In consideration of its physico-chemical properties, the elucidation of ascorbate oxidase three-dimensional structure may also contribute to the comprehension of the association-dissociation phenomena and of their biological significance.

The protein employed for the crystallization experiments was purified from green zucchini squash according to the method of Avigliano *et al.* [5], showed absorption ratios $A_{280}/A_{610} = 25 \pm 1$, $A_{330}/A_{610} = 0.8 \pm 0.05$ and had a turnover number of $5 \times 10^5$ mol/min. Several micro-buttons filled with a 15 mg/ml solution of the enzyme were used in parallel dialysis experiments against different buffers and precipitating agents, in the pH range 5–9. Under the following conditions the same characteristic blue crystals of the enzyme could be grown:

(a) 1.8 *M* ammonium sulfate, at pH values 6.7 through 8.4

(b) 1.0 *M* sodium citrate, at pH 7

(c) 1.9 *M* potassium phosphate, at pH 7

The crystals obtained were subsequently used for a preliminary crystallographic investigation. From the analysis of the diffraction pattern symmetry and of the systematic absences it was possible to conclude that ascorbate oxidase crystallizes in the orthorhombic space group $P2_12_12_1$ with unit cell edges $a = 125.4$, $b = 189.8$, $c = 112.2$ Å. The asymmetric unit can thus accommodate a dimer of the enzyme (Mr 294,000) and the (volume) solvent content of the crystals is 45%. The crystals diffract to 3.0 Å resolution; this crystalline modification is isomorphous with that reported by Ladenstein *et al.* [6] for the same enzyme, but grown under different physico-chemical conditions.

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## O8

## EPR-Detected Interaction between Cytochrome $a_3$ and Cytochrome $a$ in Cytochrome $c$ Oxidase

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A prevailing view of cytochrome *c*-oxidase's mode of action is that the cytochrome *a* moiety of the oxidase is first reduced by electrons from ferrocytochrome *c*, and then electron transfer occurs from cytochrome *a* to cytochrome $a_3$ and finally to oxygen [1]. We address the question of how this functional interaction is related to structural perturbation of the $a_3$ center by the *a* center or to simple proximity of the two centers. We have investigated this problem with NO complexed to heme $a_3$ in fully reduced oxidase ($a^{2+}\cdot Cu_a^{1+}\cdot a_3^{2+}\cdot Cu_{a3}^{1+}$) and with NO and CO complexed to heme $a_3$ in a mixed valence (MV) state oxidase ($a^{3+}\cdot Cu_a^{2+}\cdot a_3^{2+}\cdot Cu_{a3}^{1+}$).

Bovine cardiac cytochrome *c* oxidase was prepared by methods of Refs. 2 and 3. Fully reduced and mixed valence complexes were prepared in a modified Thunberg cell under strictly anaerobic conditions. EPR (electron paramagnetic resonance) spectra were recorded under non-saturating microwave powers with a Bruker ER 420 (9.0–9.8 GHz) spectrometer over a 12–77 K temperature range.

The EPR of nitrosylferrocytochrome $a_3$ (*i.e.*, $a_3$-NO) in both fully reduced and MV forms showed detailed hyperfine structure from nitrogen nuclei of NO and proximal histidine. The EPR features encompassed a g-value range of $g_x$, $g_z$, $g_y = 2.09$, 2.006, 1.98. The MV-$a_3$-NO form, but not the fully reduced, showed additional temperature-dependent spectral changes setting in below 40 K. The most striking change occurred near g = 2.09 and smaller line-broadening changes occurred near g = 2.00. In MV-$a_3$-NO there thus appears to be an internal magnetic interaction that shows rapid, temperature-dependent fluctuations down to 40 K but below 40 K slows sufficiently to allow resolution by EPR. This internal interaction has a magnitude of 12, 1.5, and 5 Gauss at $g_x$, $g_z$ and $g_y$, respectively.

At present we have two separate explanations for the phenomenon which both indicate $a$–$a_3$ interaction. Either, as a result of redox changes at cytochrome *a*, a changed ligand-binding environment, which has conformations that rapidly convert down

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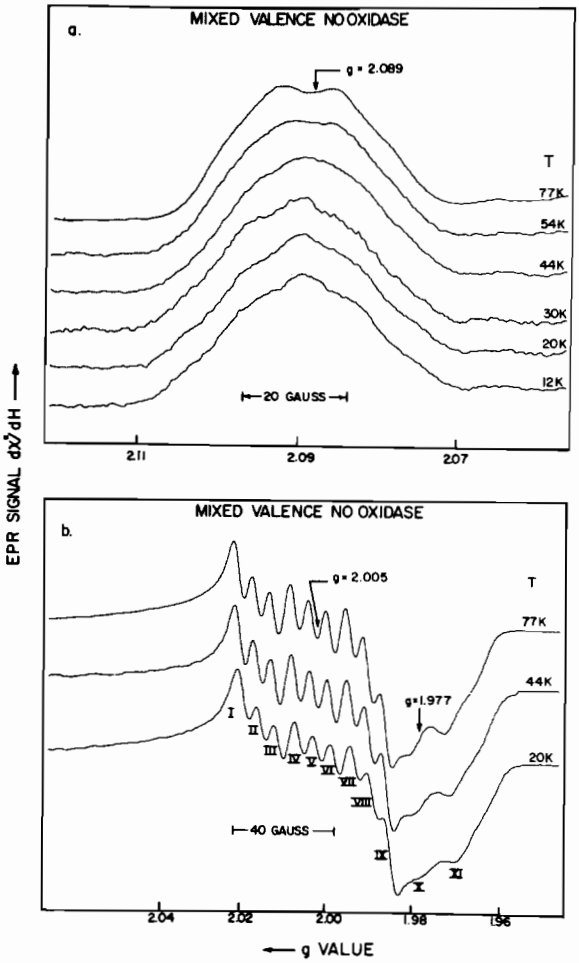


Fig. 1. (a) Shows the g = 2.09 feature of the MV-$a_3$-NO EPR spectrum from 12 to 77 K. (b) Shows the changes in the MV-$a_3$-NO EPR spectrum near g = 2.00 from 20 to 77 K.

to 40 K, occurs at NO-liganded cytochrome $a_3$. Or, a dipolar spin–spin interaction occurs between the NO-liganded $a_3$ center and a paramagnetic metal center in cytochrome *a*; this spin-spin interaction is modulated by temperature-dependent spin-lattice relaxation (which we have independently measured) of the center in cytochrome *a*. If the latter explanation is correct, the distance between $a_3$-NO and the interacting center in cytochrome *a* can be estimated at 15 Å, and the more likely interacting center in cytochrome *a* predicted to be heme *a*.

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## O9

## Crystallographic Studies on Horse Isoferritins

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Ferritin is an iron-storage protein which is found in eucariotes as well as in procariotes [1]. In mammals ferritin is present in almost every tissue, at higher concentrations in iron-rich organs such as spleen and liver. Ferritins extracted from a single tissue contain families of different isoferritins which can be distinguished according to their surface charges and/or immunological properties [2]. The prototype ferritin molecule is an oligomer of 24 subunits, arranged in 432 symmetry, which form an inner cavity of approximately 75 Å diameter. This hollow oligomeric molecule readily accommodates an inorganic matrix of hydrated ferric oxide phosphate (up to 4500 iron atoms stored) which possesses crystalline order. Ferritin heterogeneity can be related at a molecular level to the different associations of two subunit types in the protein shell. In liver ferritins the proteic shell is composed mainly of the so called L subunits (18,500 mol. weight), while H-type subunits (21.000 mol. weight) are predominant in heart ferritins [3].

Most of what is presently known of the three-dimensional structure of ferritins comes from the crystallographic investigations on spleen ferritin [4], which readily crystallizes from cadmium sulfate solutions (Space Group F432; a = b = c = 184 Å, one subunit per asymmetric unit). Much less is known about the structure of the H subunit-rich isoferritins, dealt with in this communication.

In the course of the last year we have achieved the crystallization of horse heart isoferritin (both holo and apo forms) under different physico-chemical conditions. In particular we were able to grow crystals from protein solutions containing MPD (2-methyl-2,4-pentane diol) and in the presence of polyethylene glycol (av. mol. weight 4.000 ÷ 6.000), but not from $CdSO_4$ (Fig. 1 a,b). Thus heart ferritins cannot be crystallized under the same physico-chemical conditions which are used for the crystallization of spleen ferritins. It is interesting to note that in the presence of MPD the reverse is also true.