Resonance Raman Spectra of Metal-free Porphin and some Porphyrins*

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ABSTRACT

Resonance Raman spectra have been obtained of solid porphin, protoporphyrin IX, mesoporphyrin IX dimethyl ester and the two position isomers of Coproporphyrin tetramethyl esters 3 and 4, using the rotating Raman cell technique. The various sidechains in different porphyrins have very pronounced effects on the Raman spectrum of porphin. The usefulness of the resonance Raman technique in the identification of substituted porphins and closely related position isomers is demonstrated.

INTRODUCTION

Porphyrins are of great biological importance and have already received considerable attention since they form the chromophore in hemoglobins and cytochromes. Recently, many interesting papers have appeared on the resonance Raman effect in hemeproteins (1-12) and chlorophylls (13-14) and some tentative spectra-structure correlations have been suggested. In these studies, it is also suggested that only the molecular vibrations associated with the chromophore exhibit enhanced

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Raman scattering when the exciting radiation falls within an electronic absorption band. As studies of the porphyrins contribute to the understanding of the activities and properties of the biologically important compounds, a proper understanding of the Raman spectra of the basic chromophore (porphin) and the effect of sidechain substituents is essential for a detailed interpretation of complicated systems like hemoglobin and chlorophylls etc.

Until now it has not been possible to record the Raman spectra of the dark coloured porphyrin bases because of their well known highly fluorescent characteristics and local heating effects due to absorption of exciting radiation. Using the rotating Raman cell technique (15), we have been able to record the resonance Raman spectra of porphin and some of its substituted derivatives as solids. Our preliminary results are reported here.

EXPERIMENTAL

Porphin, protoporphyrin IX, obtained from Cal Biochem. and mesoporphyrin IX dimethyl ester from K and K Laboratories, were used without further purification. The reference samples of isomeric coproporphyrins 3 and 4 tetramethyl esters were provided by Dr. S.F. MacDonald and are well characterized materials of very high purity.

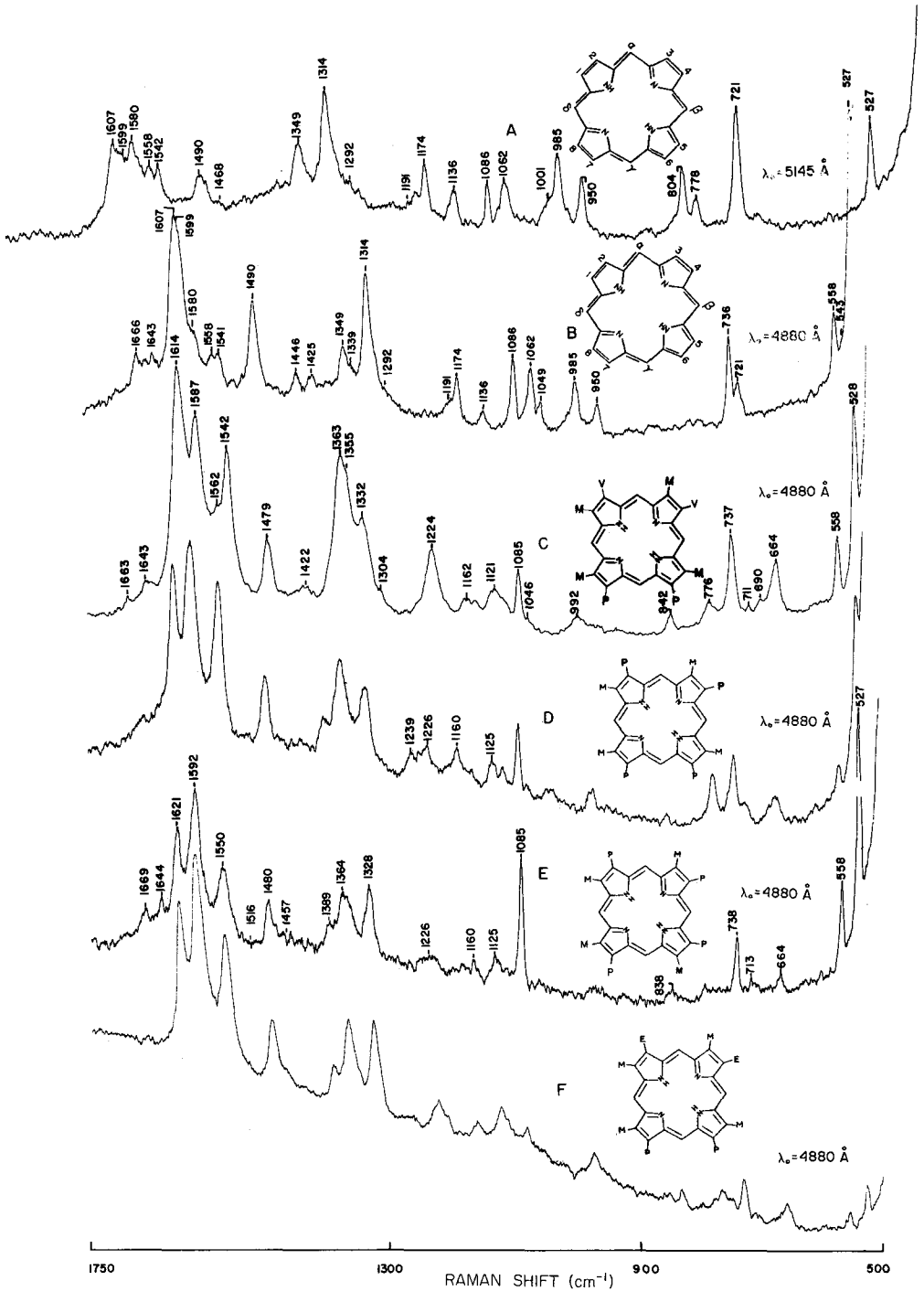
The Raman spectra were obtained with a Spex 1400 double monochromator and EMI 6256 photomultiplier and photon counting detection. Different excitation lines were provided by a Coherent Radiation 52 G Ar^+ laser. Our preliminary attempts to observe Raman spectra from aqueous or organic solutions of porphyrins were unsuccessful due to very high

fluorescent background. For this reason we tried the solid materials in a KBr pellet and indeed obtained Raman spectra of very good quality (Fig. 1). A layer of pure KBr was pressed in the groove of the rotating cell and the mixture of porphyrin in KBr (1:100 approximately) was pressed on top. In this way only a few milligrams of some of the rare samples could be handled easily. Laser powers of about 400 mW were used without any signs of destruction of the samples even after irradiation of several hours.

DISCUSSION

The original tracings of the Raman spectra of porphin, protoporphyrin IX, coproporphyrin 3 and 4 tetramethyl esters and mesoporphyrin IX dimethyl ester with 4880 Å excitation are shown in Fig. 1. Also included is the Raman spectrum of porphin with 5145 Å excitation (Fig. 1A).

There are a number of important aspects of these spectra. First of all, the spectra of porphin (Fig. 1A and 1B) with 5145 Å and 4880 Å excitations are significantly different showing that different vibrational modes are enhanced to the extent that they borrow intensity by mixing with appropriate electronic states. The optical absorption spectrum of porphin vapour (16) contains five bands located at 6275, 5750, 5115, 4835 and 3725 Å. Those at 5115 and 4835 Å are near to the exciting lines of the argon ion laser and produce large changes in the intensity of the resonance Raman spectra (Fig. 1A and 1B). The two bands at 558 cm^{-1} and 736 cm^{-1} in the Raman spectrum of porphin with 4880 Å excitation are missing from the spectrum with 5145 Å excitation, while two extra bands at 778 cm^{-1} and 804 cm^{-1} appear with 5145 Å excitation. Other changes are the disappearance of the doublet at 1446/1425 cm^{-1} from the spectrum


A
B
C
D
E
F
λ₀=5145 Å
λ₀=4880 Å
λ₀=4880 Å
λ₀=4880 Å
λ₀=4880 Å
λ₀=4880 Å
1750
1300
900
500
RAMAN SHIFT (cm⁻¹)

at 4880 Å (Fig. 1B) and the enhancement of weak features around 1580 cm^{-1} in Fig. 1A. Corresponding changes were observed with other substituted prophins by excitation at different wavelengths.

Secondly, there is a marked difference to be seen in the spectra of porphin and its substituted derivatives. The bands at 950, 1174, 1314 and 1446 cm^{-1} present in the spectrum of porphin with 4880 Å excitation are either very weak or absent from the spectra of substituted porphins while some new features in their spectra develop around 665, 775 and 840 cm^{-1}. Moreover, there are very marked changes in the relative intensities of the corresponding bands in the spectra of different porphyrins throughout the whole spectral region. For instance, the relative intensities of the bands

Fig. 1 Resonance Raman spectra of porphin and porphyrins in a KBr pellet. The scale is linear in wavelength (Å). Laser power ~500 mW, spectral slit width~5 cm^{-1} for all samples. The symbols M, V and P stand for $-CH_3$, $-CH=CH_2$ and $CH_3-CH_2-COOCH_3$ except for case (C) of protoporphyrin where P stands for CH_3-CH_2-COOH.

(A) Porphin with 5145 Å excitation.

(B) Prophin with 4880 Å excitation.

(C) Protoporphyrin IX with 4880 Å excitation.

(D) Coproporphyrin 3 tetramethyl ester with 4880 Å excitation.

(E) Coproporphyrin 4 tetramethyl ester with 4880 Å excitation

(F) Mesoporphyrin IX dimethyl ester with 4880 Å excitation.

at 1314 and 1349 cm^{-1} in the spectrum of porphin are just reversed in that of protoporphyrin IX (Fig. 1C). The very weak bands in porphin at 1541 and 1580 cm^{-1} appear as strong bands in those of porphyrins. Changes are also apparent among the spectra of protoporphyrin IX and those of copro- and meso-porphyrin methyl esters.

The absorption spectra of all these compounds are basically the same (having four bands in the visible region and a band in the near UV) with some differences in band positions. However, substituents at the porphin periphery seem to affect the electron cloud of the chromophore and, therefore, the resonance Raman spectra.

The Raman spectra of position isomers of coproporphyrins 3 and 4 tetramethyl esters are displayed in figs. 1D and 1E respectively. There are noticeable changes in the 700-800 cm^{-1}, 1050-1300 cm^{-1} and around the 1600 cm^{-1} regions. The bands at 773 and 1239 cm^{-1} present in the spectrum of isomer 3 are missing from the spectrum of isomer 4 while the bands at 1085 and 1644 cm^{-1} gain in intensity in isomer 4. The most obvious change is the disappearance of the band at 773 cm^{-1} from the spectrum of isomer 4. To check the effect of probable crystal modifications or polymorphs, spectra of both isomers were recorded with KBr pellets made by applying different pressures ranging from 1500 lbs. to 12000 lbs. per square inch. There were no apparent changes in the spectra. The differences in the Raman spectra of these isomers could be due to low symmetry in isomer 3 compared to the higher symmetry of isomer 4. It is worth mentioning that these isomers could not be dis-

tinguished by either paper chromatography (17) or infrared spectroscopy (18). Resonance Raman spectroscopy, therefore, appears to offer a valuable means of distinguishing between the closely related position isomers.

Although the elucidation of the nature of the vibrational modes of the porphin chromophore is a very complicated and difficult problem at this time, a few points are worth mentioning. In the preliminary interpretation of the spectra of hemeproteins (12, 6), it is suggested that the band around 1620 cm^{-1} arises from the C=C stretching of the vinyl groups of protoporphyrin IX which may form part of the porphyrin chromophore due to conjugation. However, a band at this position is present in the spectra of all the porphyrins studied here which do not have vinyl substituent and may equally well arise from the C=C modes of porphin skeleton itself. Raman studies of metal chelates of porphin, tetraphenyl porphin and other porphyrins in solution are expected to provide insight into the nature of vibronic coupling and identification of modes of various symmetry species. These studies are in progress and will be published shortly.

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