Iron Sulfur Clusters and the Role of Iron in Aconitase

Anne Venturelli Literature Seminar March 19, 1992

Protein bound iron sulfur clusters are found in most bacteria, plants, and mammals, primarily as electron transfer agents, e.g. cytochrome, and sulfite reductase, but also less commonly as active sites of enzymes, e.g. aconitase. Iron sulfur clusters can be mononuclear to tetranuclear in iron, with cysteinyl, sulfide, and/or histidine ligand environments about the iron.

Aconitase catalyses the isomerization of citrate to isocitrate via a dehydration/rehydration reaction, which occurs in the second and third steps of the Krebs Citric Acid Cycle.

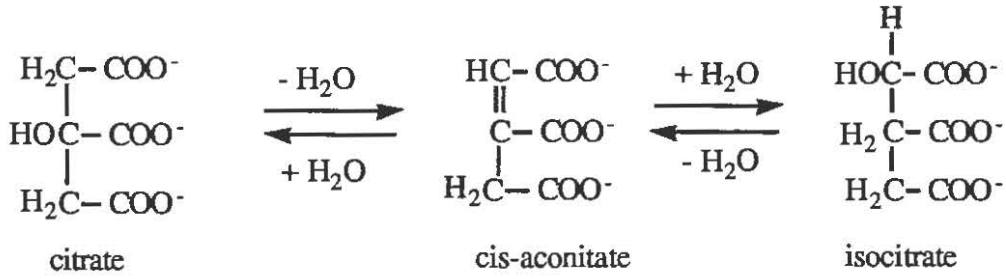

Figure 1: Isomerization of Citrate to Isocitrate via *cis*-Aconitate,

Aconitase was first identified in 1937 by Martius [1], who discovered the enzyme's ability to distinguish between the two acetyl arms of citrate. Attempts to purify the enzyme gave inactive aconitase, which could be reactivated with ferrous ion [2]. In 1968, Glusker [3] proposed the "ferrous wheel" mechanism, in which water and the carboxyl groups of *cis*- aconitate can coordinate to the iron in two orientations differing by 90° about the double bond, giving citrate or isocitrate (Figure 2).

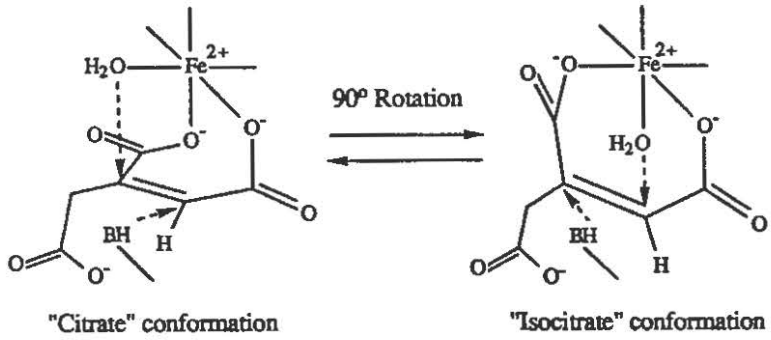

Figure 2 : "Ferrous Wheel" Mechanism

Aconitase was found to be an iron-sulfur protein in 1972 by Kennedy [4], who found by chemical analysis 2 Fe and 3 S/protein. Ruzicka and Beinert then discovered that the inactive Fe-S protein showed an EPR spectrum with g = 2.01, a characteristic value for a [Fe4S4] cluster, such as HiPIP (High Potential Iron Protein) [5]. In 1980, with the discovery of the three iron cluster in *Azotobacter vinelandii* [6], found crystallographically to be [Fe3S3] [7], and the correlation of the Mössbauer spectra [8], aconitase was assumed to have a three iron center. Beinert solved this dilemma by chemical analysis [9], in which the ratio of Fe:S was

close to 0.75 for the inactive enzyme and 1.0 for the active form, and EXAFS data, which gave Fe-Fe distances of 2.71 Å, a distance similar to Fe-Fe distances in cubanes (Figure 3). The evidence that aconitase has a cubic structure, was further supported crystallographicallly by Stout [10].

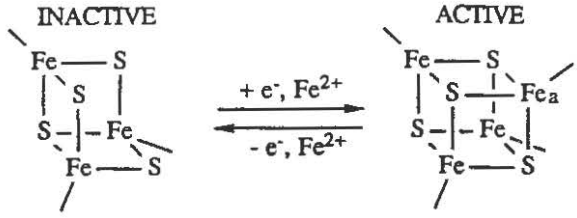

Figure 3 : Inactive to Active Aconitase

Through the use of Mössbauer, EPR and ENDOR spectroscopy [11], with labelling of the substrate, the mechanism of the isomerization of citrate to isocitrate has been developed. This mechanism involves the binding of citrate to the iron and dehydration to give the *cis*-aconitate, which then disengages from the active site, rotates 180°, reattaches, and rehydrates [12]. The iron - sulfur cluster acts as a Lewis acid by dehydrating the citrate, and then activating the adjacent carbon for hydroxyl attack.

Although there is no detailed crystal structure, the active site of aconitase has been developed through the use of various spectroscopic studies. Aconitase contains a unique iron-sulfur cluster which does not function as an electron transfer agent, but instead is involved in the binding and isomerization of citrate to isocitrate.

References

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