SYNTHESIS OF INDIUM-111 MESOPROTOPORPHYRIN IX

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SUMMARY

Indium-111 mesoprotoporphyrin IX has been prepared by refluxing suitable proportions of $InCl_3$, sodium acetate, and mesoprotoporphyrin IX in glacial acetic acid. The labeled metalloporphyrin is sufficiently water-soluble for use as a scanning agent, and can also be incorporated into heme apoproteins for perturbed gamma-gamma angular correlation measurements.

Key Words: Porphyrin, Indium-111, Scanning Agent, Perturbed Angular Correlations

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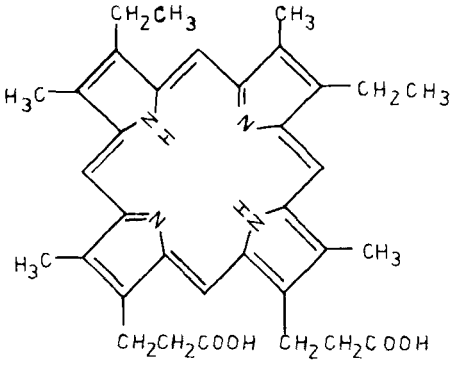
INTRODUCTION

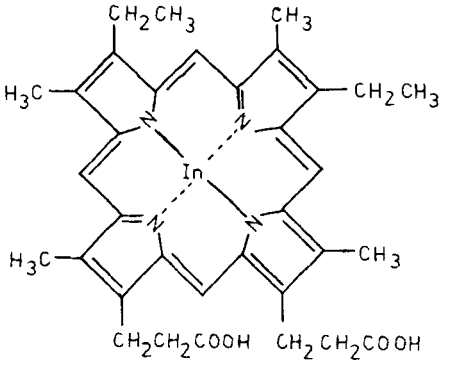
The need for a water-soluble, indium-111 labeled porphyrin has become increasingly evident. First, it is well-established that various porphyrins are taken up by lymph nodes and neoplastic tissues (1-9), based primarily on observation of fluorescence of the (metal-free) porphyrin. Attempts to characterize this tumor-localization using radio-labeled porphyrins have however largely failed (e.g., ref. 10), due to lack of suitable isotopes, presence of chemical impurities, and chemical difficulty of prompt introduction of the radio-label into the porphyrin. Second, indium-111 has near-optimal physical properties for use in tumor-scanning: convenient half-life (2.8 da), simple decay scheme with few side products, and emission of two gamma rays in high proportion (173 keV at 87%; 247 keV at 93%) to give about 180 useful photons per 100 disintegrations for ready detection by rectilinear scanners as well as the Anger scintillation camera system (11-14). However, water-soluble indium complexes stable under physiological conditions are scarce. In^{+3}, for example, is unstable in water except at acid pH. Although there have been scanning applications with indium-111 (15,16), in which the indium is chelated by an EDTA (ethylenediaminetetraacetate) group to which has been attached a chemically functional protein-binding reagent, such agents are rather difficult, expensive, and time-consuming to prepare and as a result are not yet widely used.

Several indium porphyrins have been synthesized, and the first crystal structure of an indium porphyrin, α,β,γ,δ-tetraphenylporphinatoindium(III) chloride, has just been reported (17). However, of the readily available water-soluble porphyrins, protoporphyrin IX itself is not suitable because of its reactive (to light or air) vinyl side chains. We therefore selected

mesoprotoporphyrin IX, 1, for incorporation of the indium-111 radio-label. All chemical procedures were first tested using cold indium, and the procedure modified somewhat for preparation of the radio-labeled indium porphyrin (see below).



MESO-PROTOPORPHYRIN IX, 1



INDIUM MESO-PROTOPORPHYRIN IX, 2

RESULTS AND DISCUSSION

The usual method for preparing indium mesoprotoporphyrin IX, 2, is to reflux a large excess of indium with the porphyrin (see Experimental). However, such a method is not applicable toward synthesis of indium-111 mesoprotoporphyrin IX (In:MPP), because the specific activity of the final product would be too low. The search for optimum reaction conditions was therefore conducted using cold indium, by varying the reflux time, the proportion of hot to cold indium, and the relative amount of 1.

Visible absorption spectra provide a convenient means for monitoring the reaction progress (see Fig. 1). On formation of 2, the intensity of the α-band of 1 increases markedly, along with a hypochromic shift from 592 nm to 573 nm. In addition, the β-band shifts from 548 nm to 536 nm.

Analytical thin-layer chromatography (tlc) was used to check the purity of the indium porphyrin product. The best solvent system for development of the chromatograms was found to be a 2:1 mixture of toluene/methanol. [No spraying agent is required to see the spots, since both the starting material and product are intensely colored.] With this solvent system, the MPP remains at the origin and the In:MPP migrates upward.

The final procedure gave (cold) In:MPP product in high purity, and was used to prepare indium-111:MPP. Identical procedures can obviously be used to synthesize ^{113m}In:MPP, which may be of more direct clinical value, since ^{113m}In is available from the small cyclotrons located at many hospitals. Similarly, it should be possible to prepare indium-111 protoporphyrin IX by the same procedure. The sparing aqueous solubility of In:MPP suggests that it is desirable first to dissolve the indium-111:MPP in dilute base (ca. 0.01 N NaOH) followed by 10-fold dilution with borate buffer (ca. pH 9) before clinical injection. Finally, it is possible to reconstitute indium-111:MPP into apomyoglobin (or other heme apoproteins) either for clinical use or for perturbed angular correlation studies *in vitro* or *in vivo*, as will be reported elsewhere (18).

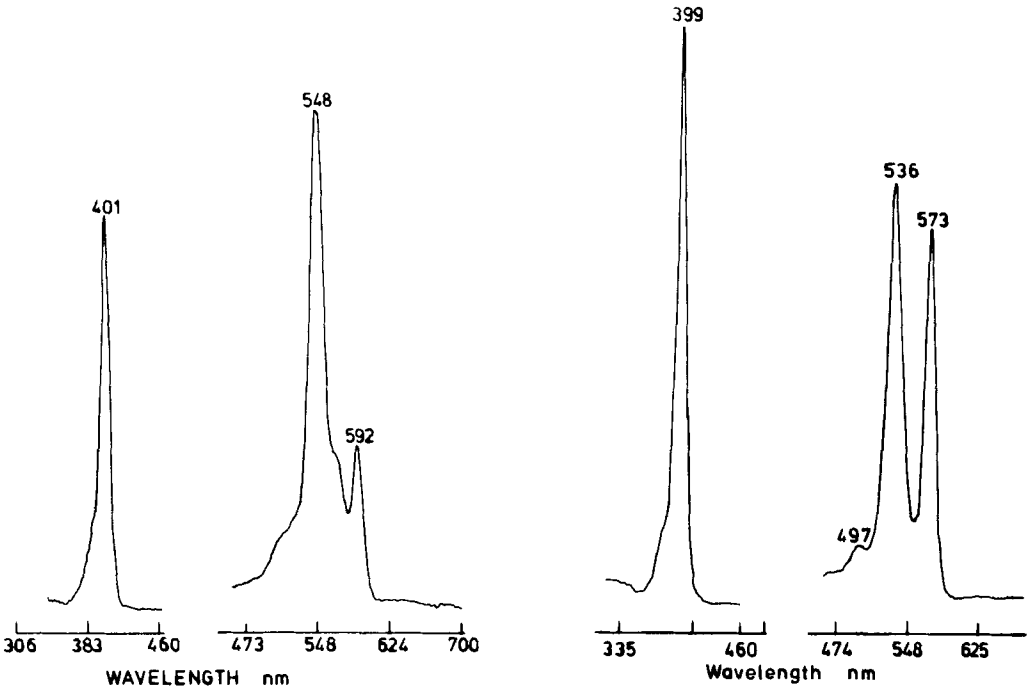


Figure 1. Visible absorption spectra (Cary 14) of meso-protoporphyrin IX (left) and indium mesoprotoporphyrin IX (right) in formic acid.

EXPERIMENTAL

Non-radioactive indium mesoprotoporphyrin IX (In:MPP).

Although mesoprotoporphyrin IX (MPP) is commercially available, it was much cheaper to produce it from ferri-protoporphyrin IX by a procedure essentially the same as that of Taylor (19). Ferri-protoporphyrin IX was obtained from Strem Chemicals, Inc.; palladium oxide ($PdO \cdot xH_2O$) from Matheson Coleman & Bell; formic acid (90%) from Fisher; ammonia from Allied Chemical Canada, Ltd.; ammonium acetate from MCB; sodium tartrate AR from Mallinkrodt; indium chloride ($InCl_3$, anhydrous, ultrapure) from Alfa; reagent glacial acetic acid from Allied Chemical; silica gel (70-140 mesh) for column chromatography from Macherey, Nagel & Co., Germany.

In a 250 ml one-necked round-bottom flask fitted with a reflux condenser, indium chloride (0.148 g) and meso-protoporphyrin IX (0.200 g) were heated to boiling in glacial acetic acid (160 ml) containing sodium acetate (0.717 g). The reaction was protected from light by aluminum foil, and allowed to reflux for 4½ hr. Progress of the metallation process was monitored spectroscopic-

ally by the disappearance of the visible spectrum (Fig. 1) of meso-protoporphyrin IX. The solution was concentrated on a rotary evaporator to a minimal volume. Addition of water to the warm, concentrated solution gave a copious precipitate of bright-red In:MPP. The solid was collected, washed with water, and dried over pellets of sodium hydroxide in vacuo. Analytical tlc was used to check the purity of In:MPP (see Results), using Eastman chromatogram sheets of silica gel with fluorescent indicator.

The crude In:MPP was purified by first dissolving the product (100 mg) in minimal methanol, followed by chromatography on a 62 x 1.6 cm column of silica gel packed in toluene. The column was eluted with toluene/methanol (2:1). In:MPP eluted first, followed by unreacted MPP and impurities. All fractions were checked spectroscopically and by tlc. The solvent was removed in vacuo to recover the bright-red In:MPP.

Indium-111 mesoprotoporphyrin IX (In-111:MPP).

All manipulations with indium-111 were carried out behind an improvized lead shield (3 mm thick) making an enclosure 50 x 50 x 40 cm, in a fume hood. A Picker Model 642081 Labmonitor was used for determining radiation levels, estimating sample activity, and checking for contamination. Because of the conveniently short half-life of indium-111, all contaminated glassware and solutions could simply be stored in the lead enclosure until detectable radioactivity had disappeared. Indium-111 was obtained commercially as carrier-free $^{111}InCl_3$ in aqueous solution containing 0.45% to 0.9% sodium chloride, from Medi-Physics Corp., Emeryville, California.

In a 25 ml round-bottom flask fitted with a reflux condenser, MPP (6 mg), cold $InCl_3$ (2.3 mg), sodium acetate (9.1 mg) were combined in glacial acetic acid (6 ml) and stirred vigorously. $^{111}InCl_3$ (1.1 ml solution; 2.2 mCi) was injected into the reaction mixture, which was then refluxed for 6-7 hr [a shorter reflux time of 3 hr is sufficient when high specific activity is not

required]. The solution was then concentrated to minimal volume by evaporation. Addition of cold deionized water to the warm, concentrated solution produced a bright-red precipitate which was washed repeatedly with cold water and air-dried. No attempt was made to purify the product, since essentially all (>99%) of the indium-111 was converted to the complex. The overall procedure can thus be completed in about 0.1 of one half-life of the indium-111 decay, for negligible loss in total activity.

ACKNOWLEDGMENTS

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REFERENCES

1. Figge F. H. J. - Cancer Research 4, 465 (1944).
2. Schwartz S. - cited by R. L. Lipson et al. (ref. 3).
3. Lipson R. L., Baldes E. J., and Olsen A. M. - J. Natl. Cancer Inst. 26, 1 (1961).
4. Barker D. S., Henderson R. W. and Storey E. - Br. J. Exp. Pathol. 51, 628 (1970).
5. Leonard J. R. and Beck W. L. - Laryngoscope 81, 365 (1971).
6. Gray M. J., Lipson R., Maeck J. V. S., Parker L. and Romeyn D. - Am. J. Obstet. Gynecol. 99, 766 (1967).
7. Lipson R. L., Baldes E. J. and Olsen A. M. - Chest 46, 676 (1964).
8. Gregorie H. B. and Green J. F. - J. SC Med. Assoc. 61, 157 (1965).
9. Gregorie H. B. Jr. et al. - Ann. Surg. 167, 820 (1968).
10. Bases R., Brodie S. S. and Rubenfeld S. - Cancer 11, 259 (1958).
11. Goodwin D. A., Sundberg M. W., Diamanti C. I. and Meares C. F. - Radiology and Other Biophysical Methods in Tumor Diagnosis, Year Book Medical Publishers, Inc. (1975), p. 57.
12. Takeda S., Uchida T. and Matsuzawa T. - J. Nucl. Med. 18, 835 (1977).
13. Goodwin D. A., Sundberg M. W., Diamanti C. I. and Meares C. F. - Radiopharmaceuticals (ed. Subramian, Rhodes, Cooper and Sodd), Society of Nuclear Medicine, New York (1975), Chapter 9.
14. Goodwin D. A., Menzimer D. and del Castilho R. - J. Nucl. Med. 11, 221 (1970).

15. Sundberg M. W., Meares C. F., Goodwin D. A. and Diamanti C. I. - J. Med. Chem. 17, 1304 (1974).
16. Sundberg M. W., Meares C. F., Goodwin D. A. et al. - Nature 250, 587 (1974).
17. Ball R., Lee K. M., Marshall A. G., and Trotter J. - submitted.
18. Marshall A. G., Lee K. M., and Martin P. W. - submitted.
19. Taylor J. F. - J. Biol. Chem. 135, 569 (1940).