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Note

Postulated tetraphenylporphyrin–zinc tetraphenylporphyrin sandwich complex

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A new method for the determination of trace metals has been developed in our laboratory[1–3], utilizing thin-layer chromatography (TLC)[1] and the mass spectrometric integrated ion current (IIC) technique[2]. This procedure is specific and sensitive in the femtomole (10^{-15} mole) range. Tetraphenylporphyrin (TPP) was chosen as the chelating reagent for the derivatization of trace metals in tissue extracts, because it possesses a high molecular weight, a large molecular ion, and its chelates are stable during TLC. Following conversion to their respective chelates, the metal TPPs are separated by TLC and characterized by their R_F value and colour. After elution from the chromatogram, they may be identified and quantitated by mass spectrometry (MS)[2,3]. As an excess of TPP is necessary in order to achieve a stoichiometric derivatization, it is necessary to maintain excess of TPP in the reaction. In certain cases it was observed that the subsequent recovery of metal TPP was lower than expected. This phenomenon has been explored using ZnTPP and as a result it is proposed that sandwich complexes possessing different chromatographic properties may be formed. Such complexes are known to exist[4–7] in the cases of mercury, uranium, and tin.

EXPERIMENTAL

The upper phase of a mixture of acetic acid, water, light petroleum (b.p. 80–100°), and toluene in the ratio of 85:15:66:33 (LT 2:1/A85) was used as the solvent system. MN Polygram Sil G (Macherey-Nagel and Co., Düren, G.F.R.) precoated TLC plates 20 cm × 20 cm in size, with layers 250 μm thick, were used without prior activation. The chromatograms were developed in glass tanks (Desaga, Heidelberg, G.F.R.) containing freshly prepared solvent added to a depth of 1 cm 1 h before the introduction of the chromatogram.

ZnTPP (10 μg), prepared as previously described[2,8] in chloroform (1 ml), was mixed with various amounts of TPP reagent (1–10 mg); 10 μl of the resultant solution was then applied to the origin zone of the MN Polygram Sil G plates. After development in the solvent system LT2:1/A85, 10 μg of deuterated ZnTPP (ZnTPP-D_{20})

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was superimposed on the separated ZnTPP zone (R_F 0.80). This zone was then outlined with a plastic stylus, scraped off the chromatograms, extracted in 50–100 μl chloroform, and an aliquot (5 μl) was introduced into the tip of a direct insertion probe. The probe was then inserted into the ion source region of an AEI MS-902S mass spectrometer. Spectra and IIC profiles were obtained as previously described[2]. A sample to which no TPP had been added was used as a standard.

In a different experiment, synthetic ZnTPP (100 ng) and TPP (60 μg) and a mixture of the two were chromatographed on MN Polygram Sil G plates in the solvent system LT2:1/A85. After separation in the first direction, the plates were allowed to dry, turned through 90°, and then developed again in the same solvent system. From each developed chromatogram several regions (see Fig. 2) were removed, extracted with chloroform, and analysed by MS for ZnTPP.

RESULTS AND DISCUSSION

Excess TPP reduced substantially (more than 50%) but never eliminated completely the recovery of ZnTPP (see Fig. 1). It would appear from this phenomenon that some of the TPP reagent must be entering into some kind of complex with ZnTPP and that this complex exhibits different chromatographic properties (probably remains associated with the TPP reagent) from those of ZnTPP.
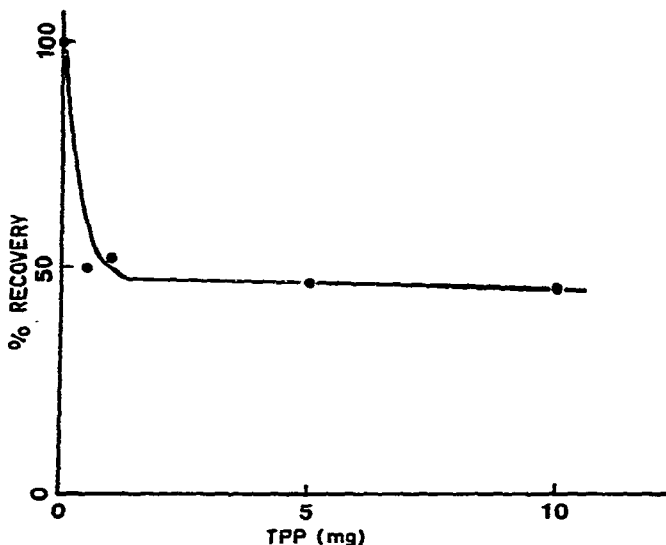

Fig. 1. Effect of added TPP on the recovery of ZnTPP separated on MN Polygram Sil G plates.

The appearance (in daylight) of two-dimensional chromatograms on which ZnTPP, TPP, or a mixture of the two have been separated is shown in Fig. 2. Only one zone (e) on chromatogram A possesses the necessary colour and the R_F value of ZnTPP. That only ZnTPP was present in this zone was proved by MS. Similarly, by MS, ZnTPP was shown to be absent from all the other zones. Two zones d and g on chromatogram B, both green in colour, exhibited the mass spectrum of TPP. In
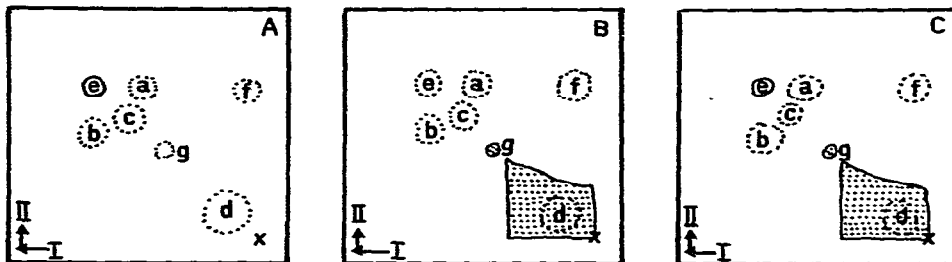
Fig. 2. Appearance in daylight of thin-layer chromatograms after the two-dimensional separation of ZnTPP (A), TPP (B) and a mixture of the two (C) in the solvent system LT2:1/A85 on MN Polygram Sil G plates. x = Origin zone; e = usual ZnTPP zone; d = usual TPP zone; g = impurity in TPP reagent; a, b, c, f = other regions examined for ZnTPP; the shaded area is the region covered by TPP when the layer is overloaded with TPP.

chromatogram C, *i.e.*, a mixture of ZnTPP and TPP, zone e was identified, by MS, as ZnTPP, zone g was identified as TPP, and in zone d, both TPP and ZnTPP were found. A search at *m/e* 1289, the molecular ion of the TPP–ZnTPP complex, revealed nothing; neither did the spectrum of the contents of this zone reveal anything other than TPP and ZnTPP.

Regions a, b, and c, which appeared on each chromatogram, were removed, extracted with chloroform, and analysed mass spectrometrically for ZnTPP. No trace was found. Such a finding, of course, means that the ZnTPP found in zone d of chromatogram C was not produced as a resultant of contamination or tailing from the ZnTPP zone and that the TPP reagent was not the origin of ZnTPP. No zinc was found in region f on any of the chromatograms.

Porphyrin sandwich complexes have been established before in porphyrin chemistry[4–7] and some examples of these are shown in Fig. 3; for the sake of clarity, only the nitrogen atoms of the porphyrin rings are shown. The mercury complex shown in Fig. 3c, a triple sandwich, is an octaethylporphyrin complex and is sufficiently stable to be isolated as a solid compound[5]. The corresponding HgTTP sandwich complex, however, cannot be isolated and we consider that this is because of steric interaction of the phenyl groups on the stacked porphyrin rings. A weak non-isolatable interconvertible mercury sandwich complex may, however, be formed and this would explain why HgTPP and TPP cannot be separated by TLC[2].

Since the hole in the porphyrin ring is not large enough to accommodate a trace metal in the plane of the ring, it must exist outside[9], thus facilitating the formation of a sandwich complex. It has been shown, for example, that the large metals Sn^{2+} and U^{2+} can form sandwich complexes[4,6,7] (see Figs. 3a and b). It is logical, therefore, to postulate that Zn^{2+} can also form a sandwich complex (Fig. 3d). In addition, Zn^{2+} and Hg^{2+} possess the same outer electron configuration and so ZnTPP, in analogy with HgTPP, would not be expected to form an isolatable complex with TPP. If the TPP molecules were skewed with respect to one another, the phenyl groups could, at least partially, fit between each other. In such a case, however, because of the interaction between phenyl groups, the bonds between the metal TPP and TPP would be quite weak. In the case of Zn the TPP–ZnTPP complex must be relatively stable since after two-dimensional separation, region f in chromatogram C
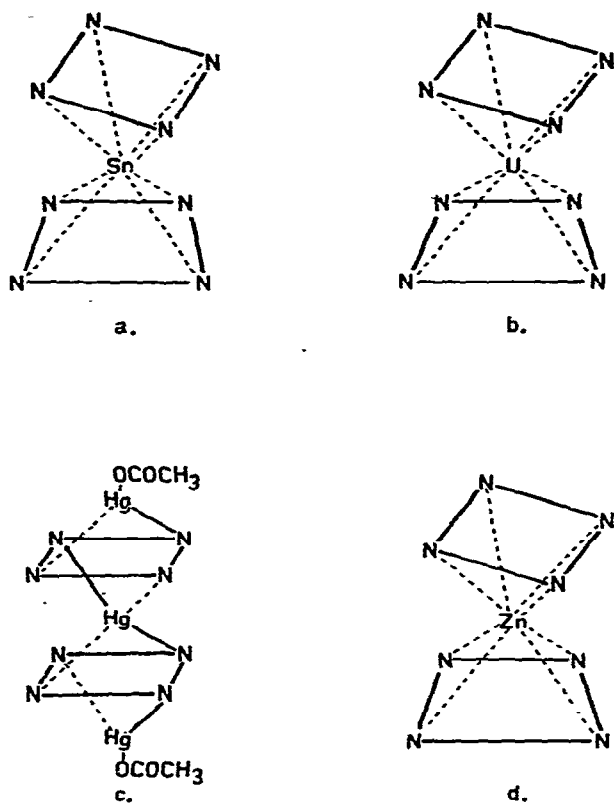
Fig. 3. Structure of some porphyrin–metal porphyrin complexes. Only the nitrogens of the porphyrin ring are shown.
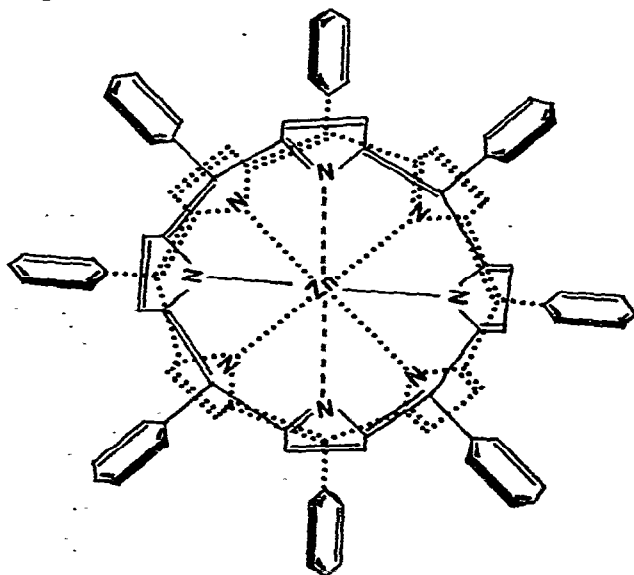

Fig. 4. Structure of the postulated TPP–ZnTPP complex (as viewed from the top).

(Fig. 2) contains no ZnTPP, thus proving that the complex does not break down after the first separation. The complex, however, is thermally unstable in the mass spectrometer, giving rise to the superimposed spectra of TPP and ZnTPP.

Fig. 4 shows the postulated TPP–ZnTPP complex as viewed from the top.

Recovery of ZnTPP from the ZnTPP zone following TLC reduces when a large excess of reagent TPP is present. If an absolute internal standard (*i.e.*, the rare stable isotope of the metal) is used[10], the loss of the ZnTPP does not affect the accuracy of the IIC quantitative procedure; it does, however, cause some loss in sensitivity. Since TPP chelates have been considered as models for the study of the mechanism of actions of metals in the body with naturally occurring porphyrins[11–14], it would be fruitful to investigate the polymers of other metal TPP chelates.

REFERENCES

1 K. S. Hui, B. A. Davis and A. A. Boulton, *J. Chromatogr.*, 115 (1975) 581.
2 B. A. Davis, K. S. Hui and A. A. Boulton, *Biomed. Mass Spectrom.*, 3 (1976) 71.
3 B. A. Davis, K. S. Hui and A. A. Boulton, in A. Frigerio and D. M. Desiderio (Editors), *Advances in Mass Spectrometry in Biochemistry and Medicine*, Vol. 2, Spectrum, New York, in press.
4 E. B. Fleischer, *Acc. Chem. Res.*, 3 (1970) 105.
5 M. F. Hudson and K. M. Smith, *Tetrahedron*, 31 (1975) 3077.
6 M. O. Tsutsui, D. Ostfeld, L. Hoffman, T. S. Srivastava, K. Suzuki and R. A. Velapodi, *Ann. N.Y. Acad. Sci.*, 206 (1973) 397.
7 D. Ostfeld and M. Tsutsui, *Acc. Chem. Res.*, 7 (1974) 52.
8 A. D. Adler, F. R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, 32 (1970) 2443.
9 B. Gonzalez, J. Kouba, S. Yee, C. A. Reed, J. F. Jirner and W. R. Scheidt, *J. Amer. Chem. Soc.*, 97 (1975) 3247.
10 K. S. Hui, B. A. Davis and A. A. Boulton, *Int. J. Neurochem. Res.*, submitted for publication.
11 A. D. Adler, *Ann. N.Y. Acad. Sci.*, 206 (1973) 7.
12 J. E. Falk, *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1964.
13 D. G. Irvine, *Int. Rev. Neurobiol.*, 16 (1974) 145.
14 V. L. Fromke, M. Y. Lee and C. J. Watson, *Ann. Int. Med.*, 70 (1969) 1007.