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B19

A Novel System Involving Exchange-Coupling between a Heme and an Iron–Sulfur Cluster

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The β-subunits of *E. coli* sulfite reductase (SiR) contain an Fe-isobacteriochlorin, termed siroheme, and a [4Fe-4S] cluster. Mössbauer and EPR studies [1] of oxidized SiR have demonstrated that the siroheme and the iron–sulfur cluster are exchange-coupled. Such a coupling implies a covalent link between the two chromophores; it is reasonable to assume that the cluster is attached to the heme iron by an as yet unspecified bridging ligand. In oxidized SiR the iron-sulfur cluster is in the 2+ oxidation state, a state in which [4Fe-4S] clusters are normally diamagnetic. Through exchange interactions with the siroheme the cluster acquires paramagnetism; the experimental observations have been explained qualitatively in a model which involves isotropic exchange between the heme iron and one iron site of the cluster [2]. Recent studies [3] of one-electron and two-electron reduced SiR, a nitrite 'turnover' (ferroheme-NO) complex, and studies of SiR in chaotropic agents show that the coupling is maintained in many oxidation-, spin-, and complexation-states of the enzyme. We have also studied SiR complexed to cyanide (in three oxidation states) and carbon monoxide. Exchange-coupling is indicated in the oxidized cyano complex; in the reduced CN^- and CO complexes the heme is low-spin ferrous and thus in a state unfavorable for the development of interatomic exchange.

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B20

Multifunctional Behavior as an Aid in Deducing Metalloenzyme and Model Reaction Mechanisms

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The ability of a substance to participate in competing reactions presents difficulties but if the problems can be resolved a clearer picture of the behavior in all reaction modes may be forthcoming. Oxalacetate is involved in a number of different biological reactions which are catalyzed by metalloenzymes. Many of these reactions are amenable to independent investigation in model systems employing metal ions.

Decarboxylation is one reaction mode of $oxac^{2-}$ which has attracted interest for many years owing to strong resemblances between the rate dependencies of the enzymic and model systems on metal ion concentration. The Steinberger–Westheimer mechanism had early been accepted as the means by which metal ions catalyzed $oxac^{2-}$ decarboxylation:

$$M^{2+} + oxac^{2-} \rightleftharpoons M(oxac) \xrightarrow{-CO_2}$$

$$M(enolatepyruvate) \xrightarrow{H^+} M(pyruvate)$$

Prevailing evidence indicates that the activation barrier is lowered by the complexation of the high energy enolate of pyruvate.

Enolization and hydration reactions of $oxac^{2-}$ procede within the same time frame and are closely entwined. Resolved rate data show that the reactions are subject to acid and base catalysis. Proton catalysis appears to be equally effective for both, but large differences are evident in base catalysis. Hydration rates are sensitive to bases possessing an oxygen donor atom. OH^- is a very efficient catalyst and even H_2O catalyzed rates are appreciable. Tertiary amines are found to be weak catalysts. Enolization appears to be more susceptible to softer bases. The rate constant for the OH^- catalyzed path is 1/6 as large as that determined for hydration, and H_2O catalysis is negligible; however, tertiary amines are potent catalysts and the more basic ones exceed OH^- in activity. Different sites are involved in catalysis. Enolization involves the removal of a $-CH_2-$ proton from