1 J. J. Roberts and A. J. Thomson, *Progr. Nucleic Acid Res. Molec. Biol., 22,* 71 (1979).
2 M. C. Poirier, S. J. Lippard, L. A. Zwelling, H. M. Ushay, D. Kerrigan, C. C. Thill, R. M. Santella, D. Grunberger and S. H. Yuspa, *Proc. Nat. Acad. Sci. U.S.A., 79,* 6433 (1982).
3 S. J. Lippard, *Science, 218,* 1075 (1982).
4 J. P. Caradonna, S. J. Lippard, M. J. Gait and M. Singh, *J. Am. Chem. Soc., 104,* 5793 (1982).
5 T. D. Tullius and S. J. Lippard, *Proc. Natl. Acad. Sci. U.S.A., 79,* 3489 (1982).
6 T. V. O'Halloran and S. J. Lippard, submitted for publication.

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.

1 J. A. Christner, E. Münck, P. A. Janick and L. M. Siegel, *J. Biol. Chem., 256,* 2098 (1981).
2 E. Münck, in 'Iron–Sulfur Proteins', T. G. Spiro, ed., Wiley–Interscience, New York, 1982, p. 147.
3 J. A. Christner, E. Münck, P. A. Janick and L. M. Siegel, submitted.

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

$oxac^{2-}$, while in hydration the base attacks the keto carbon atom through an H_2O molecule, $-C(=O)-CH_2- + HOH\cdot\cdot B \rightleftharpoons -C(O^-)(OH)-CH_2- + BH^+$. In either case, an oxyanion is formed at the 2-carbon atom.

Metal ion complex formation has a relatively small effect on the rate constants for acid catalyzed enolization, suggesting that there is little interaction between the metal ion and the site for proton attack, the keto group oxygen atom. In contrast, the rates along the base catalyzed pathways are strongly influenced by a metal ion and exhibit increases that appear more dependent on the nature of the catalyst than on the reaction mode. The presence of Mg(II) induces increases of three orders of magnitude in the rates of OH^- catalyzed enolization and hydration, while the H_2O catalyzed pathway for hydration and the tertiary amine catalyzed pathways for both reactions undergo rate increases of 20–30 fold. A charge effect is apparent in these figures, but, especially in the case of OH^-, the metal ion must interact with, and stabilize the intermediate oxyanions in ways similar to that by which decarboxylation is promoted.

Rates of ketonization of enolpyruvate are also being investigated. Consistent with the charge differences acid catalysis is slower, and base catalysis is faster with enolpyruvate than with $oxac^{2-}$. Complexing metal ions show very strong cooperativity with base catalysts. Here again, the metal ion seems to function as a stabilizer of the intermediate oxyanion.

The coordinating abilities of metal ions are paramount in their causing activation barriers to be lowered in these model reactions. However, recent evidence obtained in a detailed study of enzymic decarboxylation suggests that an enzyme may not utilize this function of a metal ion, but may achieve greater catalytic efficacy through other mechanisms, such as proton transfer.

B21

Active Site of Copper Amine Oxidases

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Copper amine oxidases catalyse the oxidative deamination of biological amines according to the following equation:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$$

It should be recalled that another class of amine oxidases was described. They contain FAD as a prosthetic group, but will not be discussed here.

All copper amine oxidases have molecular weight higher than 100,000: some of them appear to contain two equivalent subunits which are probably not linked by disulfide bridges [1, 2]. All these enzymes contain at least two copper atoms and one carbonyl group per molecule. The nature of the carbonyl cofactor is a controversial issue: many data suggest that it could be identified as pyridoxal-5-phosphate [1].

Electron spin resonance (ESR) spectra show that the two copper atoms are in an equivalent environment and in the cupric state [3, 4]. The spectrum is typical of Cu(II) in a complex of tetragonal symmetry [3]. The use of ^{15}N labeled putrescine demonstrated that the substrate is not directly bound to copper [3]. Magnetic resonance studies [3, 5] and the comparison of the absorption and ESR spectra of the bovine enzyme with a model complex [6] suggest that in these enzymes at least two nitrogen atoms and 2 oxygen (from water) are coordinated around the copper ion.

When copper amine oxidases were treated with phenylhydrazine, 1 carbonyl group/mol was titrated [1] and an enzymatically inactive irreversible adduct was formed: its formation requires the presence of cupric copper [7].

After treatment with phenylhydrazine the bovine plasma enzyme shows changes in the ESR parameters of only one copper [7], indicating that the two copper sites are not identical. The non-identity of copper sites was demonstrated also in the pig plasma enzyme by magnetic studies [5]. The copper atoms however, become equivalent after treatment with strong ligands like N_3^- and CN^- [5]. These results indicate a functional significance of the difference between the two copper atoms. Since the enzyme molecule contains two non identical copper ions and only one carbonyl group, these findings suggest that only one of the two coppers has a role in the substrate oxidation. If the second prosthetic group is pyridoxal-5-phosphate, one of the copper ligands could be the nitrogen of the pyridine ring [1]. The substrate may bind the carbonyl cofactor and copper may have a catalytic role in oxygen activation [7].

1 B. Mondovi and A. Finazzi-Agrò, 'Structure and Function Relationships in Biochemical Systems', F. Bossa, E. Chiancone, A. Finazzi-Agrò and R. Strom eds., Plenum, 1982, p. 141.

2 P. Turini, S. Sabatini, O. Begani, F. Chimenti, C. Casanova, P. L. Riccio and B. Mondovì, *Anal. Biochem., 125,* 294 (1982).

3 B. Mondovì, G. Rotilio, M. T. Costa, A. Finazzi-Agrò, E. Chiancone, R. E. Hansen and H. J. Beinert, *J. Biol. Chem., 242,* 1160 (1967).