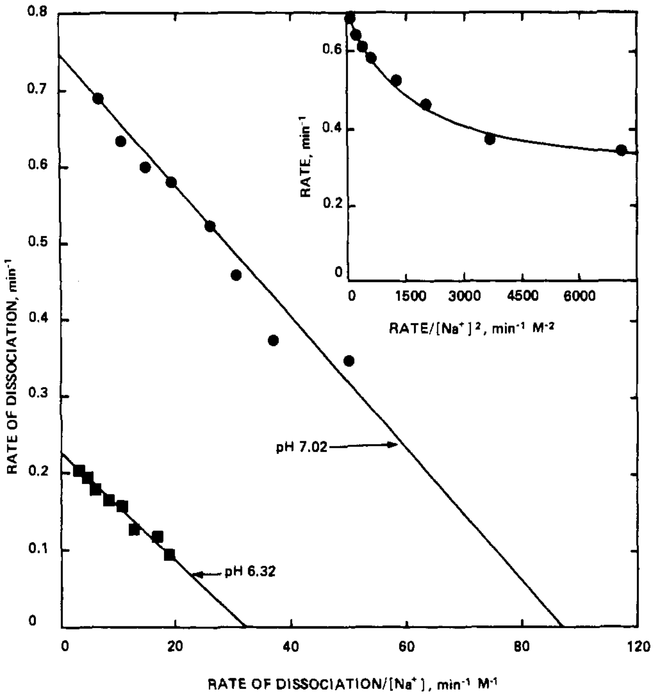

FIGURE 4: Dissociation of arginine decarboxylase as a function of Na^+ concentration. Dissociation was begun by mixing equal parts of arginine decarboxylase (in a pH 5.2 P_i buffer) and DTNB (in a pH 6.48 or 7.60 P_i buffer). Final conditions: arginine decarboxylase, 2 mg/mL; P_i, 7.3 mM at pH 6.32 and 4.6 mM at pH 7.02, except for the lowest Na^+ concentration at pH 6.32 where it is 4.1 mM; DTNB, 1.0 mM; Cl^-, 0 to 53 mM at pH 6.32 and 0 to 93 mM at pH 7.02; μ, 6 to 61 mM at pH 6.32 and 10 to 102 mM at pH 7.02; T, 37.2 °C. The measured rates have been divided by the concentration of arginine decarboxylase, using a subunit mol wt of 82 000.

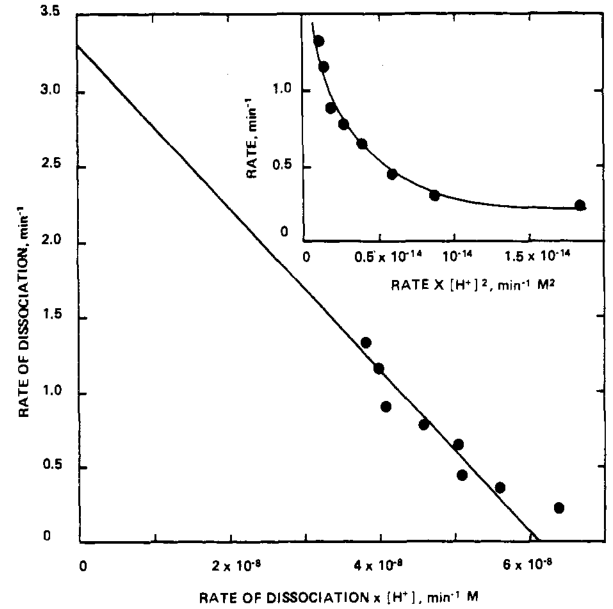

FIGURE 5: Dissociation of arginine decarboxylase as a function of pH. Dissociation was begun by mixing equal parts of arginine decarboxylase (in a pH 5.2 P_i buffer) and DTNB (in a P_i buffer of the appropriate pH). Final concentrations: arginine decarboxylase, 2 mg/mL; Na^+, 40 mM; P_i, 14 to 19 mM; DTNB, 0.5 mM; Cl^-, 14 mM; μ, 42 to 52 mM; T, 37.2 °C. The actual pH values are (left to right) 7.55, 7.46, 7.34, 7.22, 7.11, 6.94, 6.81, and 6.54. The measured rates have been divided by the concentration of arginine decarboxylase, using a subunit mol wt of 82 000.

or activators, and ordered and random sequences of Na^+ binding, H^+ dissociation, and DTNB reaction. Of the 25 unique rate equations obtained in this analysis, 16 do not fit the above. This includes all of the mechanisms where a concerted dissociation step is possible. In one of the remaining cases, the intercepts in Figure 3 should vary identically with pH. This is clearly not the case.

The eight remaining rate equations apply to mechanisms N-3, H-2, I-1, I-4, I-5, I-14, A-11, A-14, A-15, A-17, and R-1, as shown in Table II of Boeker (1978). Since all of these contain terms in $[H^+]/[\text{DTNB}]$, conditions under which it is negligible must be considered. There are two possibilities: (1) the experimental conditions resulted in saturation by one or more reactants, making some terms negligible; or (2) the dissociation mechanism contains an irreversible step, giving a simpler rate equation.

The first possibility will be true if terms in $[H^+]/[\text{DTNB}]$ are negligible with respect to terms in $1/[\text{DTNB}]$ and $1/[\text{DTNB}][Na^+]$ under the conditions of Figure 4. This appears to be extremely unlikely. The DTNB concentration was 1.0 mM while the half-maximal concentration in Figure 3 was 2.5 mM at pH 7.25 and 0.5 mM at pH 6.54. Similarly, the pH values used were 6.32 and 7.02, while the half-maximal pH in Figure 5 is 7.7.

The second possibility is to assume that one of the steps in the reaction is irreversible. Rate equations for each such case are shown in Table III of the following paper (Boeker, 1978). Only two satisfy the empirical rate law deduced above; these are the equations for mechanisms H-2 (and I-1), I-4, N-3 (and A-11), A-14, A-15, and A-17, when the step prior to reaction with DTNB is irreversible. These mechanisms, including the first dissociation step and the initial reaction with DTNB, are shown diagrammatically in Figure 6. In mechanisms H-2 and I-4, the irreversible step is the binding of a Na^+ ion; this is difficult to justify. In the remaining mechanisms, however, the irreversible step is the dissociation of subunits, which seems very likely. As can be seen in Figure 6, these mechanisms differ only with respect to the number of intermediates that can release a Na^+ ion. Since it seems very likely that this type of mechanism represents the actual case, a detailed representation of the simplest one is shown in Figure 7.

Discussion

The results presented here differ from those obtained without rapid mixing (Boeker, 1977) in one crucial respect: the rate of dissociation depends on the first power of the Na^+ and H^+ concentrations, rather than the second power. As discussed in detail in the next paper (Boeker, 1978), and as can be seen in Figure 1, the experimental requirements for measuring initial rates in this system are extremely restrictive. The differences between the two sets of data indicate that the measurement lag in the spectrophotometer was long enough that more than one step of the dissociation process was being observed.

Of the 49 possible mechanisms for which rate equations have been derived in the following paper (Boeker, 1978), most have been eliminated by such straightforward criteria as the change in the apparent maximum rate when Na^+ is varied at fixed pH, etc. The remaining six possibilities are shown schematically in Figure 6; the last four of these are simply variations on a theme. These four appear to be more likely than the others since they alone will fit the empirical rate law under a single very probable condition, irreversibility of the dissociation step. Accordingly, a simple form of this mechanism is shown in detail in Figure 7 and is used as a model in this discussion.

Na^+ and H^+ could affect the observed rate of dissociation either directly, by playing an immediate role in dissociation, or indirectly, by affecting the rate of the reaction between the

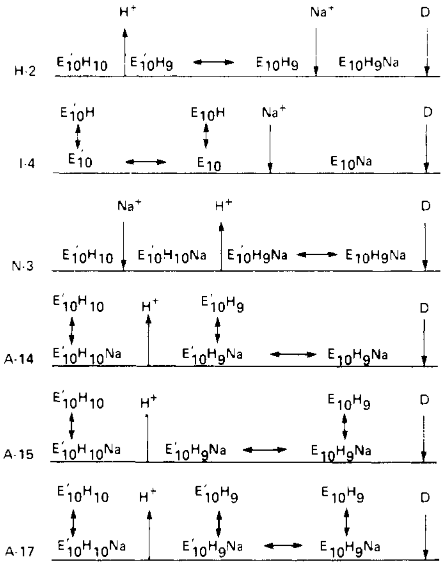

FIGURE 6: Kinetically possible dissociation mechanisms. I-1 and A-11 are also possible but are not shown because they are conceptually equivalent to H-2 and N-3, respectively. In each case the events necessary for formation of the open decamer (E_{10}) from the closed form (E_{10}') and reaction with DTNB are shown. In deriving rate equations (Boeker, 1978), the steps assumed to be irreversible were: (1) Na^+ binding in H-2 and I-4; (2) dissociation in N-3, A-14, A-15, and A-17; and (3) reaction with DTNB in all six. For the first four, the rate equation is $r = R_m/(1 + K_D/[DTNB] + K_N/[Na^+] + [H^+]/K_H + K_N'[H^+]/[Na^+]K_H)$. In the last two, the rate equation has an additional term, $K_DK_N/[DTNB][Na^+]$, in the denominator, and in A-14 and A-17, $K_N' = K_N$.

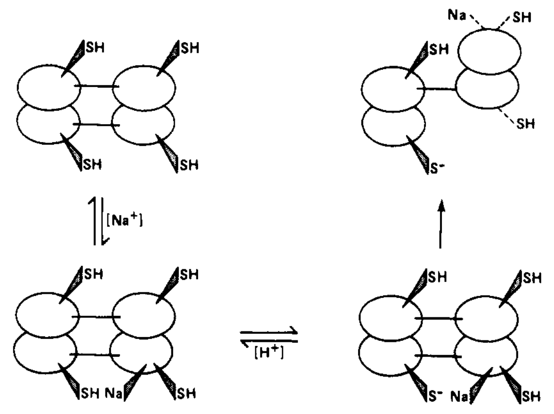

FIGURE 7: Probable mechanism for the dissociation of arginine decarboxylase. The measurements made in this paper actually describe the opening of the arginine decarboxylase decamer; a tetramer is shown only for simplicity. The H^+ and Na^+ sites have arbitrarily been assigned to different subunits.

newly available sulfhydryl group and DTNB. The effects are interpreted as direct for several reasons. The pH of the solution could increase the rate of formation of thionitrobenzoate in a trivial fashion, by increasing the amount of sulfhydryl in the reactive, ionized state. If this were the case, and if pH were not affecting dissociation in any more fundamental way, the ionization step would occur only after dissociation, and the kinetics would reflect this. As can be seen in Figure 6, this is not the case in any of the possible mechanisms.

Na^+ ions appear to play two separate roles in the dissociation process. Equilibrium experiments in the ultracentrifuge (Boeker and Snell, 1968) clearly showed that high concentrations of both protons and monovalent cations retard dissociation, while divalent cations appear to prevent it completely. On this basis, it seemed reasonable that protons and Na^+ ions would play similar roles, perhaps substituting for one another. The results presented here eliminate this possibility, since Na^+ ions increase the rate of dissociation while protons decrease it. Apparently, Na^+ ions have two separate effects: they increase the rate of dissociation and, at the same time, change the equilibrium in favor of the associated species. Since the second effect is not observed kinetically, an additional step (or steps) must occur after the reaction with DTNB. The experiments in the stopped-flow apparatus are in qualitative agreement with this; even though high Na^+ concentrations gave a very fast initial rate, the rate slowed quickly and dissociation did not proceed far at all.

It seems likely that at least one of the two cation effects is in fact related to ionic strength. In most of the experiments presented here, the ionic strength varies only slightly and is not a factor, but this was not true when Na^+ is varied. In this experiment (Figure 4), the ionic strength increases with $[Na^+]^2$ and the dissociation rate with the first power, suggesting an ionic strength effect between the negatively charge DTNB molecules and the sulfhydryl groups. However, if this increase were due only to ionic strength, it seems likely that it would continue with increasing $[Na^+]$, rather than reaching a maximum. In addition, in the spectrophotometer experiments reported previously (Boeker, 1977), the measured rate, apparently not a true initial rate, increased with $[Na^+]^2$ and $1/[H^+]^2$, indicating that two Na^+ ions are involved at the same time as two protons. It seems very unlikely that either result would be obtained from a purely ionic strength effect.

Although the quantitative results presented here are not sufficient to obtain accurate values for R_m, K_D, etc., it is possible to estimate these parameters. Four of the mechanisms shown in Figure 6 have the property that, if the data in Figure 4 are presented as a double-reciprocal plot, one of the coordinates at which the lines intersect gives a value for K_N'. For the remaining two mechanisms, this coordinate gives K_N. The observed value is 6 mM. In Figure 3, DTNB was varied at a fairly high concentration of Na^+, 20 mM. Assuming that K_N and K_N' are near 6 mM, a replot of the two apparent maximum rates as a function of pH suggests that the true R_m is around 30 min^{-1}. The estimate of pK_H obtained from this replot is 8.1. The results presented in Figure 5 are consistent with these values for K_H and R_m. If R_m is 30 min^{-1}, a value for K_D of 30 mM can be estimated from the appropriate intercept in Figure 3. The values for K_H and K_D support the earlier suggestion that terms in the rate equation in $[H^+]/[DTNB]$ cannot possibly be negligible under the experimental conditions used here.

In the detailed rate equations (Boeker, 1978) for the mechanisms in Figure 6, the expression for K_H is related to the ionization constant of this proton in the same way that a substrate's Michaelis constant is related to its dissociation constant. If, then, the dissociation step in Figure 7 is slow compared with the preceding step, K_H is an ionization constant. Since protonation reactions are ordinarily very fast, the observed pK_H, around 8, suggests strongly that it is a cysteine side chain ($pK_a = 8.3$) which is ionizing. This is consistent with the fact that one sulfhydryl per subunit becomes available during dissociation. Assuming that it is a sulfhydryl which ionizes, it must be the same as the one which reacts with DTNB, as suggested in Figure 7, since only one is available after the dimers are formed.

Since there is only one Na^+ ion and one proton involved in each dissociation step, the events which occur on the dissociating subunits must differ. The Na^+ ion can bind only to one of these, and the proton can dissociate only from one. It seems reasonable that the binding of Na^+ disrupts an electrostatic attraction between the subunits, while the ionization of the 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[†]

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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

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[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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