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Molecular Models of Metal Chelates to Illustrate Enzymatic Reactions

The conversion of citrate to isocitrate by aconitase involves a discrimination betweeen the two seemingly equivalent ends of the symmetrical citrate molecule. This discrimination was explained in 1948 by Ogston (*1*) who showed that attachment of a symmetrical molecule such as citrate to an enzyme at three points could lead to differentiation of the like groups. Schwartz and Carter (*2*) later showed that discrimination could also be accomplished non-enzymatically by a two-point contact of *meso*-substrate and asymmetric reagent. The Ogston model has long been used both as a mechanism for selective reactions and as a device for demonstrating the non-equivalence of like groups in a symmetrical molecule. This paper describes an alternative to the Ogston model which utilizes a metal ion to discriminate between the symmetrical ends of the citrate molecule. Molecular models of citrate and aconitate-metal chelates are used to illustrate a possible mechanism for the aconitase reaction.

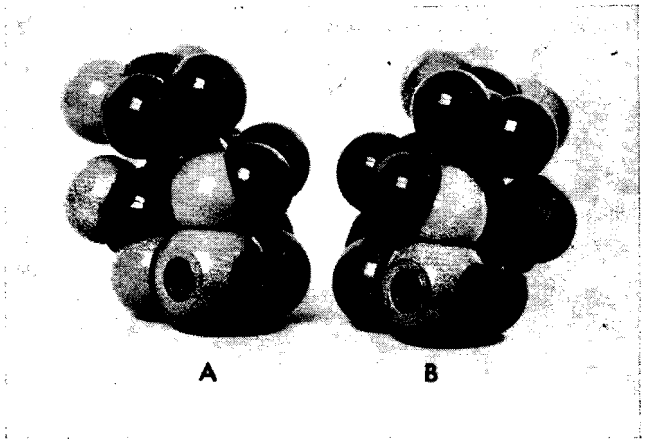

Figure 1. Molecular models of citrate-metal chelates. Left side (1a)—S isomer. Right side (1b)—R isomer. In these models the carboxymethyl group is oriented towards the top, the coordinated hydroxyl group is above the metal ion, and the coordinated carboxyl groups are on either side of the metal ion. The R isomer is shown in the same orientation in Figure 2.

Citrate forms a metal chelate with various divalent metal ions in which the middle-carboxyl group, the hydroxyl group, and one of the end-carboxyl groups are coordinated to the metal ion (*3*). The complex thus formed is asymmetric and can exist in two isomeric forms, depending on which end-carboxyl group is coordinated. It can easily be seen from the molecular models[1] of these two isomers (Fig. 1) that they are readily distinguishable and are in fact non-superimposable mirror images. These two isomers can be designated as having the R and S configurations according to the Cahn-Ingold-Prelog system (*4*). The carboxyl group of citrate which originates from acetyl-coenzyme A in the condensation reaction is chelated in the case of the S isomer and not chelated in the case of

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[1] Fisher-Hirschfelder-Taylor Molecular Models, Fisher Scientific Co.

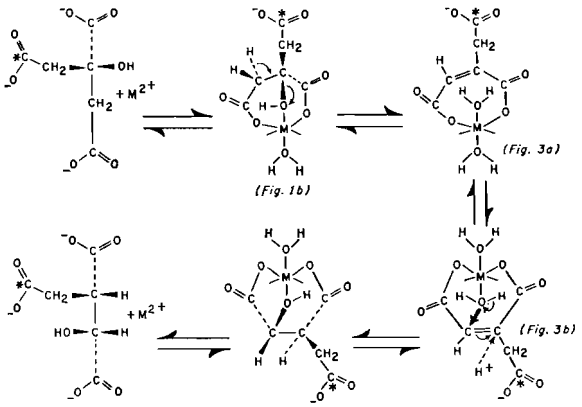

Figure 2. Stereochemistry and mechanism of the aconitase reaction.

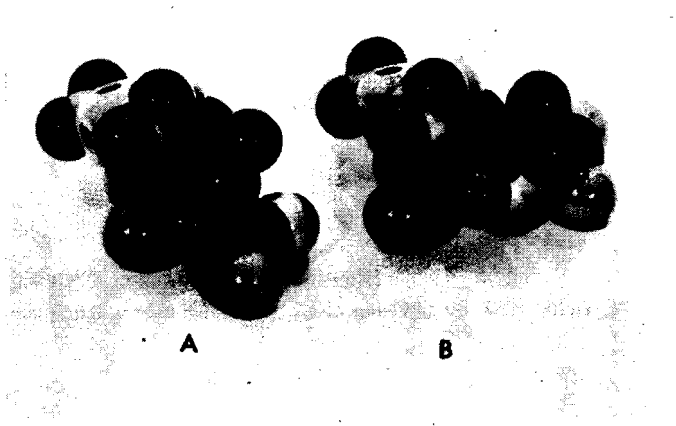

Figure 3. Molecular models of the *cis*-aconitate-metal complex. Left side (A)—Conformation for hydrotion to citrate. Right side (B)—Conformation for hydration to isocitrate. Line drawings of these two conformations are shown in Figure 2.

the R isomer. These two symmetrical ends of citrate can thus be readily distinguished through metal-complexing.

The aconitase reaction as shown in Figure 2 involves dehydration of citrate with the formation of *cis*-aconitate which is then rehydrated to the proper isomer of isocitrate. Isotopic labeling experiments have shown that citrate is dehydrated by a *trans*-elimination and aconitate is hydrated by a *trans*-addition (*5*, *6*). Furthermore, the hydroxyl group is added on one side of aconitate going to citrate, and on the opposite side going to isocitrate.

Iron(II) is required for the aconitase reaction and is thought to function as part of the active site through chelation with both the substrate and the enzyme (*7*). If this is so, a mechanism can be formulated in which only the R isomer of the citrate-Fe(II) complex fits into the active site of the enzyme. Citrate can then dehydrate as shown in Figure 2 to form a chelated aconitate. Keeping in mind the fact that hydration of aconitate to form isocitrate has to take place by attack of a hydroxyl group on the side opposite that from which the citrate hydroxyl was removed, one sees that aconitate can then swing down and become hydrated by nucleophilic attack of the coordinated water molecule on the bottom side of the metal ion. This mechanism is quite similar to that proposed by Speyer and Dickman (*7*) except for one important point. Their mechanism shows citrate being dehydrated and hydrated on the same side to form isocitrate. This mechanism can easily be illustrated by manipulation of a molecular model of a metal-complex of *cis*-aconitate. Hydration by attack of one of the coordinated water molecules leads to citrate (Fig. 3*a*) and hydration by attack of the other coordinated water on the opposite side leads to isocitrate (Fig. 3*b*). Close examination of the molecular model shows that this mechanism is entirely consistent with the known stereochemical course of the reaction as shown in Figure 2.

The use of molecular models of metal chelates thus represents a good means of illustrating the stereochemistry of citrate enzymology and a useful alternative to the classical Ogston model. Indeed, this effect of metal chelation on the symmetry of *meso*-compounds such as citrate has important implications concerning the role of metal ions in enzyme stereospecificity.

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