It appears then that the acceleration of electron transfer from cytochrome *a* to cytochrome a_3 by cytochrome *c* is not due to an allosteric interaction. Alternative possibilities include a direct involvement of the redox site of cytochrome *c* in the internal transfer of cytochrome *c* oxidase [3,4], or an as yet undefined electron transfer step between cytochrome *a* and cytochrome a_3 which is thermodynamically unfavorable. We are currently exploring these possibilities.

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PS2.9 — TH

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THE USE OF «NON-PERTURBATIVE» SITE-DIRECTED MUTAGENESIS FOR THE STUDY OF ELECTRON TRANSFER MECHANISMS IN METALLOPROTEINS

Recent advances in genetic engineering methodology have led to the design of proteins that provide new routes to the study of electron transfer mechanisms. Using site-directed mutagenesis [1-3], we have optimized yeast iso-1 cytochrome *c* [4,5] for studying intramolecular electron transfer mechanisms via the ruthenium modification method [6-9]. Each mutation was designed [10] to maintain the structural and functional integrity of the protein while providing for the efficient study of the effect of donor/acceptor distance, intervening medium, and donor/acceptor orientation on intramolecular electron transfer rates [11]. Currently, we have completed the generation, isolation, and characterization of three isostructural/isofunctional mutants of yeast iso-1 cytochrome *c*. The first two mutations, Cys-102/Ser and Ser-102/Thr, were designed to circumvent complications in the physical characterizations caused by the cysteine sulfhydryl [12,13]. And the third mutation, His-39/Lys (performed on the Thr-102 mutant), was designed to provide convenient access to the other naturally occurring surface histidine (His-33). This poster is concerned with the design rationale and experimental manifestation of the above mutations and the biological and physical characterization of these systems. It also addresses future directions in this work as well as the potential of using protein engineering and computer graphics methodologies for the design of novel, metalloprotein based, redox catalysts (for example, see [14]).

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PS2.10 — MO

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Cu(II)-LADH: A MULTI-SITE REDOX SYSTEM

In pursuit of our goal to understand the factors that govern the redox chemistry of multi-site metalloenzymes, we are investigating various metallo-substituted derivatives of liver alcohol dehydrogenase (LADH). LADH is a dimeric protein (MW ~ 80,000) comprised of identical subunits, each of which contains two Zn^{2+} ions — one at a catalytic site (c) and the other at a structural site (n) [1]. The catalytic Zn^{2+} is coordinated to 2 cysteines, 1 histidine and a water molecule and the other is bound to 4 cysteine residues.

The Cu(II)-substituted derivative, Cu_2(c) Zn_2(n)LADH, has been shown to be a spectroscopic analog of type 1, blue copper sites in proteins [2,3]. We have undertaken the electrochemical and spectroscopic characterization of this and other metal-substituted derivatives, including ones in which a coenzyme such as NAD^+ has replaced the coordinated water molecule at the active site. These data will be compared with results obtained for other blue copper proteins [4]. In the course of our investigations we have found it necessary to develop a new purification scheme for the commercially available native protein involving preparative scale isoelectric focusing. The characterization of the separated species (>6) will be presented. Moreover, a modification of a recent preparation [5] allows us to prepare electrophoretically pure derivatives of the type M_2(c)Zn_2(n)LADH. Our approach to understanding long range electron transfer has been through the use of covalent modification (using $[Ru(NH_3)_5(H_2O)]^{2+/3+}$) of surface histidines of structurally well-characterized proteins to provide specific redox pathways. The structure of LADH [6], examined using computer graphics, reveals five such groups that are available for modification. The distance between these residues and the catalytic site (containing Cu^{II} — fig. 1), including a description of the interposing medium, is given in Table I. We have been able to modify the histidines, enabling the electron transfer energetics between Ru^{II} and Cu^{II} to be evaluated.

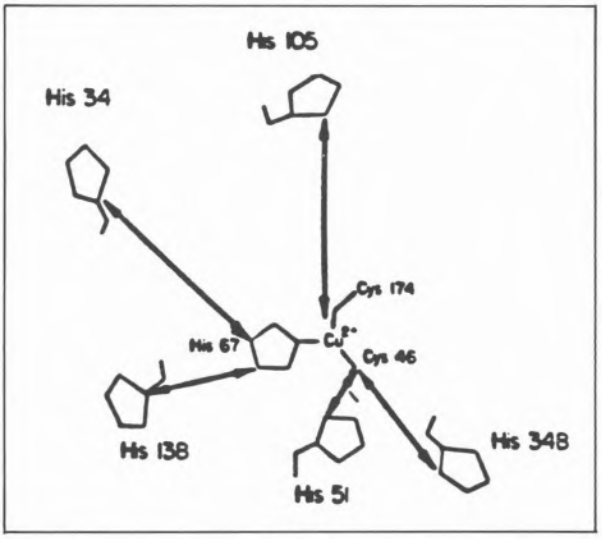

Fig. 1
Active Site of Cu^{II} substituted LADH illustrating nearest surface histidine residues

Table I
Structural Data for Cu_2(c)Zn_2LADH

Histidine Residue	Cu-histidine (A)	«medium»
51	7.4	nothing
348	14.9	Thr-370, Arg-369
138	15.1	Phe-140
105	21.7	Ash-109, Phe-319
34	22.3	Ser-156, Thr-142