The Mechanism of Aconitase Action

EVIDENCE FOR AN ENZYME ISOMERIZATION BY STUDIES OF INHIBITION BY TRICARBOXYLIC ACIDS*

JOSEPH J. VILLAFRANCA

From the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

SUMMARY

Tricarboxylic acids were shown to be both competitive and noncompetitive inhibitors of aconitase with the inhibition pattern dependent upon which substrate was used. Tricarboxylic acids were linear competitive inhibitors when citrate or isocitrate was the substrate but noncompetitive inhibitors when cis-aconitate was the substrate. trans-Aconitate was a linear competitive inhibitor with cis-aconitate as substrate and noncompetitive when citrate or isocitrate was substrate. These data support a kinetic scheme which involves an enzyme isomerization between two forms, one of which preferentially interacts with cis-aconitate and the other with citrate and isocitrate.

Aconitase (EC 4.2.1.3, citrate(isocitrate)hydrolyase) catalyzes the isomerization of citrate (I) to isocitrate (II), and the dehydration of citrate or isocitrate to cis-aconitate (III). The formation of isocitrate from citrate does not involve the obligatory formation of cis-aconitate as a distinct intermediate (1, 2).

Most of the mechanisms which were proposed for these transformations invoked the idea that cis-aconitate binds to aconitase in two configurations. These two modes of binding cis-aconitate are necessary to accommodate the trans removal of water from citrate and isocitrate, the proton (tritium) retention by the enzyme, the stereochemistry of citrate when bound to aconitase (5) and the absolute configuration of isocitrate (6).

A mechanism, consistent with the above data and based on crystallographic data of binary metal ion chelates of citrate, cis-aconitate, and isocitrate, was proposed by Glusker (7, 8) and is called the "ferrous-wheel" mechanism. Another plausible mechanism for the aconitase reaction which is consistent with the isotopic data and recently obtained NMR data (9, 10) is the "Ballar-twist" mechanism proposed by Villafranca and Milidvan (10). The present work was started to explore the interaction of aconitase with structural analogs of the three substrates by kinetic means for the purpose of clarifying the structural requirements for the binding of substrates. The major conclusion from the present work is that aconitase exists as two stable enzyme forms which are isomerizing. One enzyme form of aconitase preferentially binds cis-aconitate and structural analogs of cis-aconitate, whereas the other form preferentially binds citrate and isocitrate and structural analogs of these two substrates.

EXPERIMENTAL PROCEDURE

Materials

Aconitase was purified as described previously (11). Citric acid, L-cysteine hydrochloride, itaconic acid, citraconic acid, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, triethanolamine, and isocitric dehydrogenase (grade A) were products of Calbiochem. Monopotassium three-N(1)-isocitric acid, R- and S-citramalic acid, D- and L-malic acid, L-hydroxyglutarate, maleic acid, oxalacetic acid, α-ketoglutarate, and cis- and trans-aconitate were purchased from Sigma. Aldrich Chemical Co. supplied the tricarboxylic acid and iminodiacetic acid. Mann enzyme grade Tris was used and Fe(NH₄)₂SO₄ was Baker analyzed reagent grade. All other chemicals were the highest grade available. Fluorocitric acid was prepared by the method of Fanshier et al. (12) and an authentic sample of erythro-fluorocitrate was the generous gift of Dr. E. Kun.
Enzyme Assays—Rates of product formation with aconitase were measured in two ways: (a) by following the production of NADPH at 340 nm on a Gilford model 240 recording spectrophotometer. This method was used when citrate or cis-aconitate was the substrate and the buffer solution contained 90 mM Tris-Cl (or triethanolamine-Cl), pH 7.5, 0.5 mM MgCl₂, 0.1 mM NADP, and 1 mg of isocitrate dehydrogenase per ml (~2 units per mg). (b) by following the production of cis-aconitate at 240 nm. This method was used with isocitrate as the substrate and occasionally with citrate. Caution must be exercised when this assay is used due to spurious absorption at 240 nm from citrate and iron salts and blanks were run periodically without enzyme present to ensure that true enzymic rates were being measured from absorbance changes at 240 nm. The buffer used was 90 mm Tris-Cl (or triethanolamine-Cl), pH 7.5, various amounts of substrate, ± an inhibitor, with activated aconitase added last in a final volume of 1.0 ml. The method for activating the enzyme has been described previously (11); and (b) by following the production of cis-aconitate at 240 nm. This method was used with isocitrate as the substrate and occasionally with citrate. Caution must be exercised when this assay is used due to spurious absorption at 240 nm from citrate and iron salts and blanks were run periodically without enzyme present to ensure that true enzymic rates were being measured from absorbance changes at 240 nm. The buffer used was 90 mm Tris-Cl (or triethanolamine-Cl), pH 7.5, various amounts of substrate, ± an inhibitor, with activated enzyme added last. An enzyme unit is 1 μmole of product produced per min. All kinetic data are ±5% or less.

RESULTS

Trans-Aconitate as Inhibitor—It has always been assumed that trans-aconitate (IV) is a substrate analog of cis-aconitate and therefore should be a competitive inhibitor. Inhibition of the enzymatic rate has been noted many times (13, 14) but a thorough investigation has never been reported to conclusively demonstrate competitive inhibition. In Fig. 1, the inhibition patterns of trans-aconitate with all three substrates of the aconitase reaction are given. The first point to be made is that trans-aconitate is a linear competitive inhibitor of aconitase (see inset in Fig. 1) when cis-aconitate is used as substrate. A Kᵢ value (Table I) which represents the binding of trans-aconitate to the enzyme was calculated from Equation 1, where the symbols have their usual kinetic meaning.

\[
\frac{1}{v} = \frac{K_m}{[S]V} \left( \frac{1}{1 + \frac{[I]}{K_I}} \right) + \frac{1}{V}
\]  

(1)

The next observation from the data in Fig. 1 is that trans-aconitate is a noncompetitive inhibitor when either citrate or isocitrate is the substrate. From a replot of the slopes (S) and intercepts (I) of these data (insets of Fig. 1), trans-aconitate is found to be a linear noncompetitive inhibitor with respect to both slope and intercept. Kᵢ values (Table I) were calculated from Equation 2 where Kᵢ₈ and Kᵢ₁₁ are the inhibition constants calculated from the slope and intercept, respectively (see Cleland (15)).

\[
\frac{1}{v} = \frac{K_m}{[S]V} \left( \frac{1}{1 + \frac{[I]}{Kᵢ₈}} \right) + \frac{1}{V} \left( \frac{1 + \frac{[I]}{Kᵢ₁₁}}{1 + \frac{[I]}{Kᵢ₁₁}} \right)
\]  

(2)

The Kᵢ₈ values obtained from data with the substrates citrate and isocitrate agree very well with the Kᵢ value determined with cis-aconitate as substrate as expected if the inhibitor, trans-aconitate, is binding to the same enzyme form in each case. However, the noncompetitive component must arise from the fact that the substrates citrate and isocitrate bind to an enzyme form different than the enzyme form to which cis-aconitate binds. Thus cis-aconitate and trans-aconitate bind to an enzyme form which I will designate as F and citrate and isocitrate bind to E. To explain the results in Fig. 1, there must be an isomerization between E and F and these results are interpreted in terms of an iso mechanism for aconitase (using the terminology of Cleland (16)). These are the first such extensive studies on the interaction of inhibitors with aconitase. They provide further insight into the mechanism of action of aconitase as elucidated in earlier NMR studies (9-11) and these results will be discussed later in this paper.

Tricarballylate as Inhibitor—Tricarballylate (V) can be considered a substrate analog of both citrate and isocitrate. In Fig. 2 the inhibition patterns are shown for cis-aconitate and isocitrate as substrates. Tricarballylate is a linear competitive inhibitor (inset in Fig. 2) when isocitrate is the substrate and a Kᵢ value was calculated by using Equation 1. The same results are obtained when citrate is the substrate. When cis-aconitate is the substrate tricarballylate is a noncompetitive inhibitor and the data fit Equation 2. The replots of slopes and intercepts are given in the insets in Fig. 2 and show that tricarballylate is a linear noncompetitive inhibitor in both slope and intercept terms. Tricarballylate binds to the E form of aconitase and, as predicted, there is good agreement between Kᵢ and Kᵢ₈ inhibitor constants (Table I) in accord with the predictions of the iso mechanism (see "Discussion"). The Kᵢ value agrees with the value of 6.0 mm determined by Gavron and Bircelliher (17).

Fluorocitrate as Inhibitor—Since the early studies by Peters (18) of fluorocitrate inhibition of aconitase a large number of inhibition studies have been carried out with aconitase preparations of various degrees of purity (19-22). Extensive work from Kun's group has established that erythro-fluorocitrate (VI) is the isomer which is biologically synthesized from fluorocitrate (VI) and oxaloacetate (12, 23, 24). With purified pig heart aconitase and citrate as the substrate, fluorocitrate was found to be a linear competitive inhibitor with a Kᵢ value of 0.29 mm (25). The fluorocitrate used in this earlier study (25) was a racemic mixture. In an expansion of my earlier work (20), fluorocitrate is found to be a linear competitive inhibitor when citrate is the substrate and a linear noncompetitive inhibitor when cis-aconitate is the substrate (Fig. 3). Fluorocitrate, like tricarballylate, is a substrate analog of citrate and interacts with the E form of aconitase in a competitive manner from the present kinetic analysis. In Table I are the Kᵢ and Kᵢ₈ values obtained from these kinetic data. The agreement is not as...
Inhibition constants for aconitase and tricarboxyl acids

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_I$</th>
<th>$K_{II}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-Aconitate</td>
<td>0.13 (A)</td>
<td>0.16 (I)</td>
</tr>
<tr>
<td>Tricarballylate</td>
<td>2.5 (C)</td>
<td>0.13 (C)</td>
</tr>
<tr>
<td>Fluorocitrate</td>
<td>0.12 (C)</td>
<td>0.02 (A)</td>
</tr>
</tbody>
</table>

The symbols A, I, and C refer to the substrates which were used for the kinetic determination of the inhibitor constant, cis-aconitate (A), isocitrate (I), and citrate (C). All kinetic constants are ±10 to 20%.

good as the data reported for the two previous substrate analogs and the reason could be that fluorocitrate can act as both a competitive and "irreversible" inhibitor of aconitase (12, 25, 27). All of the kinetic data were taken as the initial slopes of the rate of product formation, but the onset of inactivation could have been present to increasing degrees as the amount of fluorocitrate increased (see Ref. 27). Thus the disagreement in $K_I$ and $K_{II}$ values may reflect this difference. The fluorocitrate used in this study was a single isomer which may account for the lower $K_I$ value reported in Table I when compared with previous data (25, 26).

Dicarboxylic Acids as Inhibitors—The following dicarboxylic acids as shown were tested as inhibitors of aconitase since they have some structural resemblance to the substrates of aconitase.

![Figure 1](https://example.com/fig1.png)

**FIG. 1.** Inhibition of aconitase by trans-aconitate. Assays with the various substrates were conducted as described under "Methods." A unit is 1 amole of product produced per min. Each solution contained a final concentration of 1.5 mg of purified and activated aconitase (II) per ml when citrate was the substrate, and 0.15 mg per ml when cis-aconitate and isocitrate were substrates. The millimolar concentrations of trans-aconitate are: A, none; B, 0.1 mM; C, 0.2 mM; D, 0.4 mM; E, 1.0 mM; F, 0.05 mM. $T = 25^\circ$.

![Figure 2](https://example.com/fig2.png)

**FIG. 2.** Inhibition of aconitase by tricarballylate. All conditions were the same as in the legend to Fig. 1 except that 1.0 mg of activated aconitase per ml was used in each assay solution. The millimolar concentrations of tricarballylate are: A, none; B, 2.5 mM; C, 5.0 mM; D, 10.0 mM. $T = 25^\circ$.

L-Malate (VII) and L-hydroxyglutamate (XI) were very weak inhibitors of aconitase ($K_I > 30$ mm) and were not studied further. The slight inhibition could have been due to changes in ionic strength of the buffer medium since high concentrations of...
FIG. 3. Inhibition of aconitase by fluorocitrate. All conditions were the same as in the legend to Fig. 1. The enzyme concentration was 0.2 mg per ml. The millimolar concentrations of fluorocitrate are: A, none; B, 0.04 mM; C, 0.08 mM; D, 0.12 mM. T = 25°.

These compounds were needed to produce even a slight inhibition. Citramalate (VIII), citraconate (X), maleate (IX), itaconate (XII), and iminodiacetate (XV) produced no detectable inhibition with any of the three substrates of aconitase. Maleate (IX) was also not an inhibitor when acetate was present, this experiment being performed to see if inhibition could be achieved with two separate “parts” of cis-aconitate present simultaneously.

Oxalacetate (XIII) produced inhibition of the rate of the enzymic reaction when isocitrate was the substrate (Fig. 4). The inhibition pattern was noncompetitive in nature but was not simple with both the slope and intercept replots being parabolic in nature when plotted against (1). Equation 3 was used to evaluate these data.

\[
\frac{1}{v} = \frac{K_m}{[S]v_0} + \frac{1}{v_0} \left( \frac{1 + [I]^2}{K_i^2} \right)
\]

In the inset of Fig. 4 both the slopes and intercepts give linear replots when plotted against (I)² as predicted from the phenomenological form of Equation 3. This inhibition appears to involve 2 molecules of oxalacetate which may preferentially interact with the F form of aconitase. Support for this conclusion can be found in the work of Britten (28) who determined that oxalacetate was a competitive inhibitor of aconitase when cis-aconitate was the substrate, but the experiment did not show whether this competitive inhibition was linear or parabolic. Oxalacetate differs from all of the other dicarboxylic acids studied since it can exist as the trinegative enolate (XVI). The end of oxalacetate is present to the extent of 15% at pH 7.5 and the third pKₐ value of this enol is 12.2 (29). The third pKₐ is lowered to ~8.1 in the presence of divalent metal ions.

Aconitase is activated by Fe(II) and a significant amount of trinegative enolate (XVI) could be present and be responsible for inhibition of aconitase. All of the other structural analogs which were good inhibitors were trinegative and perhaps the enolate of oxalacetate meets this criterion. This structure permits hydrogen bond formation via the enol oxygen to a protonated base in the active site of aconitase. Ketoglutarate (XIV) is also a parabolic noncompetitive inhibitor of aconitase when isocitrate or citrate is the substrate, but work on this inhibitor was not pursued due to the complexity of interpretation of parabolic noncompetitive inhibition.

**DISCUSSION**

The kinetic data reported in this paper represent the first thorough study of inhibitors of the enzyme aconitase. The inhibition patterns reported for structural analogs of citrate, isocitrate, and cis-aconitate gave competitive and noncompetitive inhibition patterns which are analyzed in terms of an iso-uni mechanism. It is the nature of kinetic schemes that any of several models could be accommodated within the framework of the experimental data reported in this paper. The kinetic model that is proposed involves two isomerizing forms of aconitase—one that preferentially binds citrate and isocitrate (the E form) and another form that preferentially binds cis-aconitate (the F form). From the initial velocity data one cannot exclude the possibility that citrate and isocitrate bind to F in nonproductive complexes. However, the kinetic scheme proposed on the basis of the data in this paper has as its main feature the restriction that cis-aconitate reacts with the E form and citrate reacts with the F form of aconitase to give product. This kinetic scheme is elaborated below and predictions are made based on this iso mechanism which are found in the data represented in Figs. 1 to 3.

The simplest possible kinetic scheme involving the binding of citrate (C) and isocitrate (Iso) to the E form of aconitase and cis-aconitate (A) to the F form of aconitase and allowing E and F to interconvert is given in Scheme I. In this proposed kinetic scheme it can be shown that the intermediates EC, FA, and EIso can be treated as transitory enzyme forms and their individual rate constants ignored since the form of the rate equation will be the same as in the case where EC (or any other intermediate)
is presumed to give unimolecular degradation to substrate or product (Cleland (15, 16), Plowman (30)).

The introduction of the isomerization step between the stable enzyme forms E and F gives the following rate expression, which is obtained by the method of King and Altman (31), for these limiting conditions: observation of the initial rate of conversion of C to Iso, \( \langle A \rangle = 0 \) (Iso) = 0, Equation 4,

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}[C]}
\]

which reduces to the familiar Michaelis-Menten equation (Equation 5)

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}[C]}
\]

where

\[
\frac{1}{V_{\text{max}}} = \frac{(k_i + k_j)/k_i k_j E_T}{k_i k_j E_T}
\]

\[
K_m = \frac{(k_i + k_j)(k_i + k_j)/k_i k_j}{k_i k_j E_T}
\]

The introduction of the enzyme isomerization adds extra rate constants to the two terms on the right side of Equation 4. It is therefore apparent that \( K_m \) does not necessarily represent the binding constant of citrate to aconitase.

When the interaction of inhibitors is taken into account in Scheme I one must now consider whether the inhibitors will interact with the E or F forms of aconitase or both. From the results in Figs. 1 to 3 and Table I, a minimal kinetic scheme is proposed (Scheme II) in which inhibitor \( I \) is thought to interact with the E form of aconitase preferentially (but not necessarily exclusively) and inhibitor \( I' \) with the F form of the enzyme (where \( I \) is tricarballylate or fluorocitrate and \( I' \) is trans-aconitate).

These restrictions are suggested by the data and will be tested by the predictions of kinetic Scheme II. The rate expression in Equation 6 is derived for the case where \( I' \) (trans-aconitate for example) is binding to enzyme form F and the substrate is citrate (C) and isocitrate (Iso) is being monitored. Equation 6

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}[C]} \left[ 1 + \frac{[I']}{K_{I'F}} \right] + \frac{K_{n}}{V_{\text{max}}[C]} \left[ 1 + \frac{[I']}{K_{I'F}} \right] \left[ 1 + \frac{[I]}{K_{I'F}} \right]
\]

where

\[
K_{I'F} = k_{1i}(k_i + k_j)/k_i k_j
\]

\[
K_{I'F} = k_{1i}(k_i + k_j)/k_i k_j
\]

is a rate expression predicting linear noncompetitive inhibition since the \( 1/V_{\text{max}} \) and \( K_m/V_{\text{max}}[C] \) terms both are multiplied by expressions involving \( [I'] \) from the first power. This prediction fits the data in Fig. 1 when citrate is the substrate and \( I' \) is trans-aconitate is the inhibitor. For expressions involving noncompetitive inhibition without enzyme isomerization, the term which multiplies \( K_m/V[C] \) is also \( 1 + [I]/K_{I'F} \). The added complications introduced by enzyme isomerization with no simplifying assumptions about which rate constants are larger than others is evident in Equation 6. Equation 7 reduces to Equation 5 when \( [I'] = 0 \), the simple rate expression involving enzyme isomerization.

A replot of Equation 7 of slope against \( [I'] \) gives an intercept at slope = 0 where \( -[I'] = k_{1i}(k_i + k_j)/k_i k_j \). Thus, only if \( k_i \gg k_j \) does \( K_{I'} \) represent a simple binding constant. The point at which all the lines intersect in the plot of \( 1/v \) against \( 1/[C] \) (noncompetitive) is \( [C] = (k_i + k_j)/k_i k_j \) but cannot be used in a straightforward manner to evaluate individual rate or equilibrium constants when an enzyme isomerization is part of the over-all rate expression.

The other feature of the data in Fig. 1 is the competitive inhibition by trans-aconitate when cis-aconitate is the substrate. Equation 8 gives the rate expression derived from Scheme II for this situation. Equation 8

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}[C]} \left[ 1 + \frac{[I']}{K_{I'F}} \right] + \frac{K_n}{V_{\text{max}}[A]} \left[ 1 + \frac{[I']}{K_{I'F}} \right]
\]

reduces to Equation 9

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}[C]} \left[ 1 + \frac{[I']}{K_{I'F}} \right] + \frac{K_n}{V_{\text{max}}[A]} \left[ 1 + \frac{[I']}{K_{I'F}} \right]
\]

where

\[
1/V_{\text{max}} = (k_i + k_j)/k_i k_j E_T
\]

\[
K_m = (k_i + k_j)(k_i + k_j)/k_i k_j
\]

\[
K_n = (k_i + k_j)(k_i + k_j)/k_i k_j
\]

\[
K_{I'} = k_{1i}(k_i + k_j)/k_i k_j
\]

Equation 9 has the expected form for a rate expression involving competitive inhibition between \( I' \) and \( A \) and therefore fits the data in Fig. 1. When \( [I'] = 0 \), Equation 9 reduces to a rate expression similar to Equation 5 with the exception that \( A \) is the substrate and different rate constants represent the enzyme catalyzed reaction.

It is worthwhile to consider alternatives to this mechanism and predictions from these alternatives at this point. Under the initial velocity conditions, if the isomerization between \( E \) and \( F \) is slow and citrate is reacting with \( E_1 \), then the \( F \) form would not contribute to the initial velocity. If trans-aconitate binds to \( F \), then there would be no effect on the reaction velocity. trans-Aconitate does produce an inhibiting effect when citrate is the substrate so the isomerization cannot be slow. If the isomerization is rapid, then trans-aconitate could be a competitive inhibitor with respect to citrate only if the binding constant of trans-aconitate for the \( F \) form of aconitase were very weak and not much of this enzyme form formed a complex. This prediction is not found in the data since trans-aconitate is a
noncompetitive inhibitor when citrate (or isocitrate) is the substrate. The possibility that citrate binds to both E and F can be considered and a kinetic scheme with this restriction predicts square terms with respect to citrate. There is no apparent curvature in the slope and intercept replots which means that if citrate binds to the F form of aconitase the binding constant is much weaker than the binding constant to the E form.

The predicted noncompetitive behavior of Scheme II represented in Equation 7 arises from the fact that the distribution equation for $FZ' (FZ'/E_0)$ (16) has a term which involves the prediction of the kinetic model but further experimental evidence for isomerization must be obtained.

An essential feature of a kinetic scheme which shows both competitive and noncompetitive behavior is that the $K'_{T}$ value derived from data where $I'$ is a competitive inhibitor must equal the $K_{T}$ values reported in this paper. The $K_5$ and $K_{18}$ values reported in Table I can be considered upper limits for the binding constants of both the inhibitors studied. The "true" binding constants could be lower than the reported $K_5$ values if $k_k > k_i$.

Other more complicated kinetic schemes were devised in which one of the unimolecular transformations of $EC$ to $FA$ or $E130$ was rate determining but those all reduced to kinetic expressions of the same form as Equations 4, 6, and 8 with different groupings of rate constants. Since no independent determinations of individual rate constants have been obtained for aconitase (except $k_1$ from previous NMR data (10)) the simplified Scheme II was used to evaluate all of the kinetic data. Henson and Cleland (32) evaluated kinetic data from liver aconitase and concluded that more than one central complex was involved in the over-all catalytic mechanism. However, none of these individual complexes has been observed by rapid kinetic techniques and this information will be needed before the complete sequence of intermediates can be determined for the reactions catalyzed by aconitase.

The major finding of this paper that aconitase exists as two stable forms in solution can be used to rationalize previous data gathered with this enzyme and perhaps support previously proposed mechanisms. Rose and O'Connell (1) reported that the conversion of $[3-3H]$isocitrate to $[2-3H]$citrate occurred without any tritium loss at early times in the hydration of cis-aconitate. Additional results suggested that cis-aconitate would react with the $EH^+$ form of aconitase when cis-aconitate was the substrate and the reaction $E + H^+ \rightarrow EH^+$ may not be orders of magnitude faster than the rate-determining step in the hydration of cis-aconitate. These data can be easily explained in light of the present kinetic data since cis-aconitate binds and reacts with citrate. The question that is still not answered is whether the $F$ form of aconitase is protonated or not. The $E$ and $F$ forms may interconvert slowly (Fig. 5) (not slower than the rate-determining step, however) with rapid protonation of $F$ prior to reaction with cis-aconitate. However, $E \rightarrow F$ may be fast and protonation and deprotonation of $F$ (to $EH^+$ in Fig. 5) may be slow (perhaps of the same order of magnitude as the rate-determining step).

The existence of two forms of aconitase is a reasonable suggestion when one considers the fact that aconitase can react with two different substrates which differ in their structure. cis-Aconitate has $sp^3$ hybridization at carbon atoms 2 and 3, giving a planar configuration, whereas citrate (and isocitrate) has $sp^3$ tetrahedral geometry at carbon atoms 2, 3, and 4. The active site may undergo changes in "shape" when trying to accommodate these different substrates and this hypothesis is particularly tenable when one considers that cis-aconitate must "flip-over" during catalysis to satisfy the stereochemical requirements of the interconversion of citrate and isocitrate (7, 10). The proposed mechanisms for aconitase which involve the Fe(II) in a "ferrous-wheel" or "Bailar-twist" mechanism accounts for the "flip-over" of cis-aconitate but additional constraints in the active site may be necessary to accommodate the binding of cis-aconitate in both the citrate- and isocitrate-like conformations (7, 10). More experiments must be performed before this fascinating mechanism can be understood completely.

Acknowledgment—I would like to thank Dr. W. W. Cleland for helpful discussions concerning these data and their interpretation.

REFERENCES


Fig. 5. Kinetic scheme for aconitase. The protonation of $E$ to $EH^+$ is assumed to be fast and deprotonation slow in accord with the findings of Rose and O'Connell (1). Citrate and isocitrate bind to the $F$ form of aconitase and cis-aconitate binds to the $EH^+$ (or $FH^+$) isomerized form of aconitase.
The Mechanism of Aconitase Action: EVIDENCE FOR AN ENZYME ISOMERIZATION BY STUDIES OF INHIBITION BY TRICARBOXYLIC ACIDS
Joseph J. Villafranca


Access the most updated version of this article at http://www.jbc.org/content/249/19/6149

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/19/6149.full.html#ref-list-1