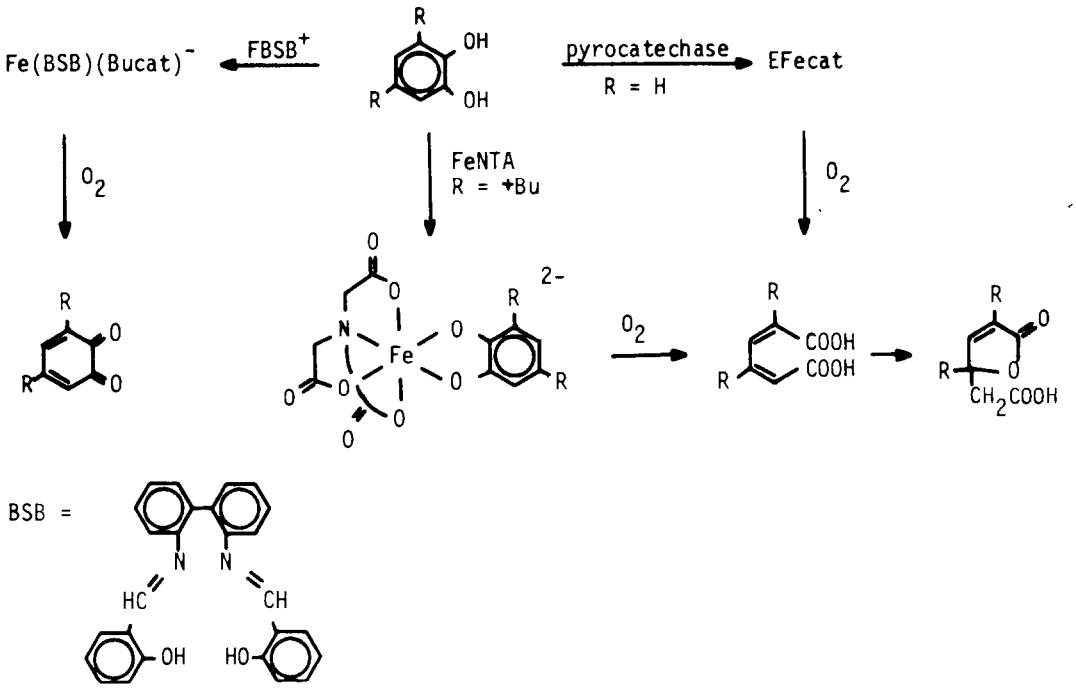

Scheme.

tyrosines [2]. Models using phenolic ligands, *i.e.* salen [3], or N,N'-bis(salicylidene)-1,1'-biphenyl-2,2'-diamine (BSB; scheme), however, failed to yield the enzymatic ring cleavage of catechols in mixed ferric complexes. *E.g.*: $[Fe(BSB)(Bucat)]^-$ ($BucatH_2$ = 3,5-di-t-butylcatechol), upon exposure to O_2, was found to undergo rapid oxidation to form 3,5-di-t-butylquinone. In this system, $[FeBSB]^+$ (identified by nmr and Raman Resonance spectra; C. Ruh, M. G. Weller and U. Weser, to be published) acts as an effective catalyst for catechol oxidation, but in a way different from the one characteristic for pyrocatechase.

The dioxygenase action is, however, exhibited by ferric nitrilotriacetate (FeNTA). We have isolated the mixed complex $Fe(NTA)(Bucat)^{2-}$ as its piperidinium salt [4]. With O_2, this system yields ring cleavage of the bound catechol, and it does so catalytically (scheme). Apparently, the coordinated catechol is attacked by dioxygen to form muconic acid, the latter then separates from the ferric centre, and another catechol enters the complex to undergo oxidation. Spontaneous cyclization of the muconic acid finally gives the lactone that is isolated as the reaction product.

A typical example: A mixture of FeNTA and $BucatH_2$, molar ratio 1:100, in aqueous DMF with borate buffer pH 8, after 7 days at room temperature, yields 80% lactone, together with traces (2%) of quinone and some unreacted catechol, *i.e.* a turnover of 80 mol catechol per mol Fe^{3+}. Without NTA present, with Fe^{3+} aq only, quinone is the oxidation product found.

Both for the epr and visible spectra of $[Fe(NTA)(Bucat)]^{2-}$ and for its reactivity towards O_2, ferric nitrilotriacetate can be viewed as an active centre analogue of pyrocatechase.

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N11

Spectroscopic Evidence for Metal–Thiolate Clusters in Complexes of Cd(II) with Dithiol Hexapeptides and in Cd–Metallothionein

HARALD WILLNER, MILAN VAŠÁK and JEREMIAS H. R. KÄGI

Biochemisches Institut der Universität Zürich, Zürichbergstrasse 4, CH-8028 Zürich, Switzerland

Metallothioneins constitute a novel family of widely occurring metal–thiolate proteins which are engaged in the metabolism and the detoxification of posttransitional metal ions (Zn(II), Cd(II), Cu(I),

etc.). All mammalian forms characterized are single chain proteins of 61 amino acid residues with binding sites for 7 metal ions [1]. The large metal-binding capacity is conditioned by the presence of 20 cysteine residues which provide thiolate ligands for the formation of two adamantane-like metal–thiolate cluster structures containing 3 and 4 metal ions, respectively [2, 3]. The unique occurrence of these clusters in metallothionein is believed to be related to the presence of 7 –Cys–X–Cys– sequences in the polypeptide chain where X stands for an amino acid residue other than Cys. To explore the metal complexing features of such dithiol sequences, we have now chemically synthesized by the Merrifield procedure the hexapeptides Ser–Cys–Val–Cys–Ala–Ala, Ala–Cys–Lys–Cys–Ala–Ala and Ala–Cys–Ser–Cys–Ala–Ala and have examined the spectroscopic features of their complexes with Cd(II) and related metal ions. Stepwise addition of Cd(II) to aqueous solutions of these peptides yields complexes of 1:2, 2:3 and 1:1 metal-to-peptide stoichiometry (Fig. 1(a)). The first two complexes display features

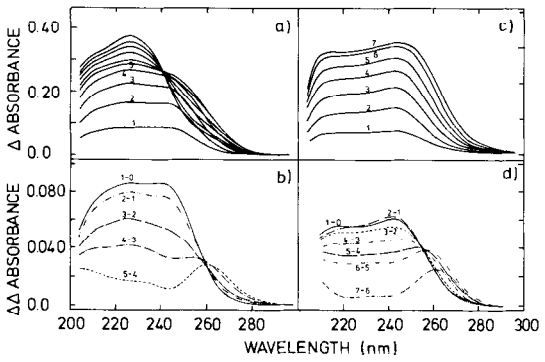

Fig. 1. Complex formation of Cd(II) with Ser–Cys–Val–Cys–Ala–Ala and with apometallothionein (=metal-free form of metallothionein). (a) Difference absorption spectra (peptide absorption subtracted) of a 30 μM solution of the peptide following successive additions of Cd(II) in 5 mM sodium phosphate, pH 7.0. At each titration step the concentration of Cd is increased by about 3.5 μM. (b) Difference–difference spectra showing absorption increments at each titration step (same data as in a)). (c) Difference absorption spectra (absorption of apometallothionein subtracted) of a 3.5 mM solution of apometallothionein following successive additions of Cd(II), in 5 mM Tris chloride buffer, pH 8. At each titration step the concentration of Cd is increased by about 3.5 μM. (d) Difference–difference spectra showing absorption increments at each titration step (same data as in c)).

typical of tetrahedral tetrathiolate coordination also seen in Cd(II)–metallothionein [4] (Fig. 1(c)). The 2:3 complex is thought to be a binuclear cluster complex composed of two tetrahedral cadmium–thiolate units connected via two bridging thiolate ligands. It differs from the mononuclear 1:2 complex by a slight red shift of the absorption envelope which can be attributed to the greater polarization of the bridging sulfur ligands by the metal. In addition, its formation is accompanied by a nearly complete loss of the strong circular dichroism denoting the greater symmetry of its structure. The spectral shift signalling the formation of the ligand-bridged binuclear complex is most clearly indicated by the emergence of a maximum near 260 nm in the difference–difference absorption spectrum (Fig. 1(b)).

Spectral changes entirely analogous to those accompanying the successive formation of the mononuclear and binuclear tetrahedral Cd(II)–peptide complex also occur in the course of reconstituting Cd(II)–metallothionein from Cd(II) and apometallothionein (Figs. 1(c) and (d)). Under the conditions employed (pH 8), Cd(II) binds at first to separate tetrathiolate sites. However, with all thiolate ligands becoming occupied, a red shift develops signifying the change-over to the clustered structure containing 40% bridging ligands. Interestingly, these spectral changes are much less pronounced when Cd(II) is incorporated at lower pH. Below pH 4, the binding of successive equivalents of Cd(II) is, in fact, accompanied by a blue shift of the Cd(II)–thiolate absorption envelope indicating an initial preferential formation of thiolate-bridged structures. Hence, it would appear that depending on pH the building-up of the metal–thiolate clusters in metallothionein proceeds through different pathways.

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N12

Modeling Studies of the Iron/Copper Binuclear Active Site of Bovine Cytochrome *c* Oxidase

LON J. WILSON*, VINAI CHUNPLANG, B. KAYE LEMKE, CONNIE L. MERRILL, ROBERT J. SAXTON and MARK L. WATSON

Department of Chemistry, William Marsh Rice University, P. O. Box 1892, Houston, Tex. 77251, U.S.A.

In attempts to model possible μ-imidazolato [1], μ-oxo [2, 3], and μ-mercapto [4] active site structures for resting (beef heart) cytochrome *c* oxidase