VANADIUM-SUBSTITUTED HEMOPROTEINS (I)

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Summary

The vanadium containing myoglobin and horseradish peroxidase were synthesized by recombination of apoproteins and various 2,4-modified vanadyl porphyrins.

Optical as well as electron paramagnetic resonance spectra were examined in detail, and it is concluded that vanadium-porphyrin can be used as a sensitive probe for the heme-environment in various hemoproteins.

Introduction

The metal substitution technique of iron-porphyrin has proved its usefulness for elucidating the structure-function relationship of various hemoproteins. One of the successful approaches of this line is the recent study of cobalt substitution of hemoglobin, myoglobin(1-6) and cytochrome c(7,8).

The present communication aims to demonstrate the possible use of vanadium ion as a probe for the study of heme-environment in various hemoproteins. The possibility that vanadium porphyrin may have some biological significance should not be completely excluded, since it occurs in petroleum(9).

Materials and Methods

The 2,4-modified vanadyl porphyrins were synthesized by the slightly modified methods of Erdman et al.(10) from vanadyl sulfate($VOSO_4$) and 2,4-modified porphyrins. The crude product was subjected to the silica-gel column chromatography, developed by the mixed solvent of methanol, chloroform and n-hexane as a ratio of approximately 1:1:1. After more than 5-times rechromatography, the crystallization was achieved from the dimethylformamide(DMF) solution under reduced pressure. The purity was checked by thin layer chromatography and optical absorption spectra. The details of the preparation and analytical data of those vanadyl porphyrins will be

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reported elsewhere(11). Sperm whale oxymyoglobin was prepared from the meat according to Yamazaki et al.(12). Horseradish peroxidase was purified by DEAE and CM cellulose column chromatography according to Shannon et al.(13) from the materials of Toyobo(JAPAN) product(R.Z. = 0.7). The enzyme used(R.Z. = 3.1) was the main fraction adsorbed on a CM cellulose column. All other reagents were analytical grade.

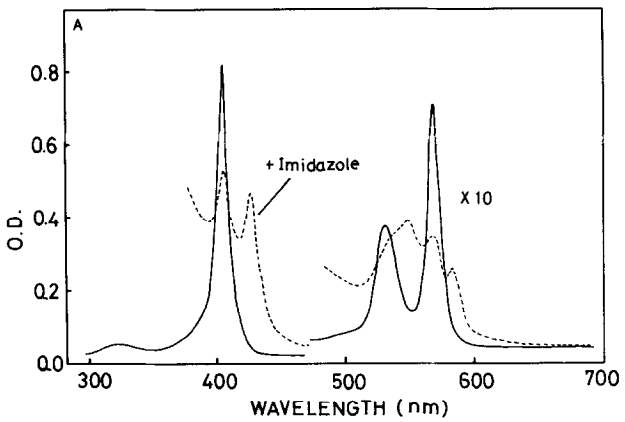
Recombination of vanadyl porphyrins and apoproteins was carried out as reported previously(14,15), where DMF was used for the solvent of vanadyl porphyrins. After dialysis against distilled water, the product was purified by DEAE cellulose column chromatography(15). The concentrations of apoproteins and vanadyl porphyrins were determined spectrophotometrically by use of the extinction coefficients at 280 nm of apoproteins (14,15) and the Soret band of vanadyl porphyrins(10,11), respectively.

Optical absorption spectra were measured with Cary-118 and Shimadzu UV-200 spectrophotometers. A Varian E-line spectrometer was used for electron paramagnetic resonance(EPR) measurements, operated with 100 KHz field modulation. The microwave frequency and power were 9.301 GHz and 10 mW, respectively. Modulation amplitude was 4 gauss.

Results

Figure 1-A shows the absorption spectra of vanadyl meso-porphyrin in DMF in the absence and presence of imidazole. The typical hemochromogen type spectrum of base-free vanadyl porphyrin, having absorption maxima at 405, 532 and 569 nm, is disappeared with the coordination of imidazole. Since the 6-th coordination position of vanadyl porphyrin has been occupied by an oxygen atom, imidazole coordinates only the 5-th position. Such coordinations were confirmed by titration experiment(11). The dissociation constant was found to be of the order of 1 M, showing the extremely low affinity as compared with the case of iron-porphyrin(16).

Figure 1-B shows the absorption spectra of vanadyl meso-myoglobin(Mb) in the absence and presence of sodium dodecyl sulfate(SDS). In native vanadyl-Mb at pH 7.5, the overall absorption spectrum resembles to the imidazole-coordinated spectrum in Fig. 1-A, not to the base-free spectrum of the hemochromogen type. However, the α-band around 590 nm splits



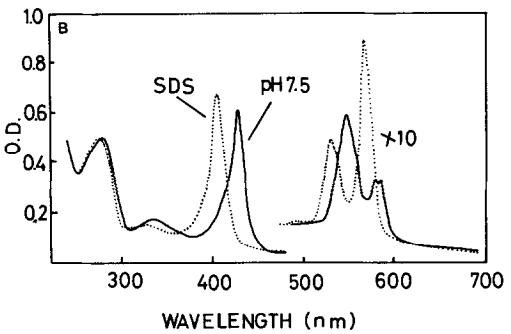


Fig.1: Optical absorption spectra of vanadyl meso-porphyrin(A) in DMF and vanadyl meso-Mb(B). Concentrations; vanadyl meso-porphyrin, 28 μM. Imidazole, 3 M. Vanadium meso-Mb, 25 μM. SDS, 0.1%. 0.1 M phosphate buffer, pH 7.5. Room temperature.

into small twin peaks and the β-band is larger than the α-band. The Soret absorption peak is also shifted from 427 nm to 430 nm. The molar ratio of apoprotein to vanadyl porphyrin was found to be 1:1, determined by the re-extraction of vanadyl porphyrin from the synthesized vanadyl-Mb. Upon addition of SDS, the spectrum turns into the typical hemochromogen type characteristic of base-free vanadyl porphyrin(cf. Fig. 1-A). Tables 1 and 2 summarize the absorption maxima of various vanadyl porphyrins and vanadyl porphyrin-Mb's.

Table 1

Porphyrins	- Imidazole	+ Imidazole
Proto	415, 540, 578	432, 554
Meso	405, 532, 569	427, 550, 584
Hemato	410, 539, 575	430, 545
Deutero	403, 534, 568	

The absorption maxima in nm of 2,4-modified vanadyl porphyrins in DMF. The spectra of imidazole complexes were obtained in the presence of 3 M imidazole in DMF.

Table 2

Proteins	pH 7.5	pH 4	+ SDS
Meso-Mb	430, 549, 583, 590	406, 537, 572	406, 533, 571
Hemato-Mb	414, 556, 588, 598	405, 543, 578	413, 538, 577
Meso-HRP	407, 539, 574	(a)	(a)

The absorption maxima in nm of vanadyl-Mb and -HRP. (a): No distinct optical absorption was obtained.

Figure 2 illustrates the EPR absorption spectra of vanadyl-Mb(meso- and hemato-) and vanadyl porphyrin itself. Though the optical characteristics markedly differ between vanadyl-Mb and porphyrin, the EPR absorptions little.

The pH-dependent absorption change of vanadyl meso-Mb is shown in Fig. 3, where the presence of isosbestic points is clearly seen. Lowering the pH, the absorption at 430 nm decreases with an increase of the absorption at 406 nm and the disappearance of the twin peaks around 590 nm. The spectrum at pH 4 is similar to the hemochromogen type spectrum

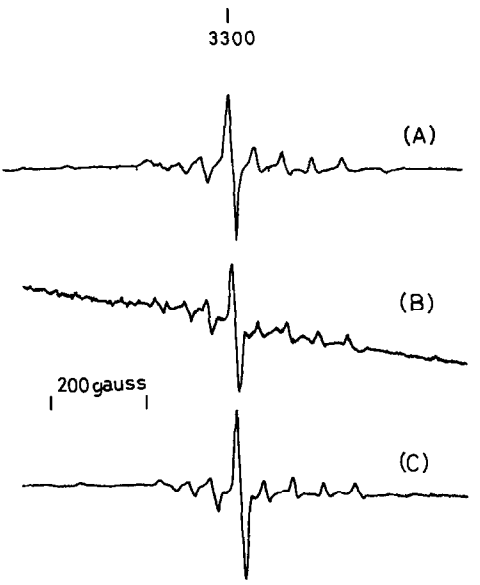


Fig.2: EPR absorption spectra of vanadyl meso-porphyrin in DMF(A), vanadyl meso-Mb(B) and vanadyl hemato-Mb(C) at the liquid nitrogen temperature. 0.1 M phosphate buffer pH 7.5. Concentrations were approximately 40 μM for all cases.

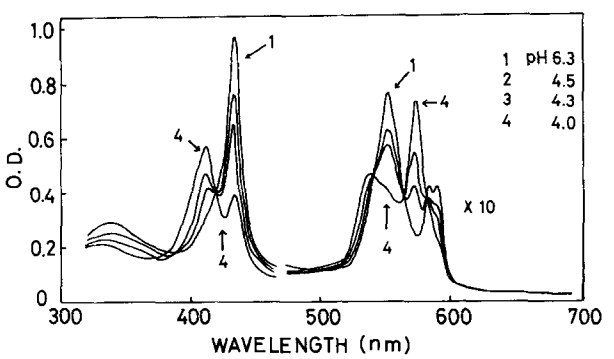


Fig.3: The pH-induced spectral changes of vanadyl meso-Mb. Adequate amounts(0.01~ 0.03 μl) of 0.1 M HCl were successively added to the optical cuvette containing vanadyl meso-Mb, with the simultaneous measurement of pH.

as is seen in Fig. 1-A. The pK value of this transition is approximately 4.4, similar to that of acid-denaturation of metmyoglobin(11). Between pH 5.5 and 11, the spectral change

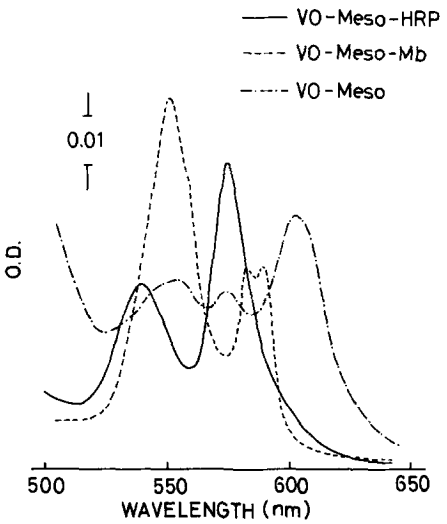


Fig.4: Comparison of the absorption spectra of vanadyl meso-Mb, -HRP and -porphyrin in aqueous solutions. The spectrum of vanadyl meso-HRP was taken at about 4°C, while others at room temperature. 0.1 M phosphate buffer pH 7.5. Concentrations were 27 μM for all cases.

does not occur, in contrast to the presence of acid-base transition in native metmyoglobin(15).

Figure 4 compares the optical absorption spectra of vanadyl meso-Mb and -horseradish peroxidase(HRP) in the visible region. The overall absorption spectrum of vanadyl meso-HRP resembles the base-free hemochromogen type spectrum(cf. Fig. 1) and also that appeared at pH 4.0 of vanadyl meso-Mb, though it differs from that of vanadyl porphyrin in aqueous solution. In the range from pH 5 and 11, the spectrum was found to be pH-independent. Here, it may be noted that the vanadyl-HRP was found to be extremely unstable under some conditions. For example, when it is stood at room temperature, the absorption spectrum turns gradually into that of a protein-free aqueous solution of vanadyl porphyrin within several minutes.

Discussion

The optical absorption spectrum of vanadyl meso-Mb has unique characteristics, especially the twin peaks of the α-band. When vanadyl hemato-porphyrin is used, similar twin peaks are also appeared. The optical characteristics may originate from the specific interaction between apoprotein and vanadyl porphyrin, since the denaturation of protein by the addition of SDS or by acidification(pH 4) causes the disappearance of this characteristic spectrum. In contrast to the optical absorption, the EPR absorption was found to be rather insensitive to the difference of such an interaction (cf. Fig. 2).

In spite of the successful preparation of vanadyl meso- and hemato-Mb, the vanadyl proto-Mb could not be obtained yet. This seems puzzling, since the presence of 2,4-vinyl groups of porphyrin ring might be suited for the recombination with apoprotein. The question remains unsolved.

The marked difference in the spectra between vanadyl-Mb and -HRP(Fig. 4) shows that vanadyl porphyrin is a sensitive probe for the study of heme-environment. Thus, the base-free hemochromogen type spectrum appeared in HRP may suggest the very weak or absence of the linkage between the 5-th ligand and the vanadyl ion as compared with that in myoglobin. Here, it is not likely to say that vanadyl-HRP is "denatured", since the absorption maxima of these hemochromogen type spectra differ in each other(cf. Table 1 and 2). Indeed, the denaturation of protein gave different absorption spectra which are similar to that of protein-free aqueous solution of vanadyl porphyrin. So far examined by the addition of various ligands, such as cyanide, azide and fluoride, distinct

complexes of those vanadyl-Mb and -HRP were not obtained. No complexes were also obtained by the addition of reductant, such as $Na_2S_2O_4$ (11).

The unique optical characteristics and the enzymic properties of those vanadium containing heme-proteins are now under more extensive investigations (11).

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