

Table I

Successive formation constants of Cu^{2+} -protein complexes. 0.1 M KNO_3 or KCl, pH 5.8-6.0 in Mes or acetate buffer. For dopamine β -monooxygenase the constants refer to binding to enzyme subunits

		CuP logK ₁	Cu ₂ P logK ₂	Cu ₃ P logK ₃	Cu ₄ P logK ₄
Dopamine β -mono- oxygenase	apo native	11.2 6.4	7.1 5.5	6.4 5.5	
Bovine serum albumin		11.2	8.7	7.0	6.0
Apo carbonic anhydrase		10.4	7.1	5.8	
Ovotransferrin*		11.2	11.4	9.1	7.8

* pH 7.9, 15 mM NaHCO_3 .

Regarding dopamine β -monooxygenase, the results establish the stoichiometry of four high affinity binding sites for Cu^{2+} ($\log K_f \sim 11$) per enzyme tetramer, and more binding sites of lower affinity ($\log K_f \sim 5-7$). While the first four Cu^{2+} represent binding to a separate class of binding sites, the next four Cu^{2+} and so forth have the same affinity as for binding of excess copper to the other three proteins analysed. Additional copper ions in excess of four per enzyme tetramer may still be necessary for maximal activity under the conditions of catalysis (presence of substrates and a reducing agent), but they should then be regarded as activating copper ions rather than being an integral part of the enzyme.

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PS3.8 — TU

STEFAN DAHLIN
BENGT REINHAMMAR
JONAS ÅNGSTRÖM

Department of Biochemistry and Biophysics
University of Göteborg and Chalmers Institute of Technology
S-412 96 Göteborg
Sweden

NMR STUDIES OF Ni(II)- AND Co(II)-SUBSTITUTED STELLACYANIN

Stellacyanin is a small metalloprotein which, as it occurs in nature, contains a single copper(II) ion. The metal has a distorted tetrahedral coordination that gives the protein its intense blue colour. This so-called «type 1 copper» is also found in various other small proteins and in the more complex copper oxidases; laccase, ceruloplasmin and ascorbate oxidase. It is now widely accepted that the structure of the type 1 site in all proteins is essentially the same and that the copper is coordinated to two histidine imidazole nitrogens, a cysteinyl sulfur and, at least in two small proteins, to a methionine sulfur. Stellacyanin, however, contains no methionine and the nature of the fourth ligand is still enigmatic. Since stellacyanin is known to deviate considerably in many spectroscopic and chemical properties compared to the other blue copper-containing proteins, it has attracted much interest and a great number of spectroscopic methods have been used in attempts to identify the fourth ligand and to explore the complicated structure of this copper site.

To derive more information from spectroscopic measurements, it is useful to study metal substituted proteins in which the native copper is replaced by a suitable metal ion [1]. Popular candidates for this replacement are cobalt(II) and nickel(II) and optical absorption studies of stellacyanin derivatives of these metals have been reported earlier [2]. In this work, NMR studies of Co(II)- and Ni(II)-substituted stellacyanin have been performed.

The majority of resonances are found between about 10 and -10 ppm but, as can be seen in Fig. 1, several resonances exhibit large hyperfine shifts. Such large shifts indicate that both Co(II) and Ni(II) are in their high-spin state. As expected, the shifts are temperature dependent and Curie-plots for both derivatives show that several of the resonances in Fig. 1 deviate from linearity, indicating some conformational changes at about 30°C that occur in, or near, the metal site. In order to understand this temperature dependent transition, further NMR experiments are needed and the results of such experiments together with the results of magnetic susceptibility measurements will be presented on the poster.

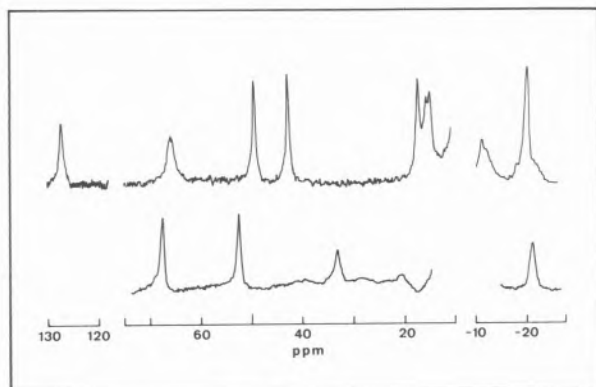


Fig. 1

Hyperfine resonances in the NMR spectra of Co(II)-stellacyanin (upper spectrum) and Ni(II)-stellacyanin (lower spectrum). The part appearing at negative ppm in the upper spectrum has been attenuated twice. Interjacent parts are omitted for clarity. For both derivatives, the concentration was 2 mM in D₂O, pH 7.4. Spectra were recorded using a Bruker WH 270 MHz spectrometer. A «water elimination Fourier transform» (WEFT) pulse sequence was employed

Varying the pH between about 4 and 9 indicated that none of the resonances in Fig. 1 are pH dependent.

It is useful to compare these spectra with the NMR spectra of Ni(II)- and Co(II)-substituted azurin obtained earlier [3]. The differences spotted in the out-shifted regions, in spectra of the two proteins reconstituted with the same metal, might give some clues to the structure of the site in stellacyanin since the fourth ligand in azurin is believed to be a methionine. The NMR spectrum of the Ni(II) derivative does not reveal any hyperfine shifted methyl resonances, thus confirming the absence of methionine in the coordination

sphere of the metal in stellacyanin. However, no clear candidates for the fourth ligand are obvious from the spectra.

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PS3.9 — TH

PETER P. CHUKNYISKI

JOSEPH M. RIFKIND

Laboratory of Cellular and Molecular Biology

Gerontology Research Center

National Institute on Aging

National Institutes of Health

DHHS, Baltimore, Maryland 21224

U.S.A.

KENNETH ALSTON

Department of Natural Sciences

Benedict College

Columbia, South Carolina 29204

U.S.A.

CONFORMATION AND TEMPERATURE DEPENDENT SPECTRAL CHANGES IN NICKEL HEMOGLOBIN

INTRODUCTION

It has been shown [1] that normal human adult hemoglobin (HbA) reconstituted with Ni(II) protoporphyrin IX does not bind oxygen and is in a T-like conformation. It was recognized that the value of studies on this stable non-reactive T-like hemoglobin would be greatly enhanced by the pre-