Biochimica et Biophysica Acta, 484 (1977) 453—464

BBA 68248

STRUCTURAL BASIS FOR ACONITASE ACTIVITY INACTIVATION BY BUTANEDIONE AND BINDING OF SUBSTRATES AND INHIBITORS

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(Received May 20th 1977)

Summary

Aconitase (citrate(isocitrate)hydro-lyase, EC 4.2.1.3) prior to activation demonstrates a single binding site for substrates and inhibitors. On the basis of kinetic experiments, at pH 8.5 and 37°C, with monomeric butanedione in borate, this binding site was found to contain a single arginine residue. Dissociation constants at pH 8.5 and 37°C, determined from inhibitory effects on butanedione inactivation rates are: citrate, 0.74 mM; D-isocitrate, 0.33 mM: *cis*-aconitate, 0.52 mM; tricarballytate, 0.42 mM; *trans*-aconitate, 0.025 mM. Corresponding dissociation constants for the active enzyme are: tricarballylate, 0.39 mM; *trans*-aconitate, 0.14 mM. Active site Fe^{2+} added to the enzyme on activation is therefore not required for binding. K_m values are: citrate, 0.23 mM and *cis*-aconitate 0.012 mM. Binding to active enzyme is considered to be transition state binding.

Introduction

The availability [1,2] of pure pig heart aconitase [citrate(isocitrate)hydrolyase, EC 4.2.1.3] makes structural studies on this enzyme possible and preliminary accounts of an active site thiol [3] and a binding site containing arginine [4] have been given.

We now report in detail (1) kinetic evidence for the reaction of butanedione-borate with an arginine residue at the binding site and (2) utilization of kinetic methodology for determination of substrate and inhibitor binding constants with a-aconitase *. Since a-aconitase lacks active site Fe^{2+} necessary for activity [5], comparison of the latter binding constants with those obtained with active enzyme itself may be expected to reveal the effect of active site

* The enzyme during the course of isolation loses iron and becomes inactive. Activity is restored by addition of ferrous ion and a reductant [5]. Activatable enzyme may thus be referred to as a-aconitase.

Fe^{2+} on binding. Present concepts suggest on mainly hypothetical grounds a binding role for Fe^{2+} as well as a catalytic role [6].

Materials and Methods

NADP, cysteine hydrochloride, ascorbic acid and triethanolamine were obtained from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Butanedione was a product of Aldrich Chemical Co. (Milwaukee, Wisconsin, U.S.A.), the pure liquid monomer being used as such for preparation of reagent solution in borate-chloride buffer prior to use. Borate-chloride buffers were prepared by neutralization of boric acid-sodium chloride solutions (reagent grade, Fisher Chem. Co., Pittsburgh, Pennsylvania, U.S.A.) with sodium hydroxide to pH 8.5: Tricarballylic acid and trans-aconitic acid, obtained from Eastman Chemicals (Rochester, N.Y., U.S.A.), were recrystallized from water, yellow impurities being removed by treatment with charcoal. Citric acid, D-*threo*-isocitrate and *cis*-aconitic anydride were obtained from Sigma Chemical Co.

Enzyme

a-Aconitase was prepared and stored as previously described [1,2,5]. Before use tricarballylate stabilizer was removed by dialysis in the cold with 45 mM sodium acetate, pH 8.0, dialysis being conducted under N_2 pressure in a 10 ml Amicon (Lexington, Massachusetts, U.S.A.) cell, a PM-10 membrane being employed. For dialysis 0.5 ml enzyme solution, 7—12 mg protein/ml, was diluted to 10.0 ml with acetate solution, then concentrated to 1.0 ml in the cell. The operation was repeated three times. The extensive dialysis lowered the specific activity by 15 to 25%. When required activated enzyme, free of activating reagents, was prepared as previously described [5], tricarballylate buffer being replaced by acetate buffer.

Assay

a-Aconitase was activated and assayed as previously described [1,2,5]. Typically a 0.1 ml reaction aliquot, approx. 30 μg protein, was activated at 23°C for 5 min with 1.4 ml activation solution containing 5 mM Fe^{2+}, 10 mM cysteine, pH 7.5, 30 mM ascorbate, pH 7.5. A 0.1 ml aliquot, 2 μg protein, was then added to 1.4 ml assay solution, pH 8.0, containing 20 mM triethanolamine, 0.2 mM Mg^{2+}, 1 mM *cis*-aconitate or 1 mM citrate, 0.75 mg/ml NADP, 0.75 units/ml isocitrate dehydrogenase in a 0.5 cm cuvette and the rate of formation of NADPH was monitored at 340 nm with a Gilford (Oberlin, Ohio, U.S.A.) recording spectrometer with an expanded scale.

Kinetics runs

Kinetics runs were conducted at pH 8.5, and 37°C in sodium borate-sodium chloride buffer. In a typical run (Fig. 1a) reaction mixtures, one ml, contained 310 μg of a-aconitase and varying concentrations of butanedione in 48 mM sodium borate-48 mM sodium chloride. At intervals 0.1 ml aliquots were pipetted into 1.4 ml of activating solution. After 5 min at 23°C a 0.1 ml aliquot (2.05 μg protein) was assayed at 23°C with 1.4 ml *cis*-aconitate assay solution.

Results and Discussion

Butanedione inactivation

Positively charged arginine residues, in general, serve as active site binding groups for enzymes catalyzing the reaction of substrates and coenzymes carrying negatively charged carboxylate and phosphate groups [7]. Such arginine residues may be detected by reaction with butanedione or by reaction with other a-dicarbonyl compounds. Both dimeric and trimeric butanedione species as well as the monomeric species react with the guanidinium group. The monomer, however, requires no special preparation and in borate buffer provides a well-explored and specific reagent for arginine residues [8,9,10]. It thus is a reagent of choice for kinetic studies.

Fig. 1 presents the effect of butanedione concentration on the rate of inactivation of a-aconitase at constant borate concentration. The individual runs give plots (Fig. 1a) that are first order in enzyme sites and a plot of slopes (k') vs. butanedione concentration (Fig. 1b) reveals a first order dependency on butanedione concentration. This dependency is confirmed by the double reciprocal plot, $1/k'$ vs. 1/(butanedione), presented in Fig. 2a. The inactivation is, therefore, also first order in butanedione concentration. The double log plot [12,13] presented in Fig. 2b confirms this dependency, the initial slope, $n = 1$, indicating the stoichiometry of reaction.

Borate affects the inactivation rate. The rate of reaction at constant butanedione concentration, 3 mM, increases with borate concentration and the slopes (k') of the individual first order plots yield a straight line when plotted (Fig. 3) against the borate concentration.

The above kinetic results are readily interpretable in terms of current understanding of the nature of the reaction of arginine residues with α-dicarbonyl reagents [8,14,15]. Briefly, reaction proceeds via initial formation of a reversible *cis*-diol adduct between the guanidinium group and the α-dicarbonyl com-

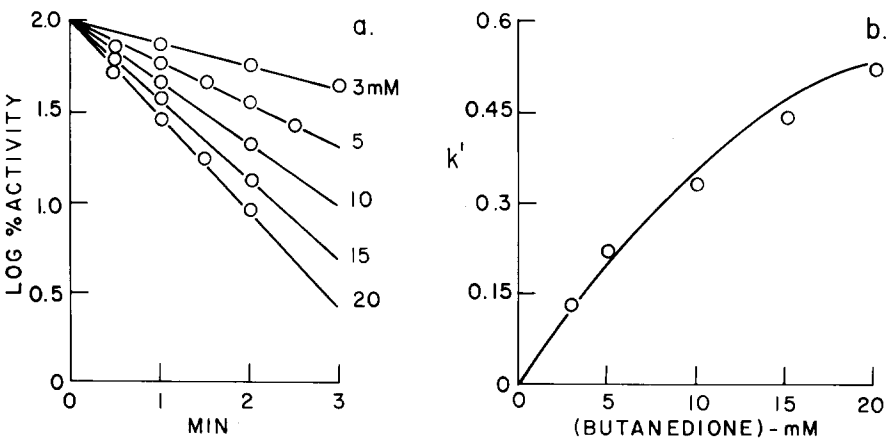


Fig. 1. Inactivation of a-aconitase by butanedione, pH 8.5, 48 mM sodium borate/48 mM sodium chloride buffer, 37°C, 310 μg a-aconitase in 1.0 ml reaction volumes. At indicated intervals 0.1 ml aliquots were removed and activated and assayed as described in the experimental section. (a) First order plots. (b) Slopes k', of first order plots vs. butanedione concentration.

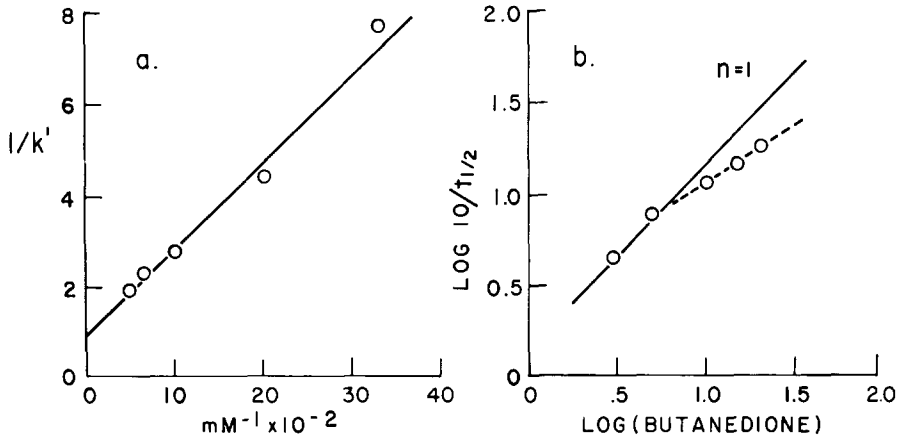


Fig. 2. (a) Double-reciprocal plot of slopes versus butanedione concentration, data from Fig. 1 (a); (b) Plot of logarithm of reciprocal of reaction half times, data from Fig. 1, against logarithm of butanedione concentration, initial slope = 1.

pound followed by stabilization of the initial adduct by reaction with another mole of α-dicarbonyl, as is the case with phenyglyoxal [14], or with a mole of borate, as is the case with cyclohexanedione [15], and as, by inference, is also the case with butanedione-adduct and borate [8].

The reactions may then be formulated,

$$\text{E} + \text{butanedione} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{E-butanedione} \tag{1}$$

$$\text{borate} + \text{E-butanedione} \xrightarrow{k_3} \text{E-butanedione-borate} \tag{2}$$

and assuming rapid equilibration with butanedione prior to reaction with borate, the overall rate for inactivation becomes

$$v = \frac{k_3 E_0 \,[\text{butanedione}][\text{borate}]}{K + [\text{butanedione}]} \tag{3}$$

with K equal to the dissociation constant for enzyme-butanedione adduct. Eqn. 3 is consistent, of course, with the observed first order Michaelis-Menten dependency on butanedione concentration and the observed first order dependencies on active sites and borate concentration. From these results it is clear that reaction of one arginine residue per mole leads to inactivation. Determination of possible simultaneous reaction of other arginine residues is not feasible with butanedione, [^{14}C]butanedione not being readily available. In this connection, both [^{14}C]phenylglyoxal and [^{14}C]cyclohexedione are available [14,15]. Of these two α-dicarbonyl reagents, only phenylglyoxal reacts with a-aconitase (Jones, L. and Gawron, O., unpublished) and further studies with this reagent are in progress.

Of added interest in connection with the butanedione-borate inactivation of a-aconitase, is the low rate of inactivation in the absence of borate (Fig. 3). While the butanedione reaction is reversible in the absence of borate, reversibility is time dependent. In the reported experiments activation and assay are

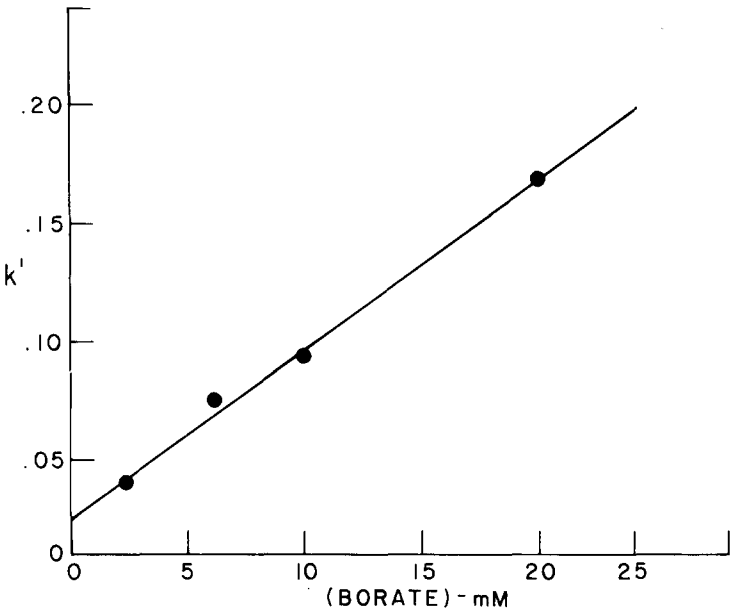


Fig. 3. Effect of borate concentration on inactivation rates at 37°C: 1.0 ml reaction volumes containing 365 μg a-aconitase, 3.0 mM butanedione 22.5 mM sodium acetate, pH 8.3.

carried out within 10 min after aliquot removal. Reversal of reaction, if occurring during this period, is apparently not extensive.

It may also be noted that under control conditions at 37°C, active aconitase is, as expected, quite unstable (Fig. 4), 80% of the activity being lost in 12 min and 90% being lost in 6 min under the same conditions but with the addition of

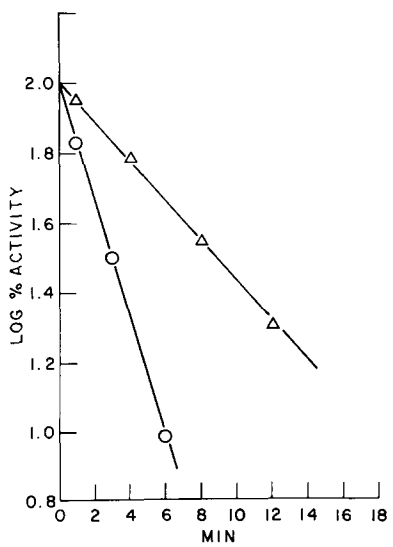


Fig. 4. Instability of active aconitase at 37°C: 1.0 ml reaction volumes containing 100 μg active aconitase prepared according to references [5]; ○, 31.5 mM sodium acetate, pH 8.3; △, 22.5 mM sodium acetate, 6.25 mM borate.

6.25 mM borate. The borate effect on active enzyme is similar to that exerted by phosphate, phosphate rapidly inactivating the active enzyme (ref. 16; Jaklitsich, A. and Gawron, O., unpublished). The active enzyme thus does not lend itself under these conditions, as well as under other conditions, to a direct study of butanedione inactivation.

Substrate and inhibitor effects

Fig. 5 depicts the effect of the competitive inhibitor, tricarballylate, on the rate of inactivation of a-aconitase at 37°C by 1 mM butanedione in the presence of 48 mM borate/48 mM sodium chloride pH 8.5. As can be seen from Fig. 5a, the inactivation rate is decreased by the presence of tricarballylate, slopes of the first order plots decreasing with increasing concentration of tricarballylate. A plot of the reciprocal of these slopes vs. tricarballylate concentration give the linear relationship noted in Fig. 5b. This relationship

$$1/k' = 1/k'_0 + [I]/k'_0K'_I \tag{4}$$

is that expected on the basis of competition between tricarballylate [I] and butanedione for the reactive arginine. With the rate of inactivation

$$v = \frac{k_3}{K}[E][\text{butanedione}][\text{borate}] \tag{5}$$

under the given experimental conditions $K \gg$ [butanedione], and

$$K'_I = \frac{[E][I]}{[EI]} \tag{6}$$

and

$$E_0 = E + EI \tag{7}$$

derivation yields Eqn. 4, k_0 representing the first order slopes in the absence

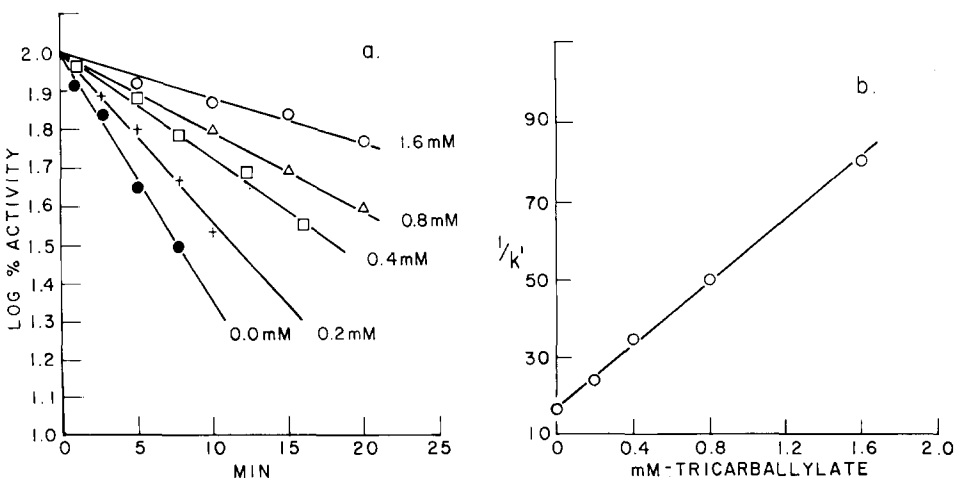


Fig. 5. Inhibition of inactivation rates at pH 8.5, 37° C by tricarballylate: 1 ml reaction volumes containing 410 μg a-aconitase, 1 mM butanedione, 48 mM sodium borate/48 mM sodium chloride, pH 8.5; (a) first order plots at indicated tricarballylate concentrations; (b) reciprocal of first order slopes vs. tricarballylate concentration.

of tricarballylate and K'_I the dissociation constant for enzyme tricarballylate complex.

Similar results are obtained with the inhibitor, *trans*-aconitate and with the substrates, citrate, D-*threo*-iso-citrate and *cis*-aconitate, the dissociation constants for the individual compounds being listed in the first column of Table I. The substrates and inhibitors of aconitase are thus reversibly bound by a-aconitase at the site that reacts with butanedione. Consequently, reaction at this site with butanedione prevents the binding of enzyme conformers to a-aconitase. Further, after reaction with butanedione, a-aconitase does not manifest enzyme activity on activation. It must be concluded, therefore, that the conformer binding site is the same in a-aconitase and aconitase. From the specificity of the butanedione reaction and the observed kinetics, it may be concluded that a single arginine residue is present at this binding site.

Binding comparisons with aconitase

For comparison purposes Michaelis-Menten constants and inhibitor dissociation constants have also been determined at pH 8.5 and 37°C in the presence of borate buffer. Representative data are given in Fig. 6a, $1/v$ vs. $1/s$ plots for the effect of *trans*-aconitate with citrate as substrate, and in Fig. 6b, a similar plot with *cis*-aconitate as substrate. From the intercepts and slopes of the corresponding plots, the values of K_m and K_i presented in Table I have been calculated. The K_m values are in agreement with those reported by Villafranca and Mildvan [17] at 25°C under different conditions, the latter values being 0.62 mM, citrate and 0.015 mM, *cis*-aconitate. The *trans*-aconitate K_i value, 0.14 mM, is the same as that, 0.13 mM, reported by Villafranca [18] while the tricarballylate K_i value, 0.39 mM is considerably smaller than that reported, approx. 3.0 mM, by the latter author [18]. It may also be noted that the non-competitive inhibition component observed [18] for tricarballylate with *cis*-aconitate as substrate and for *trans*-aconitate with citrate as substrate was not observed in this study. The essential difference this study and the study [18] referred to is the high concentration of protein used in the latter study, 0.15—1.5 mg/ml, the specific activity of the preparation being low. For this study nanogram quantities of enzyme were used.

It is instructive to initiate comparison of binding to a-aconitase and to aconitase by consideration of tricarballylate binding. The K_i values (Table I)

TABLE I

KINETICALLY DERIVED CONSTANTS (mM)

Compound	K'_i [a]	K_i [b]	K_i [c]	K_m
Trans-Aconitate	0.025	0.14	0.13	—
Tricarballylate	0.42	—	0.39	—
cis-Aconitate	0.53	—	—	0.012
Citrate	0.74	—	—	0.23
Isocitrate	0.33	—	—	—

[a] From butanedione inhibition, a-aconitase.
[b] Competitive kinetics vs. citrate, active enzyme.
[c] Competitive kinetics vs. *cis*-aconitate, active enzyme.

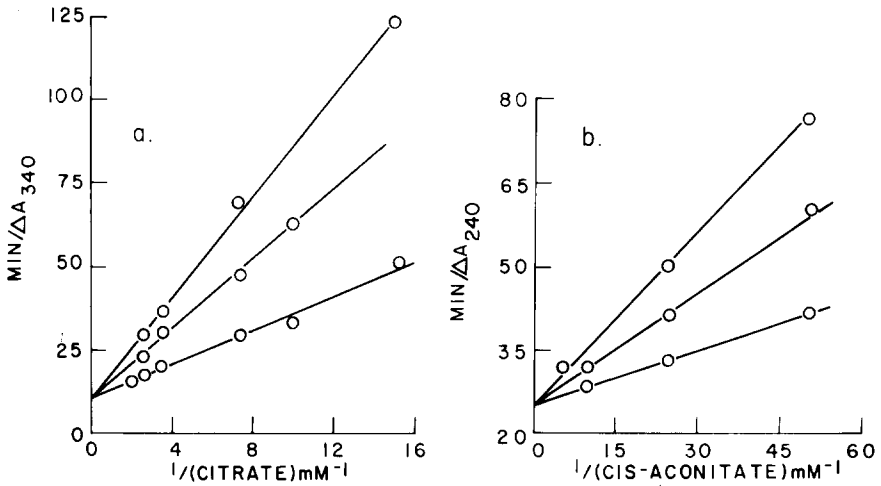
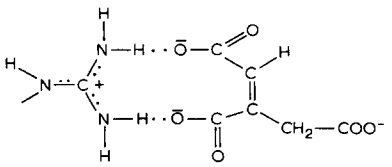


Fig. 6. Determination of K_m and K_i values at pH 8.5, 37°C: (a) 1.5 ml reaction volumes in 0.5 cm cuvettes containing 3 μg active enzyme, 0.74 units isocitric dehydrogenase, 35 mM sodium borate/35 mM sodium chloride, 0.14 mM magnesium chloride, 0.8 mg $NADP^+$, and indicated concentrations of *trans*-aconitate and citrate; (b) 1.0 ml reaction volumes in 1.0 cm cuvettes containing 0.2 μg active enzyme, 35 mM sodium borate/35 mM sodium chloride, and indicated concentrations of *trans*-aconitate and *cis*-aconitate.

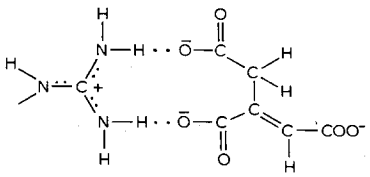
of 0.42 mM and 0.39 mM, respectively, demonstrate that both inactive and active enzyme bind tricarballylate equally well. Two conclusions may be deduced from this binding equivalence: (1) Fe^{2+} of the active enzyme does not participate in binding of unsubstituted saturated tricarboxylic acid and (2) the fit of this inhibitor to the binding site of a-aconitase and the active site of aconitase is the same. Extending the comparison to *trans*-aconitate from values of K'_i 0.025 mM and K_i 0.14 mM, it is clear that binding of trans-aconitate to aconitase is considerably weaker than to a-aconitase. In the above terms the existence of Fe^{2+} in the binding site, if anything, weakens binding of trans-aconitate and the fit of *trans*-aconitate to active enzyme is worsened. It may be suggested that the geometry of the active site enzyme is less planar that the corresponding binding site of a-aconitase and that Fe^{2+} at the active site contributes to this non-planarity. The suggestion of a three-dimensional active site also follows on the basis of a common intermediate [6] for all three substrates, citrate, D-*threo*-isocitrate and *cis*-aconitate. This intermediate may be considered as a three dimensional transitional state.

Since it is evident from the results with tricarballylate and trans-aconitate that active-site Fe^{2+} does not contribute to binding, proposed structures [19, 20], for binding of *cis*-aconitate and *trans*-aconitate as Fe^{2+} chelates may be questioned. These structures have been proposed on the basis of crystallographic studies [20] and on the basis of studies on the effect of iron on substrate and inhibitor NMR signals in the presence of aconitase [19]. These are indirect studies and in the case of the latter study, the effect of excess iron on NMR signals is interrupted in terms of binding. The present study deals directly with the question of binding in the absence of excess iron, only active site Fe^{2+} being present, and avoids thereby, extraneous effects of excess iron. The

Glusker crystallographic model [20] for *cis*-aconitate and *trans*-aconitate may, nevertheless, be taken as a point of departure for considering binding of these compounds to the guanidinium group of the active site arginine. These models demonstrate hydrogen bonding between vicinal carboxyl groups and the guanidinium group and lead to the structures given below for *cis*-aconitate and *trans*-aconitate binding.



cis-Aconitate



trans-Aconitate

The evidence and considerations given above suggest that active site Fe^{2+} is a catalytic group rather than a binding group. Ferrous-wheel [20] and Bailor twist [19] mechanisms may then also be questioned, these mechanisms for aconitase activity requiring the participation of ferrous chelates. In this connection, it may be pointed out that active site Fe^{2+} is tightly bound to enzyme and is slowly released in the presence of tricarballylate to the ferrous chelating agent, ferrozine [5]. While the addition of citrate almost completely inhibits this release to ferrozine, it is clear that active site Fe^{2+} does not require citrate for binding [5]. In this connection the enzyme, chymotrypsin, although structurally unrelated to aconitase, provides a similar example for non-participation of a catalytic group in binding. For this enzyme, conversion of active site serine to dehydroalanine does not alter binding [21].

While comparison of inhibitor binding to a-aconitase and to aconitase is possible by comparing K'_i and K_i values, such comparison is not possible for substrates, as K_m values represent kinetic composites rather than equilibrium constants. It may, however, be noted that the three substrates (Table I) bind to a-aconitase with K'_i values close to the K'_i value for tricarballylate and that the K_m values for the two substrates, *cis*-aconitate, 0.012 mM, and citrate, 0.23 mM, are lower than their corresponding K'_i values, being 0.53 mM, and 0.74 mM, respectively. It is of interest to consider an interpretation of these differences. The steady state derivation applied to the simple scheme

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \tag{8}$$

yields the well known Haldane composite constant.

$$K_S = \frac{k_2 + k_3}{k_1} \tag{9}$$

For those cases where binding and transition state formation are considered synonomous, as is the case for lysozyme [22], K_m may be conceivably a composite constant representing transition state binding, i.e.,

$$K_m = \frac{k_2^*}{k_1^*} + \frac{k_3^*}{k_1^*} \tag{10}$$

the starred constants referring to the transition state complex. Assuming this is the case for aconitase and provided $k_1^* >> k_3^*$, K_m values are expected to be smaller than corresponding K'_i values, since transition state binding is tighter than nontransition state binding [23,24]. In terms of the constants involved, k_1^* would be expected to be greater than k_1 and k_2^* smaller than k_2 because of the contribution of active site Fe^{2+} to transition state binding, a contribution which is necessarily lacking in a-aconitase. With citrate, K_m 0.23 mM K'_i 0.74 mM and *cis*-aconitate, K_m 0.012 mM, K'_i 0.59 mM, this may be the case for aconitase, the data not being in disagreement with transition state binding by active enzyme.

Considering a structural basis for the Pauling dictum [23] that maximum binding of substrate to enzyme occurs in the transition state, it would seem, as implied above for aconitase, that active site binding groups functionally distinguished from active site catalytic groups do provide such a basis, initial binding being enhanced by interaction of bound substrate with the catalytic groups. Enzymes of known (X-ray) structures do, of course, exhibit binding groups and catalytic groups at the active site and it would seem that the Pauling dictum is a consequence of such structure.

Mechanism

The arginine binding site, the contribution of active site Fe^{2+} as a catalytic group and the possible involvement of an SH group [3,25] as the proton acceptor and donor [26] suggest the mechanism depicted in Fig. 7 in terms of transition state complexes. The mechanism accounts for the *trans* mode of reversible hydration and for the sterochemistry of D-*threo*-isocitrate and citrate, but does not specify the mechanism of equilibrium for the reversible citrate to isocitrate conversion. In this connection, it is interesting to note that the arginine binding residue provides a side arm for rotation and 180° rotation around an axis through the alkylated nitrogen and the carbon of the guanidinium group would result in interconversion of citrate and isocitrate. Considering this rotation to take place with *cis*-aconitate, one would not

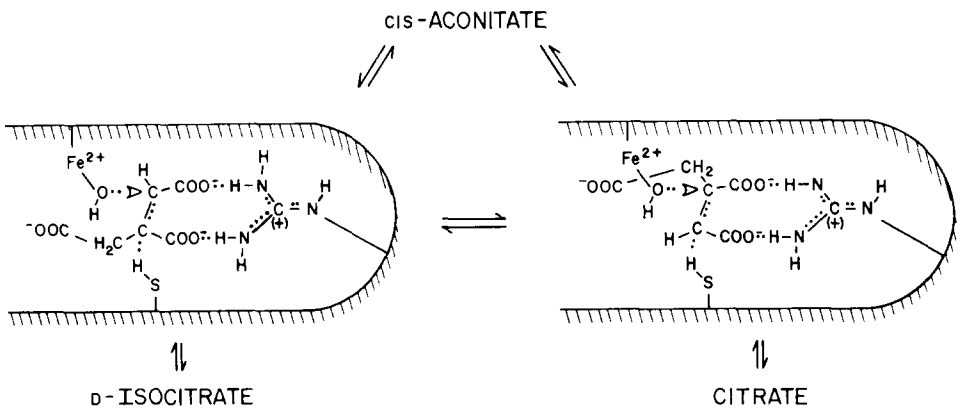
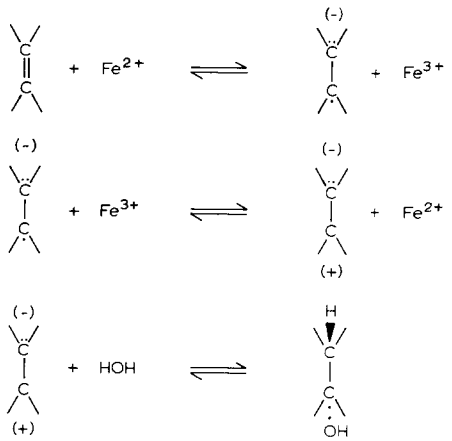


Fig. 7. Mechanism of enzyme activity with arginine as a binding group, and active site Fe^{2+} and cysteine-SH as catalytic groups, the former as hydrosyl donor, the latter as proton donor. Interconversion between transition states by rotation as discussed in the text.

expect that dissociation would have to take place prior to rotation. Thus a proton could be reversibly transferred between citrate and isocitrate, and water exchange of the proton would be inhibited by the presence of substrate. This view of the mechanism of interconversion is, of course, derived from the previously advanced flip-over hypothesis [27].

Finally, it may be noted that the enzyme contains 2 gatoms Fe^{3+} per mol [1,2] and that this structural feature which as yet has not been investigated in connection with activity, suggests, as given below, the possibility of a polarization process prior to reversible hydration.



Acknowledgements

Support of this research by Grant GM-06245 from the National Institute of General Medical Science, National Institute of Health, and by Grant BMS-7506881 from the National Science Foundation is gratefully acknowledged.

References

1 Kennedy, C., Rauner, R. and Gawron, O. (1972) Biochem. Biophys. Res. Commun. 47, 740—745
2 Gawron, O., Kennedy, Sr., M.C. and Rauner, R. (1974) Biochem. J. 143, 717—723
3 Gawron, O., Waheed, A. and Glaid, A.J. (1974) Fed. Proc. 33, 859 Abst
4 Gawron, O. and Jones, L. (1976) Fed. Proc. 35, 1393 Abst
5 Gawron, O., Waheed, A., Glaid, A.J. III and Jaklitsch, A. (1974) Biochem. J. 139, 709—714
6 Glusker, J.P. (1971) The Enzymes, 3rd edit., 5, 413—439
7 David, M., Rasched, I. and Sund, H. (1976) FEBS Lett. 62, 288—292
8 Riordan, J.F. (1973) Biochemistry 12, 3915—3923
9 Yang, P.C. and Schwert, G.W. (1972) Biochemistry 11, 2218—2224
10 Lange, L.G., Riordan, J.F. and Vallee, B.L. (1974) Biochemistry 74, 4361—4370
11 Rauner, R. (1972) Ph.D. Thesis, Duquesne University
12 Levy, H.M., Leber, P.D. and Ryan, E.M. (1963) J. Biol. Chem. 238, 3654—3659
13 Marcus, F., Schuster, S.M. and Lardy, H.A. (1976) J. Biol. Chem. 251, 1775—1780
14 Takahashi, K. (1968) J. Biol. Chem. 243, 6171—6179
15 Patthy, L. and Smith, E.L. (1975) J. Biol. Chem. 250, 557—564
16 Morrison, J.F. (1954) Biochem. J. 56, 99—106
17 Villafranca, J.J. and Mildvan, A.S. (1971) J. Biol. Chem. 246, 5791—5798
18 Villafranca, J.J. (1974) J. Biol. Chem. 249, 6149—6155
19 Villafranca, J.J. and Mildvan, A.S. (1972) J. Biol. Chem. 247, 3454—3463
20 Glusker, J. (1968) J. Mol. Biol. 38, 149—162

21 Weiner, H., White, W.N., Hoare, D.G. and Koshland, D.E. Jr. (1966) J. Am. Chem. Soc. 88, 3851–3858
22 Dickerson, R.E. and Geis, I. (1969) Structure and Action of Proteins, Harper and Row, 69—77
23 Pauling, L. (1948) American Scientist 36, 51—58
24 Lienhard, G.E., Secemski, I.I., Koehler, K.A. and Lindquist, R.N. (1972) Cold Spring Harbor Symposia Quant. Biol. 36, 45—51
25 Johnson, P., Waheed, A., Jones, L., Glaid, A.J. III and Gawron, O. (1976) Biochem. Biophys. Res. Comm. in press.
26 Rose, I.A. and O'Connell, E.L. (1967) J. Biol. Chem. 242, 1870—1879
27 Gawron, O., Glaid, A.J. III and Fondy, T.P. (1961) J. Am. Chem. Soc. 83, 3634—3640