LANTHANIDE PORPHYRIN PROBES OF HEME PROTEINS.

INSERTION OF YTTERBIUM(III)MESOPORPHYRIN IX INTO APOMYOGLOBIN

William DeW. Horrocks, Jr.[+#], Robert F. Venteicher[≠], Curtis A. Spilburg[#], and Bert L. Vallee[#]

[#]The Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts and [≠]The Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania

Received March 19,1975

Apomyoglobin has been reconstituted with the lanthanide porphyrin complex, ytterbium(III)mesoporphyrin IX. The reconstituted material exhibits absorption and magnetic circular dichroic spectra significantly different from those of the ytterbium porphyrin itself. The sizeable, positive extrinsic Cotton effect in the Soret band of Yb-mesoporphyrin IX induced by the interactions with the globin indicates that the lanthanide porphyrin complex occupies the heme crevice.

Introduction

The substitution of metal ions of the first transition series for others native to metalloenzymes (1,2) and heme proteins (3,4) has allowed the study of structure-function relationships by a variety of physical methods not applicable to the metal ions found in the native species. Owing to their unique electronic and magnetic properties, as a class trivalent lanthanide ions represent environmental probes potentially even more versatile than transition metal ions. Use of lanthanide ions as probes has been restricted largely to calcium dependent macromolecular systems, where they have been employed as calcium replacements in, e.g., proteins. For these, moreover, the study of lanthanide derivatives by a variety of methods has proved to be extremely fruitful (5).

+ J.S. Guggenheim Memorial Fellow, 1974-75

Abbreviations: CD, circular dichroism; MCD, magnetic circular dichroism; Yb-mesoIX, ytterbium(III)mesoporphyrin IX; DME, dimethylester; acac, 2,4-pentanedionate ion.

We report here an entirely new method for the introduction of lanthanide ion probes into protein molecules. The synthesis in our laboratories of the first lanthanide porphyrin complexes (6) has made feasible the reconstitution of apoheme proteins with lanthanide derivatives of naturally occurring porphyrins. The incorporation of a metal ion probe into a metalloporphyrin prosthetic group offers a number of advantages over and above those pertaining to free metal ion - protein interactions alone. Thus, the porphyrin provides a fairly rigid, known structure in the immediate vicinity of the metal and resistance to both metal exchange and loss. Furthermore, metalloporphyrins occupy a unique and specific binding site in heme proteins. While heme proteins have been reconstituted with a variety of free-base natural porphyrins (7) and various metal derivatives (3,4), the present work represents the first insertion into an apoprotein of a metalloporphyrin other than one containing a metal of the first transition series.

Experimental

Yb-mesoIXDMEacac was synthesized from free-base mesoporphyrin IX-DME and hydrated $Yb(acac)_3$ by the method of Wong *et al.* (6) except that the solvent used was 4-phenylpyridine rather than 1,2,4-trichlorobenzene. This material (3 mg) was rendered water soluble by dissolution in 0.5 ml pyridine to which 0.5 ml of 0.1 M NaOH was then added to hydrolyze the propionic acid side chain ester linkages. This solution was evaporated to dryness and dissolved in a minimum volume ($\sim$1 ml) of 0.1 M NaOH. The concentration of the resulting Yb-mesoIX solution was estimated using $\varepsilon_{570} = 14900\ cm^{-1}\ M^{-1}$ which, in turn, was determined by quantitative conversion to the di-cation of mesoporphyrin IX in 0.1 M HCl which has $\varepsilon_{547} = 15100\ cm^{-1}\ M^{-1}$ (8). Apomyoglobin was prepared from whale skeletal muscle metMb (Sigma, lot 14C-0710) by the procedure of Rossi Fanelli, Antonini and Caputo (9). The apoMb was stored at 4° and used within ten days of its preparation. The concentration and purity of the apoMb were determined spectrophotometrically using the extinction coefficients previously reported (10) (metMb: $\varepsilon_{408} = 1.79 \times 10^5$; $\varepsilon_{280} = 3.45 \times 10^4$; apoMb: $\varepsilon_{280} =$

1.59 x 10^4 cm^{-1} M^{-1}). The apoMb thus prepared contained less than 3% native metMb. Reconstitution of apoMb with hemin (Sigma, crystalline, Type 1), reduction of the resulting material with sodium dithionite, removal of the dithionite on a G-25 column followed by air oxygenation produced a species with an absorption spectrum identical to oxyMb (11). The following instruments were used for the measurements indicated: absorption spectra, Cary 14; absorbance measurements, Gilford 240; CD and MCD spectra, Cary 61.

Results and Discussion

Reconstitution was carried out by adding aliquots of solutions of (∿3 x 10^{-3} M) Yb-mesoIX in 0.1 M NaOH to solutions of apoMb in 0.05 M Tris hydrochloride, pH 8.0. All experiments were performed using this buffer. (It should be noted that the acac ligand present in the Yb-mesoIXDMEacac starting material was very likely replaced by OH^- (or μ-oxo moiety) in the basic ester hydrolysis step; in any case, it provides no obstacle to reconstitution.) Upon interaction with the apoprotein the α and β bands of Yb-mesoIX at 532 nm and 570 nm intensify and shift slightly to 534 nm and 572 nm respectively, while the Soret band shifts from 397 nm to 403 nm (Figure 1B). This shift in the Soret band and the corresponding bathochromic Soret shift upon reconstitution of apoMb with hemin form the basis for the

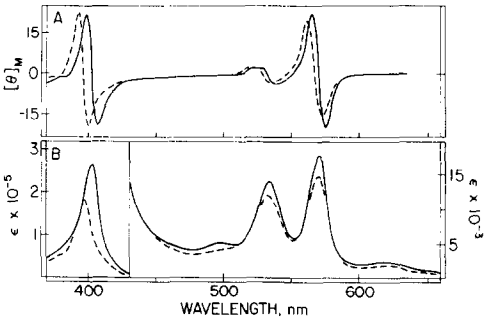

Fig. 1. MCD spectra (A) and absorption (B) ($[\theta]_M$, deg cm^2 $dmol^{-1}$ $gauss^{-1}$) of Yb-mesoIX (----) and Yb-mesoIX-globin (——) in 0.05 M Tris chloride, pH 8.0.

spectrophotometric titration of these metalloporphyrins with apoMb (Figure 2A) The interaction is stoichiometric, approximating 1:1 in both cases.

The MCD spectra of Yb-mesoIX and Yb-mesoIX-globin (Figure 1A) are noteworthy in that the ellipticities of the α and Soret bands are very similar although the extinction coefficients differ by factors of more than ten. Figure 2B shows the ellipticity at 398 nm as successive aliquots of Yb-mesoIX are added to apoMb. At this wavelength the ellipticity of Yb-mesoIX is zero, but that of Yb-mesoIX-globin is appreciable. Again, a 1:1 stoichiometry of interaction is evident.

The CD spectra provide important evidence that Yb-mesoIX has been inserted into the heme site of the globin. Free-base porphyrins (12) and metalloporphyrins (13) exhibit sizeable extrinsic Cotton effects, particularly in the Soret region, when situated in the heme crevice of this class of proteins. Such extrinsic Cotton effects have been found to be of either sign and of variable magnitude depending on the nature of the transition involved and its interaction with nearby coupled oscillators (13). A dominant positive extrinsic Cotton effect is induced in the Soret band for all Mb and Hb derivatives (13) including the apoproteins reconstituted with free-base porphyrins (12). For Yb-mesoIX-globin we observe a $[\theta]^{23°}_{404}$ of +54400 deg cm^2

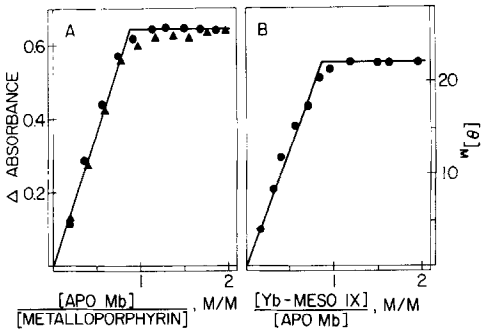

Fig. 2. A: Plot of absorbance change as apoMb is added to Yb-mesoIX monitored at 405 nm (●) or as apoMb is added to hemin monitored at 408 nm (▲). B: Plot of $[\theta]^{23}_{M}$, deg cm^2 $dmol^{-1}$ $gauss^{-1}$ at 398 nm as Yb-mesoIX is added to apoMb.

$dmol^{-1}$. The CD spectra in this region for Yb-mesoIX and hemin interaction with globin are shown in Figure 3A along with the results of titrations monitored at 404 and 407 nm, respectively (Figure 3B). A 1:1 stoichiometry of interaction is evident in both the Yb and Fe systems in agreement with the results of the absorption and MCD titrations. The sign and approximate magnitude of the 404 nm extrinsic Cotton effect correspond to those to be expected for the Yb-mesoIX moiety if it occupies the heme pocket.

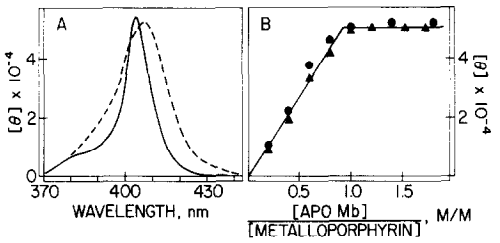

Fig. 3. A: CD spectra of Yb-mesoIX-globin (——) and reconstituted Mb (----) in 0.05 M Tris chloride, pH 8.0. B: Plot of $[\theta]^{23}$ as apoMb is added to Yb-mesoIX monitored at 404 nm (●) or hemin monitored at 407 nm (▲).

The existence of lanthanide porphyrin-globin species, demonstrated here by several spectroscopic techniques provides a new method for the introduction of lanthanide ion probes into macromolecules. Additionally, it provides evidence that highly non-planar metalloporphyrins are capable of occupying the heme crevice, since the Yb(III) ion is likely to be at least 1.4 Å from the plane of the pyrrole nitrogen atoms. Moreover, Yb-mesoIX, which appears to be indefinitely resistant to hydrolysis to the free base in 0.1 M NaOH, exists as a racemic mixture of chiral molecules (owing to the out of plane position of the Yb atom and the unsymmetrical positioning of the mesoporphyrin IX peripheral substituents). The apoprotein appears to accept both enantiomers, unless racemization occurs during reconstitution, which we consider unlikely.

We are currently investigating the nuclear magnetic resonance, fluorescence and other physical properties of Yb-mesoIX-globin and analogous species, as well as possible functional properties.

Acknowledgements

This research was supported by Grants GM-15003 and GM-02123 from the National Institutes of Health of the Department of Health, Education and Welfare, and by the National Science Foundation through Grant GP37577X.

References

1. Vallee, B.L. and Wacker, W.E.C., Metalloproteins in Neurath, H., ed., The Proteins, Academic Press, New York, 2nd ed., Vol. V (1970).
2. Vallee, B.L., Riordan, J.F., Johansen, J.T. and Livingston, D.M., Cold Spring Harbor Symp. Quant. Biol. 36, 517 (1971).
3. Spilburg, C.A., Hoffman, B.M. and Petering, D.H., J. Biol. Chem. 247, 4219 (1972).
4. Yonetani, T. and Asakura, T., J. Biol. Chem. 244, 4580 (1969).
5. Nieboer, E., Struct. Bonding (Berlin), in press.
6. Wong, C.-P., Venteicher, R.F. and Horrocks, W. DeW., Jr., J. Amer. Chem. Soc. 96, 7149 (1974).
7. Teale, F.W.J., Biochim. Biophys. Acta. 35, 289 (1959).
8. Falk, J.E., Porphyrins and Metalloporphyrins, Elsevier, N.Y. (1964), p.236.
9. Rossi-Fanelli, A., Antonini, E. and Caputo, A., Biochim. Biophys. Acta. 30, 608 (1958).
10. Harrison, S.C. and Blout, E.R., J. Biol. Chem. 240, 299 (1965).
11. Antonini, E., Burnori, M., Caputo, A., Chiancone, E., Rossi-Fanelli, A. and Wyman, J., Biochim. Biophys. Acta. 79, 284 (1964).
12. Ruckpaul, K., Rein, H. and Jung, F., Naturwissenschaften 57, 131 (1970).
13. Hsu, M.-C. and Woody, R.W., J. Amer. Chem. Soc. 93, 3515 (1971) and references therein.