Monomeric Dioxygen Complex of Cobalt(II) Substituted Cytochrome *c* Peptides in Aqueous Solution

Shosuke Kawanishi and Seiyo Sano*

Department of Public Health, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

As an artificial oxygen carrier, cobalt-substituted cytochrome *c* peptide was prepared, reaction of which with dioxygen yielded a stable monomeric oxygenated complex at room temperature; the e.s.r. signal ($g_\perp$ = 2.276) resulting from the deoxygenated form disappeared upon oxygenation and concomitantly a strong axially symmetrical e.s.r. spectrum with $g\perp$ = 2.003 appeared, suggesting the structure $Co^{III}O_2^{\cdot-}$ even in aqueous solution.

In the last decade many synthetic metalloporphyrin derivatives have been prepared as models for oxygen-binding hemoproteins.[1—3] However, most of these compounds can reversibly bind dioxygen in aprotic, but not aqueous solution. Cytochrome *c* peptides in which the histidine residue is co-ordinated with the central metal ion are interesting model compounds for studying the nature of metal–dioxygen binding in aqueous solution, and cobalt-substituted derivatives were used for the present study (Figure 1).

Cytochrome *c* peptide (1—65) and undecapeptide (11—21) were prepared by cyanogen bromide cleavage[4] and pepsin-catalysed hydrolysis[5,6] of horse heart cytochrome *c*, respectively. Iron was removed by hydrogen fluoride according to the method of Robinson and Kamen[7] and subsequently cobalt was inserted by heating the porphyrin cytochrome *c* peptides with $Co(OAc)_2 \cdot 4H_2O$ in 15% acetic acid.[8] All experimental operations were carried out under strictly anaerobic conditions using a vacuum line, according to the method of Sano *et al.*[9] The e.s.r. spectrum of the deoxygenated cobalt-substituted cytochrome *c* (Co–Cyt *c*) peptide (1—65) in aqueous acetic acid solution at 77 K exhibited a strong axially symmetrical signal at $g_\perp$ = 2.276 [Figure 2(a)], indicating that the cobalt(II) is a low-spin specimen with an unpaired electron in the d_{z^2} orbital. When the deoxygenated cobalt(II) complex was exposed to air in 15% acetic acid, the e.s.r. signal at $g_\perp$ = 2.276 disappeared and concomitantly a strong axially symmetrical e.s.r. spectrum with $g_\perp$ = 2.003 appeared [Figure 2(b)]. This implies that a cobalt–dioxygen complex was formed, having an unpaired electron residing essentially in a dioxygen π^* orbital, formally as $Co^{III}O_2^{\cdot-}$.[10,11] The deoxygenated cobalt complex in 0.2 M potassium phosphate buffer, pH 7.0 exhibited an axially symmetrical e.s.r. spectrum with $g_\perp$ = 2.268 and $g_\parallel$ = 2.030 (Figure 3), which closely resembles those of deoxy cobalt mesoporphyrin-substituted myoglobin (Mb) and hemoglobin (Hb).[12,13] The electronic spectra of both deoxygenated and oxygenated Co–Cyt *c* peptides were also identical with those of deoxy and oxy forms of cobalt mesoporphyrin-substituted Mb.[14]

It has been reported that most water-soluble cobalt–dioxygen complexes are binuclear and can be correctly described as μ-peroxo low spin cobalt(III) complexes;[2] the use of protonic solvents results in an irreversible oxidation of the cobalt complexes with dioxygen. The exceptions reported are cobalt-substituted Mb and Hb, which are capable of reversible oxygenation. It is generally accepted that the hydrophobic nature of the heme cleft is responsible for preventing the oxidation of the Fe^{II} and Co^{II} ion of Mb and Hb in the presence of dioxygen.[15] It is noteworthy that although the Co–Cyt *c* peptide (11—21) has little hydrophobicity around the Co–porphyrin, the cobalt–dioxygen complex is very stable in aqueous solution at room temperature. This implies that in a finely controlled ligand environment, even in aqueous solution, an imidazole group is capable of occupying the fifth axial position and facilitating the formation of a monomeric cobalt–dioxygen complex.

We thank the Ministry of Education, Science and Culture, Japan for a research grant, the Fujiwara Foundation of Kyoto

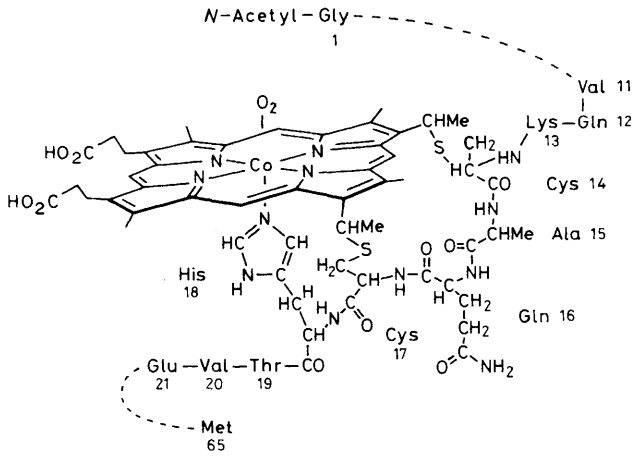

Figure 1. Dioxygen complexes of Co–Cyt *c* peptide (11—21) and Co–Cyt *c* peptide (1—65).

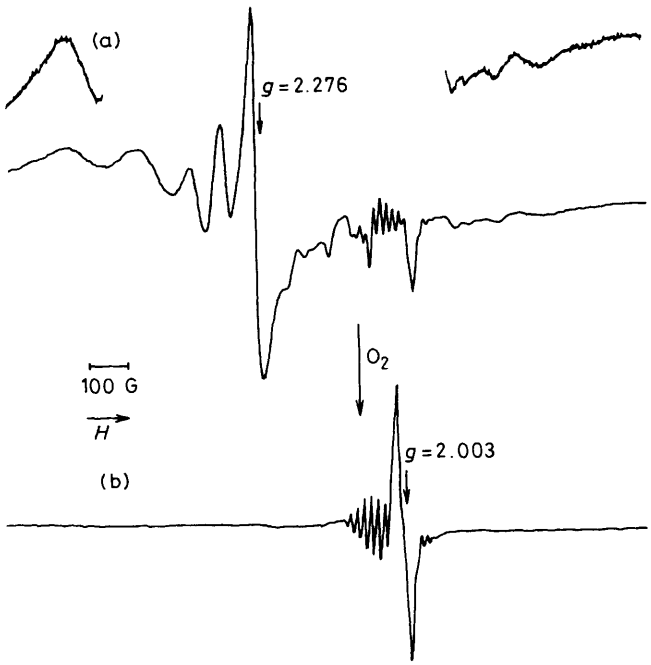

Figure 2. E.s.r. spectra of Co–Cyt *c* peptide (1—65) in 15% acetic acid at 77 K. (a) Deoxygenated sample prepared by mixing porphyrin Cyt *c* peptide (1—65) and $Co(OAc)_2$ in 15% acetic acid under deaerated conditions at 65 °C for 20 min, and then frozen in liquid nitrogen. (b) Sample (a) oxygenated by exposure to air and frozen rapidly in liquid nitrogen.

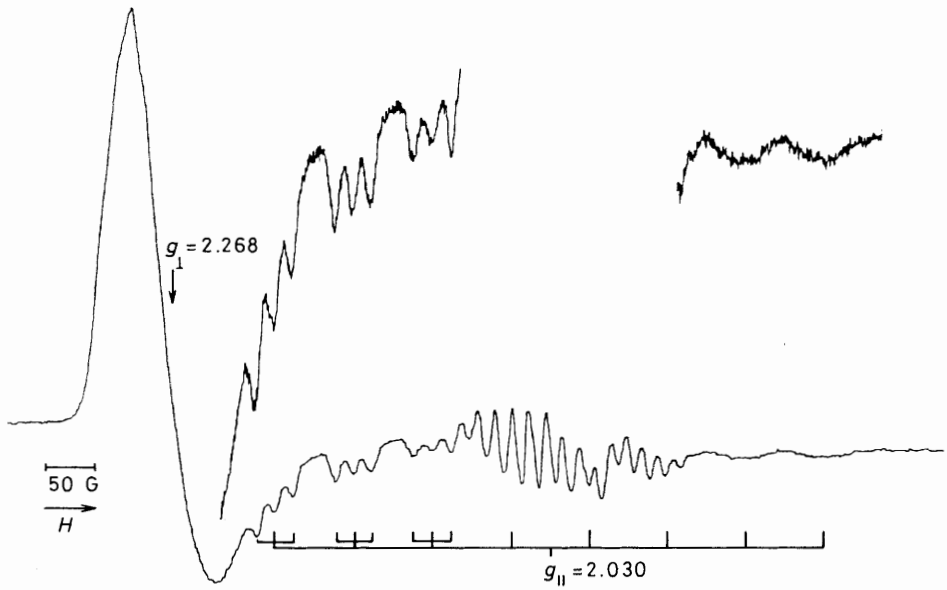

Figure 3. E.s.r. spectrum of deoxygenated Co–Cyt *c* peptide (1—65) in 0.2 M phosphate buffer, pH 7.0 at 77 K. The deoxygenated sample was prepared by reduction of the oxygenated complex with sodium dithionite in 0.2 M phosphate buffer, pH 7.0, and frozen in liquid nitrogen.

University for partial support, and Dr. Y. Sugiura for recording e.s.r. spectra.

Received, 30th July 1984; Com. 1126

References

1 J. P. Collman, *Acc. Chem. Res.*, 1977, **10**, 265.
2 R. D. Jones, D. A. Summerville, and F. Basolo, *Chem. Rev.*, 1979, **79**, 139.
3 T. G. Trayor, *Acc. Chem. Res.*, 1981, **14**, 102.
4 G. Corradin and H. A. Harbury, *Biochim. Biophys. Acta*, 1979, **221**, 489.
5 N. Nanzyo and S. Sano, *J. Biol. Chem.*, 1968, **243**, 3431.
6 H. Tuppy and S. Paléus, *Acta Chem. Scand.*, 1955, **9**, 353.
7 A. B. Robinson and M. D. Kamen, in 'Structure and Function of Cytochromes,' eds. K. Okunuki, M. D. Kamen, and I. Sekuzu, University of Tokyo Press, 1967, p. 383.
8 L. C. Dickinson and J. C. W. Chien, *Biochemistry*, 1975, **14**, 3526.
9 S. Sano, Y. Sugiura, Y. Maeda, S. Ogawa, and I. Morishima, *J. Am. Chem. Soc.*, 1981, **103**, 2888.
10 D. Getz, E. Melamud, B. L. Silver, and Z. Dori, *J. Am. Chem. Soc.*, 1975, **97**, 3846.
11 Y. Sugiura, *J. Am. Chem. Soc.*, 1980, **102**, 5216.
12 B. M. Hoffman and D. H. Petering, *Proc. Natl. Acad. Sci. USA*, 1970, **67**, 637.
13 T. Yonetani, H. Yamamoto, and T. Iizuka, *J. Biol. Chem.*, 1974, **249**, 2168.
14 T. Yonetani, H. Yamamoto, and G. V. Woodrow, III, *J. Biol. Chem.*, 1974, **249**, 682.
15 J. H. Wang, *Acc. Chem. Res.*, 1970, **3**, 90.