1. Majumdar, A. K., *N-Benzoylphenylhydroxylamine and its Analogues*, Pergamon Press, Oxfo d, 1972.
2. Bauer, L. and Exner, O., *Angew. Chem. Intl. Ed.*, 1974, **13**, 376.
3. Das, M. K. and Majumdar, A. K. *Anal. Chim. Acta*, 1970, **50**, 243.
4. — and Chakravorti D., *Indian J. Chem.*, 1974, **12**, 773.
5. — and Ghosh, M. R., *Ibid.*, 1975, **13**, 515.
6. — and —, *J. Indian Chem. Soc.* (in press).
7. Ryan, D. E. and Lutwick, G. D., *Can. J. Chem.*, 1953, **31**, 9.
8. Lutwick, G. D. and Ryan, D. E., *Ibid.*, 1954, **32**, 949.
10. Zuckerman, J. J., *Adv. Organomet. Chem.*, 1970, **9**, 21.
11. Debye, N. W. G., Fenton, D. E., Ulrich, S. E. and Zuckerman J. J., *J. Organomet. Chem.*, 1971, **28**, 339.
12. Greenwood, N. N. and Ruddick, J. N. R., *J. Chem. Soc. A*, 1967, p. 1679.
13. Fitzsimmons, B. W., Seeley, N. J. and Smith, A. W., *Ibid.*, 1969, p. 143.
14. Parish, R. V. and Platt, R. H., *Ibid.*, 1969, p. 2145.
15. Ali, K. M., Cunningham, D., Frazer, M. J., Donaldson, J. D. and Senior, B. J., *Ibid.*, 1969, p. 2836.
16. Harrison, P. G., King, T. J. and Richards, J. A., *J. Chem. Soc., Dalton Trans.*, 1976, p. 1414

GADOLINIUM(III) MYOGLOBIN: INTERACTION OF Gd(III) MESOPORPHYRIN IX WITH APOMYOGLOBIN

CERTAIN lanthanide cations are excellent spectroscopic probes of their immediate environment in enzymes and proteins[1]. Recently, the formation of ytterbium(III) myoglobin was detected by the interaction of ytterbium(III) mesoporphyrin IX with apomyoglobin using spectroscopic techniques[2,3]. In this paper, we describe the first report on the detection, synthesis and spectral properties of gadolinium(III) myoglobin. Its comparative studies with europium(III) myoglobin have also been briefly described.

The hydroxy derivative of gadolinium(III) mesoporphyrin IX [Gd(III)–MP–IX)] was prepared by heating anhydous gadolinum trichloride (250 mg) and mesoporphyrin IX dimethyl ester (50 mg) in 2 g of imidazole melt at 210°–215° C for 2½ hours[4]. The imidazole was sublimed under reduced pressure to get a residue of gadolinium trichloride and dimethyl ester of Gd(III)–MP–IX. The Gd(III)–MP–IX was prepared from its dimethyl ester by hydrolyzing the ester with 30% potassium hydroxide in methanol. The Gd(III)–MP–IX was furtl er purified on silica gel (grade V) column equilibrated with methanol. The Gd(III)–MP–IX was eluted from the column using methanol as eluting solvent. The concentrated methanol solution of Gd(III)–MP–IX was stored at 5° C.

The preparation of gadolinium(III) myoglobin [Gd(III)–Mb] is as follows: The Gd(III)–MP–IX (1·5 moles) in a small quantity of pyridine was mixed with apomyoglobin[5] (1 mole) in 0·1 M Tris-HCl buffer of pH 8. The mixture was immediately passed through a long column of Sephadex G–25 equilibrated with 10 mM potassium phosphate buffer of pH 7·2. The column was eluted with the same buffer. The middle fraction was collected and further concentrated in collodian bag (Schleicher and Schuell, UH 100/10). The Gd(III)–Mb was stored at 5° C.

The hydroxy derivative of europium(III) mesoporphyrin IX [Eu(III)–MP–IX] and europium(III) myoglobin [Eu(III)–Mb] were prepared in a manner similar to the preparations of Gd(III)–MP–IX and Gd(III)–Mb respectively. The 1:1 complex formation between gadolinium(III) mesoporphyrin IX and apomyoglobin was established by difference spectroscopy in the visible region. The difference spectrum of Gd(III)–MP–IX and apomyoglobin in molar ratio of 1:1 against the same concentration of Gd(III)–MP–IX shows a negative peak at 398·5 nm and a positive peak at 406·5 nm. This 1:1 complex formation between Gd(III)–MP–IX and apomyoglobin was further established by passing a mixture of Gd(III)–MP–IX–pyridine complex and apomyoglobin in molar ratio of 1·2:1 through a long Sephadex G–25 column. The various Gd(III)–MP–IX–protien complex fractions obtained from the column show only a characteristic Soret, α and β bands and thus point to the existence of only 1:1 complex of Gd-(III)–MP–IX and apomyoglobin.

The absorption spectra of Gd(III)–Mb and Eu(III)–Mb are given in Table I. The spectra of Gd(III)–MP–IX and Eu(III)–MP–IX in various solvents are also given in the table for comparison. The Soret, α, and β bands of metal porphyrins in various solvents are red shifted in the order: pyridine > methanol > Tris-HCl buffer. The Soret bands of the metal(III)–Mb are about 4 nm red shifted as compared to the corresponding values of metal porphyrins in Tris-HCl buffer of pH 8. These results show that the Gd(III)–Mb and Eu(III)–Mb have very similar environment at the metal site.

In order to establish the site of binding of Gd(III)–MP–IX in Gd(III) Mb, the Gd(III)–MP–IX was displaced from Gd(III)-Mb by Fe(III)-protoporhyrin IX–chloride (hemin) using difference spectroscopy. The spectrum shows two positive peaks at 399·5 and 413·5 nm and a trough at 406 nm. This result shows that the Fe(III) Mb is formed by displacing Gd(III)-MP IX from Gd(III)-Mb by hemin in the sample

cuvette. Thus, the site of binding of Gd(III)–MP–IX is the heme pocket in the Gd(III)–Mb.

TABLE I

Visible absorption maxima of Gd(III)–MP–IX, Eu(III)–MP–IX, Gd(III)–Mb and Eu(III)–Mb[a]

Compound	Solvent	Soret (nm)	*a* (nm)	*β* (nm)
Gd(III)–MP–IX	Pyridine	410·5 (269)[b]	577 (19·6)	540 (18·4)
,,	Methanol	404·5 (251)	574 (19·4)	537 (16·0)
,,	Tris–HCl[c]	401·5 (277)	571 (14·4)	534·5 (12·2)
Gd(III)–Mb	,,	405·5 (310)	572·5 (19·1)	535 (14·7)
Eu(III)–MP–IX	Pyridine	409 (208)	577 (15·8)	541 (14·8)
,,	Methanol	405 (176)	574·5 (14·1)	538 (13·7)
,,	Tris–HCl	401 (181)	572 (10·0)	535·5 (8·6)
Eu(III)–Mb	,,	405·5 (254)	573·5 (17·1)	537 (14·4)

[a] Concentrations of metal porphyrin and metal protein complex are in the range 3 to 8 μM.

[b] The values in paranthesis are millimolar extinction coefficients ϵ_{mM} (mM^{-1} cm^{-1}).

[c] Tris–HCl is 0·1 M buffer of p H8.

The electron paramagnetic resonance (EPR) spectrum of Gd(III)–Mb was recorded in Fig. 1 at liquid nitrogen temperature. The signal of Gd(III) in the Gd(III)–Mb shows nitrogen hyperfine structure with about 14 peaks (*hyperfine splitting* ≃ 15 gauss). These 14 peaks of nitrogen hyperfine structure are interpreted in terms of Gd(III)-MP.IX in the heme pocket where the four nitrogen atoms of the porphyrin and fifth nitrogen atom of proximal histidine[6] are coordinated to gadolinium(III). Thus, the EPR spectrum of Gd(III)–Mb further supports the site of binding of Gd(III)–MP–IX in the heme pocket of the Gd(III)–Mb. The splitting of the nitrogen hyperfine structure in the Gd(III)–Mb is expected to vary with change in structure of Gd(III)–Mb and, thus, these structural changes can be probed using Gd(III) in the Gd(III)–Mb.

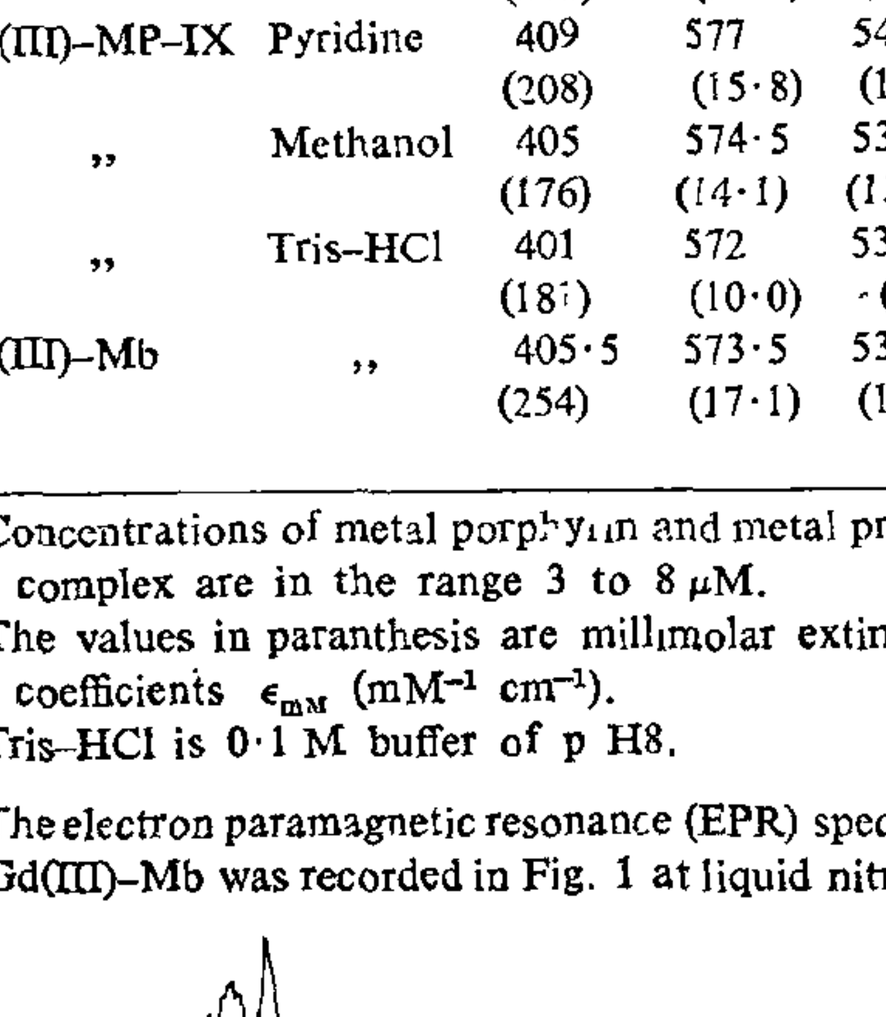

FIG. 1. Electron paramagnetic resonance spectrum of gadolinium(III) myoglobin in 0·1 M potassium phosphate buffer of pH 8 at 77° K. Concentration of Gd(III)–Mb was 1 mM.

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1. Nieber, E., *Sruct. and Bonding*, 1975, **22**, 1.
2. Srivastava, T. S., Hershberg, R. and Yonetani, T. *Biophys. J.*, 1975, **15**, 145 *a*.
3. Horrocks, Jr., W. D., Ventiecher, R. F., Spilburg, C. A. and Vallee, B. L., *Biochem. Biophys. Res. Commun.*, 1975, **4**, 317.
4. Srivastava, T. S., *Bioinorg. Chem.*, 1978, **8**, 61.
5. Teale, F. W J., *Biochim. Biophys. Acta*, 1959, **35**, 543.
6. Perutz, M. F., *British Med. Bull.*, 1976, **32**, 195.

CRYSTALLOGRAPHIC STUDY OF SOME MIXED PYROCHLORES

ANALYSIS[1] of natural pyrochlore minerals suggest that wide variety of both cationic and anionic substitutions are possible; however, literature survey revealed that very little work is reported[2–4] on pyrochlore compounds where substitution of cations is carried out at A as well as B sites. In the present studies, mixed pyrochlores containing cations of different oxidation states at A and B sites have been prepared. Pyrochlore structure has the general formula $A_2B_2O_7$ where A is a larger cation coordinated by eight oxygen ions while B is a smaller cation octal edrally surrounded by six oxygen ions, The space group is $Fd3MO_h^7$.

All the compositions mentioned in Table I were prepared by using standard ceramic technique and X-ray diffractometer patterns were taken on Philips machine using CuK*a* radiation with Ni filter.

From Table I it is observed that the compound $PbXTiNbO_7$, where X = Sm, Gd, Dy, Y and Bi posses pyroschlore structure while the compound containing neodymium is outside the limit of pyrochlore formation. If the compounds mentioned in Table I are considered on the basis of radius ratio,