Journal of Chromatography, 445 (1988) 429–432
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 588

Note

Stability-indicating high-performance liquid chromatographic analysis of tin protoporphyrin and other free acid metalloporphyrins

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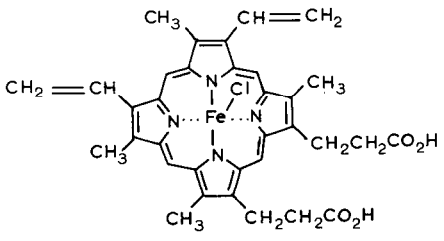
(First received February 17th, 1988; revised manuscript received April 22nd, 1988)

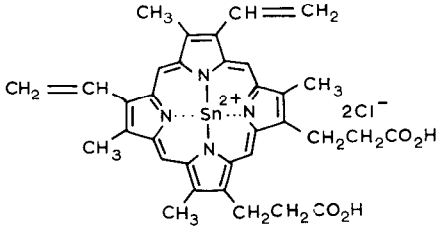
In recent years, there has been increased interest in metalloporphyrins as therapeutic agents. The iron porphyrin, hemin (I), has been found to be useful in the treatment of acute porphyria[1], while tin protoporphyrin (II) shows therapeutic activity against hyperbilirubinemia in infants[2].

Many metalloporphyrins are subject to degradation in aqueous solution due to solution pH or exposure to light. As a result, the need for a stability-indicating assay of metalloporphyrins has increased. Although there are a number of high-performance liquid chromatographic (HPLC) methods reported in the literature[3–8] for porphyrins and metalloporphyrin esters, a majority of these are not widely applicable to free acid metalloporphyrins.

This work reports a stability-indicating HPLC analysis for tin protoporphyrin; however, the procedure may be applicable to a variety of free acid metalloporphyrins.



I



II

EXPERIMENTAL

The tin protoporphyrin used in these studies was obtained from Abbott Labs. (North Chicago, IL, U.S.A.). The other metalloporphyrins were obtained from Northwestern University (Evanston, IL, U.S.A.). Protoporphyrin IX was from Porphyrin Products (Logan, UT, U.S.A.); biliverdin was from Sigma (St. Louis, MO, U.S.A.).

The HPLC system consisted of a Model 8700 solvent delivery system operated at 2.0 ml/min (Spectra-Physics, San Jose, CA, U.S.A.); a 25 cm × 4 mm I.D. PRP-1

0021-9673/88/$03.50

column, which is a 10-μm macroreticular poly(styrene–divinylbenzene) gel (Hamilton, Reno, NV, U.S.A.); and a detector operated at 400 nm (DuPont, Wilmington, DE, U.S.A.). Sample injections of 20 μl were made with a WISP 710B autosampler (Waters Assoc., Milford, MA, U.S.A.). A Spectra-Physics Model 4270 data handling system was used for data collection.

HPLC-grade acetonitrile and methanol (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and 1.0 *M* tetra-N-butylammonium hydroxide (tBAOH) in methanol (Southwestern Analytical Chemicals, Austin, TX, U.S.A.) were used to prepare the eluent. Tin protoporphyrin was eluted from the column with 0.02 *M* tBAOH, pH 12–acetonitrile (65:35). The eluent was changed to 0.02 *M* tBAOH, pH 12–acetonitrile–methanol (20:50:30) immediately after the elution of tin protoporphyrin in order to elute protoporphyrin IX, biliverdin and other degradation products.

Tin protoporphyrin and the other metalloporphyrins were dissolved at a concentration of 0.1 mg/ml in 0.02 *M* tBAOH, pH 12. Due to the possibility of photodegradation, all solutions were protected from light.

RESULTS AND DISCUSSION

The metals in the metalloporphyrins are complexed by four nitrogens, leaving two coordination sites above and below the ring system. The central metal can coordinate rapidly and reversibly with a large number of ligands, depending on the pH and counter-ion concentrations. This causes a so-called "ligand uncertainty". This is demonstrated in Fig. 1, with tin protoporphyrin. At neutral (b) and acid (c) pH, a series

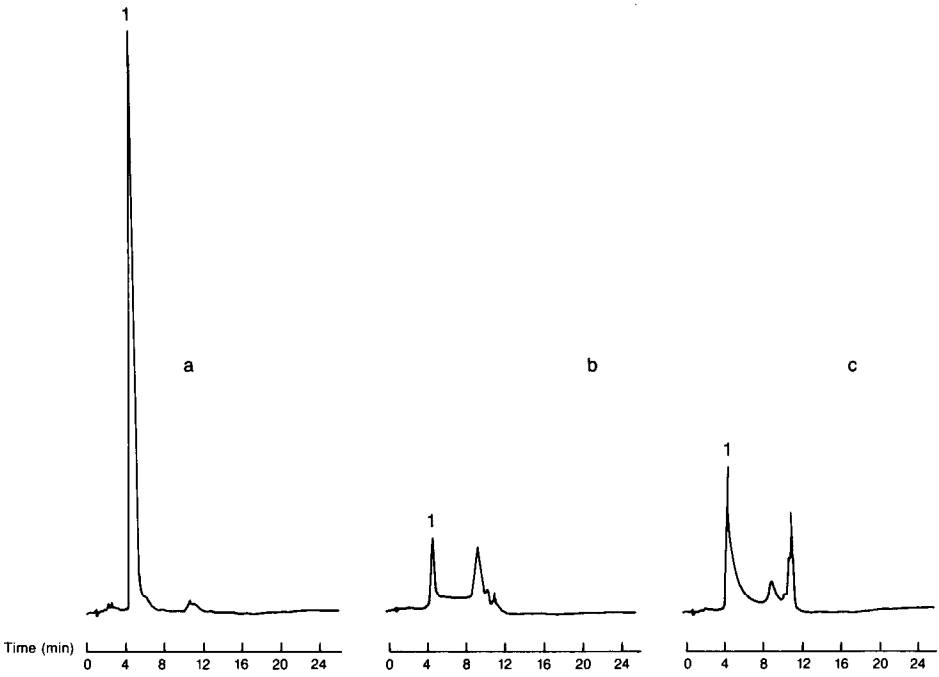


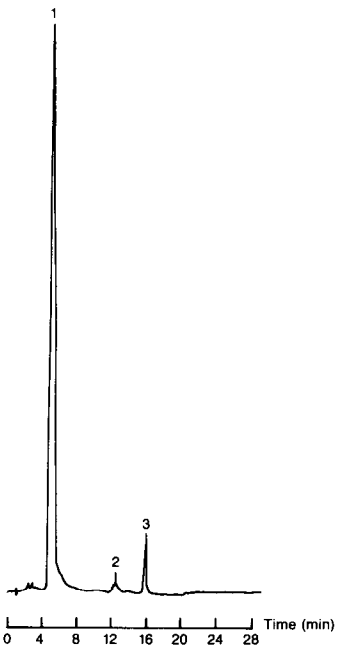
Fig. 1. Chromatograms of tin protoporphyrin (1) solutions of different pH. (a) pH 12, (b) pH 7.5, (c) pH 3.5. Total run time of chromatograms, 30 min. For chromatographic conditions, see text.

of reversible equilibria are established, resulting in the presence of several species; however, at pH 12 (a), a single species is present. For this reason, pH 12 was chosen for the chromatographic eluent and sample solvent. This requires the use of a polymeric reversed-phase column stable at high pH.

This chromatographic system separates tin protoporphyrin from protoporphyrin IX which would be formed by demetallation, biliverdin which would result from ring cleavage, and several photodegradation products of tin protoporphyrin as yet unidentified. Most of these compounds elute after tin protoporphyrin. In order to reduce the retention of these late-eluting compounds, the eluent is changed after the elution of tin protoporphyrin. Fig. 2 shows a typical separation of a sample preparation of tin protoporphyrin (1) containing 5% (w/w) protoporphyrin IX (3) and biliverdin (2).

The detector response is linear with respect to tin protoporphyrin concentration in the range of 10 to 200 μg/ml. The correlation coefficient was 0.9995 and the line essentially passes through the origin. The precision of analysis of a sample preparation of tin protoporphyrin gave a relative standard deviation of $\pm 0.81\%$ ($n = 10$).

The "available" tin protoporphyrin concentration of neutral or acid solutions can be determined by adjusting the pH to 12 with tBAOH and allowing sufficient



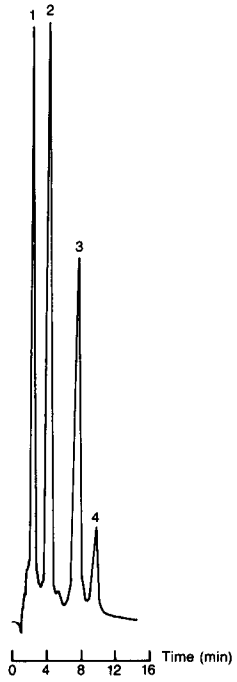


Fig. 2. Chromatogram of a tin protoporphyrin (1) solution containing 5% (w/w) biliverdin (2) and protoporphyrin IX (3). Total run time of chromatogram, 30 min. For chromatographic conditions, see text.

Fig. 3. Chromatogram of a mixture of metalloporphyrins: manganese(III) protoporphyrin (1), zinc deuteroporphyrin (2), and zinc protoporphyrin (3), and unidentified impurity (4). Chromatographic eluent, 0.02 *M* tetra-N-butylammonium hydroxide, pH 12–acetonitrile (60:40). Total run time of chromatogram, 15 min. For chromatographic conditions, see text.

time for conversion to the single dominant species present at high pH. In the case of tin protoporphyrin, this requires approximately 12 h and should be carried out in the dark, since neutral solutions of tin protoporphyrin are photochemically labile.

To demonstrate the broader application of this chromatographic system, other free acid metalloporphyrins have been examined, with minor eluent adjustment to affect retention. A chromatogram of a mixture of manganese(III) protoporphyrin IX (1), zinc deuteroporphyrin (2) and zinc protoporphyrin (3) is shown in Fig. 3. This chromatographic system cannot be used for the analysis of hemin [iron(III) protoporphyrin IX] due to its poor stability at high pH.

CONCLUSION

Tin protoporphyrin can be successfully analyzed after conversion to a single ligand species (presumably the hydroxide) by using the HPLC system described. The method is stability-indicating and can be applied to a variety of free acid metal porphyrins.

ACKNOWLEDGEMENTS

We would like to thank Dr. Brian Hoffman of Northwestern University whose research group supplied the additional free acid metalloporphyrins.

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