Resonance Raman spectroscopy study of protonated porphyrin

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\textbf{ABSTRACT:} Resonance Raman microscopy was used to study the resonance Raman scattering of the diacid (diprotonated form) of free-base porphyrin (21H,23H-porphine) in a crystal powder and KBr pellets. Intensive lines in the spectral range between 100 ÷ 1000 cm\textsuperscript{-1} have been detected and assigned as spectral manifestation of out-of-plane modes. The Raman spectra were simulated by means of DFT methods and compared with the experimental data. It is evident from experimental and theoretical results that the activation of out-of-plane modes arises from \textit{saddle} distortion of the porphyrin macrocycle upon formation of its diprotonated form.

\textbf{KEYWORDS:} Raman microscopy, protonated porphyrin, distortion of porphyrin macrocycle, out-of-plane modes, DFT analysis and modeling
INTRODUCTION

The molecule of porphyrin belongs to an interesting and important class of organic compounds. The porphyrins and their constitutional isomers have been used in the design of artificial photosynthetic systems [1, 2], sensors [3], molecular optoelectronic devices [4], photoswitches [5], optical memories [6], and in photodynamic therapy [7]. Parallel to the application-oriented studies, the details of the chemical and physical properties of porphyrin still constitute a fruitful area of fundamental scientific research. The high importance of porphyrins for current science and technology has led to intensive studies of these compounds by different spectral methods. However, there still exist free-base porphyrin spectral features for which the detailed studies have not yet been carried out.

One of the most informative and sensitive tools for investigating of vibrational states of porphyrins is Resonance Raman Scattering (RRS) [8, 9]. RRS methods have been used for detection, identification, quantification, and structural studies of porphyrins, porphyrazines and other compounds comprising the porphyrin chromophore in solvents, KBr pellets, and solid powder at ambient and low temperatures [10-13].

There is ample evidence that porphyrin moieties in their natural protein environment are highly distorted from planarity. Out-of-plane distortions of the porphyrin macrocycle lead to significant changes in their spectral properties and have been investigated because of their possible functional roles in heme- and chlorophyll-containing proteins. Because of the interest in the spectral consequences of non-planarity, numerous synthetic models of porphyrins have been prepared which mimic these distortions. The effect of distortion has been observed with the help of fine-line luminescence spectroscopy study of metallocomplexes with Mg(II), Zn(II) and Pd(II) ions [14, 15]. The RRS aspects of non-planarity in the highly substituted metalloporphyrins and the effect of the central metal ions have also been previously discussed [16-22].

In general, the simplest distorted forms of porphyrin can be produced upon protonation of free-base, i.e. without metallic ions in the center of porphyrin macrocycle. From the early days of study of the acidic forms of porphyrin it is well known that free-base porphyrin is transformed into mono- and diprotonated forms in the acid medium [15, 23-32]. Protonation occurs on one or two available nitrogen atoms, resulting in the positively charged monoacid form or the dicationic diacid form. At ambient temperature [24, 26, 28, 31] mono- and diprotonated forms are easily formed upon addition of a small amount of different kinds of commonly used acids. At low temperatures
only the diprotonated forms of tetrapyrrolic compounds are observed [24]. The interest in the investigation of the protonated forms of porphyrins is attributed to the fact that the diprotonated species are considered as useful model compounds for studying the features of nonplanar distortion of tetrapyrrolic macrocycles and their molecular flexibility [30, 33-35].

X-ray crystallography studies revealed that the diacids generally undergo saddle-type distortions, although other geometrical shapes can also be produced [15].

The first fluorescence fine-line spectra of porphyrin (H$_2$P) diacids (H$_4$P$^{2+}$) have been obtained in a solid solution of trifluoroacetic acid matrix at 4.2 K [36] using fluorescence line narrowing (FLN) technique [37], but the data have not been interpreted. Several years ago the fine-line spectrum of H$_4$P$^{2+}$ in inorganic tetraethoxysilane matrix at 4.2 K was detected [38]; these results reveal essential differences compared with the previous report [36] and the interpretation of the spectral data is also absent. Remeasured FLN spectra of H$_4$P$^{2+}$ have been recorded recently [39] and, on the basis of experimental data and theoretical treatment (DFT calculations) the manifestation of out-of-plane vibrations in the FLN spectra has been demonstrated. The relationship between the activity of out-of-plane modes and the geometry of the chromophore was discussed.

The RRS technique has also been used for investigating of structural issues in the acidic forms of porphyrin derivatives [40-42]. The photochemical formation of di- and monoacids of octaethylporphyrin in several solvents has been realized in [40] and the RRS data of the acidic forms (with the excitation wavelength of 441.6 nm) were recorded in the spectral range of 1100-1650 cm$^{-1}$. Later on, the Raman and IR spectra of H$_4$OEP$^{2+}$ and its N-deuterated analogue (D$_4$OEP$^{2+}$) were measured [41], but in the spectral range from 500 to 1700 cm$^{-1}$ the recorded Raman spectrum was of very low quality. The set of free base tetraphenylporphyrin, $p$-sulfonatophenyl, and meso-phenyl-substituted porphyrins and their N-diprotonated derivatives have been studied by Raman and IR methods. The observed vibrational bands were assigned on the basis of the deuteration shifts and compared with those observed in the structural analogues of these compounds [42, 43].

We have decided to study the RRS of the diacid form of free-base porphyrin in the solid state in a range from 100 to 1650 cm$^{-1}$, in order to compare the results of the experiment with the data obtained from FLN spectra and with the results of DFT calculations for the same compound. The interest in the investigation was motivated by the possibility to examine in detail the relation.
between *saddle* conformation of the acidic form and activation of out-plane modes in the vibrational spectra.

In this work, the acidic form of porphyrin has been with the help of RRS in a crystal powder and KBr pellets coupled with theoretical modelling in order to elucidate spectral manifestation in Raman spectra of structural changes characteristic for the diacid form. In particular, the influence of the acidic form formation on the vibrational modes and the activity of out-of-plane and in-plane modes in the Raman spectra have been examined in detail.

**EXPERIMENTAL**

**Chemicals**

Porphyrin (H$_2$P) was synthesized according to well known procedures [44, 45]. For purification and identification of the structure, standard procedures were used. The structural formulae of H$_2$P and the diacid form are shown in Fig.1. Concentrated trifluoroacetic acid, HCl and methanol were used to produce the acidic form. These chemicals were purchased from Sigma-Aldrich Co. and used without further purification.

![Fig. 1. Free-base porphyrin (left) and its diacid form (right) with bond lengths and angles obtained by calculations.](image)

**Preparation of the diacid form**

The acidic form H$_4$P$^{2+}$ was prepared using a methodology somewhat modified with respect to a previous reference [28]. MeOH was added to 100 mg of free-base porphyrin in 20 ml of concentrated trifluoroacetic or HCl acid until the free-base dissolved completely. The solution was concentrated to a small volume and deep purple crystals were obtained upon standing at room temperature, washed with diethyl ether and dried in air. The presence of the diacid form was confirmed by the appearance of the characteristic lines in IR absorption spectrum [46].
Raman setup

Renishaw InVia microscopic system was used for the measurements of Raman spectra. Ar⁺ 514.5 nm (Stellar Pro Modu-Laser, LLC) laser line was used as the excitation source. The laser light was focused on a sample with a 100x microscope objective; the laser power at the sample was less than 5 mW to prevent the transformation of the diacid into the free-base porphyrin due to heating of the sample. The Raman-scattered light was collected by the same objective through a cut-off filter to block Rayleigh scattering. A grating of 1800 grooves/mm was used. The resolution was 5 cm⁻¹, with the wavenumber accuracy of 2 cm⁻¹, both calibrated with the Rayleigh line and the 520.6 cm⁻¹ line of silicon. The Raman-scattered light was recorded by a 1024x256 pixel Peltier-cooled RenCam CCD detector.

Theoretical treatment

Geometry optimizations, vibrational frequencies, and Raman activity calculations were performed using density functional theory (DFT). Becke three-parameter functional [47] with the Lee-Yang-Parr correlation functional (+) [48] was the model of choice. The basis set was cc-PVTZ, as implemented in Gaussian 09 [49]. In order to convert the calculated Raman activities into quantities comparable with the experimental results, they were multiplied by a factor of \((x - \nu)^4/(\nu(1 - \exp(-hc\nu/kT)))\) [50], where \(x\) is the wavenumber corresponding to the laser wavelength used to record the spectra and equal to 19436.3 cm⁻¹ for the 514.5 nm laser line, \(c\) is the speed of light, \(h\) and \(k\) are Planck’s and Boltzmann’s constants, \(T\) is the temperature, \(\nu\) (in cm⁻¹) is the calculated transition frequency (after applying the scaling factor).

RESULTS AND DISCUSSION

Raman spectra of H₂P and H₄P²⁺

In order to explain the influence of diprotonation on spectral properties, the experimental data and the results of quantum-chemical calculations of H₄P²⁺ were compared with the data for H₂P. Fig. 2 exhibits the RRS spectra of H₂P and H₄P²⁺ obtained for a crystal powder. The vibrational frequencies of H₂P and H₄P²⁺ observed in the Raman spectra are summarized in Table 1. The frequencies and the symmetry of the normal modes of H₂P and H₄P²⁺ were previously assigned on the basis of quantum-chemical calculations and the results of fluorescence line narrowing (FLN) experiments [39] (Table 1). The relatively high symmetry of H₂P and H₄P²⁺
results in the similar set of vibrations observed in the FLN spectra [39] as well as in the RRS spectra.

Fig. 2. Raman spectra of porphyrin (top) and its diacid form (bottom) obtained for 514.5 nm excitation.

H$_2$P and H$_4$P$^{2+}$ belong to the D$_{2h}$ and the D$_{2d}$ symmetry groups, respectively. The metalloporphyrin belongs to the D$_{4h}$ symmetry in the case of in-plane position of central ion. The D$_{2d}$ and D$_{4h}$ symmetry groups are characterized by the presence of the axes of fourth order (S$_4$ and C$_4$, respectively), which results in similar vibrational types for both groups. Based on group theory, different modes of D$_{2d}$ symmetry can be correlated with the following modes of D$_{4h}$ symmetry:

- fully symmetric modes of A$_1$ (D$_{2d}$) correspond to A$_{1g}$ in-plane modes and B$_{2u}$ out-of-plane modes of D$_{4h}$;

- A$_2$ modes (D$_{2d}$) correspond to A$_{2g}$ and B$_{1u}$ (D$_{4h}$);

- B$_1$ modes (D$_{2d}$) correspond to B$_{2g}$ and A$_{1u}$ (D$_{4h}$);

- B$_2$ modes (D$_{2d}$) correspond to B$_{1g}$ and A$_{2u}$ (D$_{4h}$);

- E modes (D$_{2d}$) correspond to E$_u$ and E$_g$ (D$_{4h}$).
The comprehensive discussion of the experimental data and the results of quantum-chemical calculations of the normal modes for the studied compounds starts from the comparison of the Raman data for H₂P and H₄P²⁺ molecules.

Table 1. Vibrational assignments of the Raman spectral features of H₄P²⁺, comparison with FLN spectra, and the results of calculations for H₄P²⁺.

| Raman H₂P | FLN H₂P | FLN H₄P²⁺ | Raman H₄P²⁺ | Symm. | Vibrational assignment |
|-----------|---------|-----------|-------------|-------|-----------------------|
| ν exp., cm⁻¹ | ν exp., cm⁻¹ | ν exp., cm⁻¹ | ν exp., cm⁻¹ | | |
| [39] | [39] | | | | |
| 109 | - | - | 111 | 128 | B₁ | δ(NH), C₆C₇C₈, br |
| 134 | - | - | 136 | 143 | B₂ | δ(C₆H), C₆C₇N, til |
| - | - | - | 236 | 207 | A₁ | til |
| 309 | 310 | 307 | 311 | 296 | A₁ | C₆C₇C₈, br |
| - | - | 337 | 337 | 318 | B₂ | ρ(C₆H), ρ(NH) |
| 416 | - | - | 419 | 415 | B₁ | C₆C₇C₈, δ(C₆H) |
| - | - | 479 | 474 | 467 | A₂ | ρ(C₆H), ρ(C₆H) |
| - | - | - | 640 | 639 | A₁ | Fs, ρ(C₆N) |
| - | - | 675 | 671 | 690 | B₂ | Fs, ρ(C₆N) |
| 695 | - | 695 | 691 | 698 | A₁ | C₆C₇C₈, C₆C₇N |
| 723 | 723 | 713 | 717 | 702 | B₂ | C₆NC₈ |
| 786 | 790 | 800 | 785 | 794 | B₁ | C₆C₇C₈, C₆C₇N |
| 805 | - | - | 806 | 799 | B₂ | C₆N₇C₈, NC₆N₇m |
| - | - | 882 | 889 | 893 | B₂ | ρ(C₆H), ρ(C₆H) |
| 953 | - | - | 953 | 966 | A₁ | C₆N₇, C₆C₇, C₆NC₈ |
| 987 | 976 | 994 | 997 | 977? | B₁ | C₆N₇, C₆C₇C₈, C₆C₇N |
| 1062 | 1066 | 1072 | 1073 | 1074 | A₁ | δ(C₆H), C₆C₇ |
| 1138 | - | - | 1138 | 1145 | A₂ | δ(C₆H) |
| 1176 | - | - | 1176 | 1184 | B₂ | δ(C₆H), C₆C₇, C₆N |
| 1191 | 1182 | 1201 | 1197 | 1191 | B₁ | δ(C₆H), C₆C₇, δ(NH) |
| 1316 | 1318 | 1318 | 1315 | 1347 | A₂ | C₆N₇, C₆C₇, δ(C₆H), δ(C₆H), δ(NH) |
| 1353 | - | - | 1343 | 1354 | 1361 | B₂ | C₆NC₈, C₆C₇, δ(C₆H) |
| 1379 | 1388 | 1385 | 1379 | 1374 | A₂ | δ(C₆H), δ(C₆H), δ(NH), C₆N |
| 1423 | - | - | 1409 | 1390 | A₁ | C₆C₇, C₆NC₈ |
| 1490 | 1498 | 1483 | 1481 | 1459 | B₂ | C₆C₇, δ(C₆H) |
| 1543 | - | 1536 | 1532 | 1509 | A₁ | C₆C₇m, C₆C₇b, C₆NC₈ |
| 1596 | 1602 | 1597 | 1591 | 1562 | A₂ | C₆C₇m, δ(C₆H), δ(NH) |
| 1607 | 1615 | 1618 | 1614 | 1575 | B₂ | C₆C₇m, δ(C₆H), C₆NC₈ |

δ – deformation mode.
Symbols at carbon atoms: a, b - positions in the pyrrolic rings; m – carbon atom at the meso-positions.
Symbols for out-of-plane modes: til – tilting of the pyrrolic ring; br – breathing motion; ρ(XX) – mode with out-of-plane macrocycle motion of the XX bond.
The formation of porphyrin diacid with four hydrogen atoms in the center of the porphyrin macrocycle leads to significant changes in the Raman spectra of $\text{H}_2\text{P}$ (see Fig. 2 and the data in Table 1). In the Raman spectrum of $\text{H}_4\text{P}^{2+}$, a set of new lines with frequencies 236, 337, 474, 640, 671, 889 cm$^{-1}$ is revealed in the spectral range below 900 cm$^{-1}$. The corresponding lines are absent in the Raman and FLN spectrum of $\text{H}_2\text{P}$, whereas in the FLN spectrum of $\text{H}_4\text{P}^{2+}$ the lines with frequencies 337, 479, 675 and 882 cm$^{-1}$ have been detected [39].

According to the experimental results presented in Table 1 and based on the results of quantum-chemical calculations, the line at 236 cm$^{-1}$ has been assigned to an out-of-plane (oop) mode with the calculated frequency of 207 cm$^{-1}$. The line with the frequency of 337 cm$^{-1}$ corresponds to oop displacements of the meso-carbon atoms and the nitrogen atoms of the pyrrolic rings. The mode is active due to a symmetric tilting oop motion of the four rings. The line at 474 cm$^{-1}$ belongs to the practically pure (oop) displacement of the $\beta$-carbon atoms of the pyrrolic rings and the carbon atoms of the methine bridges. The line at 640 cm$^{-1}$ was assigned to an oop mode with the calculated frequency of 639 cm$^{-1}$, active due to a twisting oop motion of the four pyrrolic rings with oop pyrrole ring deformations. The line at 671 cm$^{-1}$ was assigned to an oop mode with the calculated frequency of 662 cm$^{-1}$. The mode appears due to an envelope oop motion of the four pyrrolic rings. The last out-of-plane mode in the series, with the frequency of 889 cm$^{-1}$ (one of the most intensive lines in the spectral range below 1000 cm$^{-1}$) corresponds to an oop motion of the hydrogen atoms, both at the meso-positions and on the pyrrolic rings. All the above-mentioned lines are absent in the Raman spectrum of $\text{H}_2\text{P}$ with a planar structure.

In the same spectral range of 100 ÷ 1000 cm$^{-1}$ one observes in the Raman spectrum of $\text{H}_4\text{P}^{2+}$ the lines corresponding to in-plane modes (111, 311, 357, 419, 717, 785, 806, 953, 997 cm$^{-1}$). All these lines have analogues in the Raman spectrum of $\text{H}_2\text{P}$ (see Fig. 2 and Table 1).

Evidently, the main reason of spectral manifestation and activation of the oop modes in the Raman and fluorescence spectra of the diacid of porphyrin (spectral range below 1000 cm$^{-1}$) is the result of the saddle type distortion of the porphyrin macrocycle [15, 36, 39]. The distortion of the diacid macrocycle induces alterations in the ring kinematics as well as in the electronic structure of the absorption spectra (see, for example, [24, 26, 31]). As a result of distortion of the porphyrin macrocycle the oop vibrational modes become active in the resonance Raman spectra [51-54]. These modes are forbidden in the resonance Raman spectra of planar porphyrins because the resonant $\pi-\pi^*$ electronic transitions are polarized in the plane of the porphyrin macrocycle. The saddle distortion provides a nonzero $z$-component of the electronic transition moment, thereby
inducing the RR intensity of the out-of-plane modes. Similar effects have been earlier investigated systematically for nickel porphyrins which are distorted to accommodate the short Ni-N (pyrrole) bonds and the experimental intensity pattern was predicted quite well [55, 56].

In other words, the distortion of the saddle type should lead to realizing of 3D character of the absorption oscillator [39]. In this case, all electronic transition moments in $\text{H}_4\text{P}^{2+}$ can have three components ($x$, $y$, and $z$), contrary to the situation with only in-plane $x$ and $y$ in-plane components for the planar structure of $\text{H}_2\text{P}$. The 3D oscillator of the electronic (vibronic) transitions may be expressed as the superposition of the planar oscillator ($x$ and $y$) and out-of-plane $z$ oscillator with the orientation of the corresponding transition moment components.

As evidently follows from Fig. 2, the formation of the diacid form of porphyrin leads to spectral manifestation of the out-of-plane modes in the spectral range $100 \div 1000$ cm$^{-1}$ and also to essential changing of porphyrin ring dynamics. Due to the location of two additional hydrogen atoms in the center of the porphyrin macrocycle the lengths of bonds and the values of angles in $\text{H}_4\text{P}^{2+}$ are significantly changed compared to $\text{H}_2\text{P}$. For example, based on the results of the geometry optimization, the length of the $\text{C}_a\text{C}_b$ bonds for $\text{H}_4\text{P}^{2+}$ is smaller than for $\text{H}_2\text{P}$. As a result, the vibration manifested in the spectrum of $\text{H}_2\text{P}$ at 987 cm$^{-1}$ (with participation of $\text{C}_a\text{C}_b$ bonds) will be blue-shifted, occurring at 997 cm$^{-1}$ in the case of $\text{H}_4\text{P}^{2+}$. In comparison with the spectrum of $\text{H}_2\text{P}$, the values of the experimental and calculated frequencies of $\text{H}_4\text{P}^{2+}$ for the deformation modes $\delta(\text{C}_b\text{H})$ - 1062 cm$^{-1}$ and $\delta(\text{C}_m\text{H})$ - 1191 cm$^{-1}$ are increased to 1073 and 1197 cm$^{-1}$, respectively. This effect depends on increasing force constants of the CCH angles, because the lengths of $\text{C}_a\text{C}_b$ bonds taking part in the forming of this mode are elongated and the corresponding force constants are decreased. The lengths of bonds and the values of the angles are essentially changed when four hydrogen atoms are positioned in the center of the porphyrin macrocycle, producing $\text{H}_4\text{P}^{2+}$. For example, the longest CN bond in $\text{H}_2\text{P}$ has a value 1.368 Å, whereas for $\text{H}_4\text{P}^{2+}$ the length of the all CN bonds is the same and increases to 1.389 Å. The length of the $\text{C}_a\text{C}_b$ bond is increased, on the average, by 0.011 Å in the diacid. The $\text{C}_a\text{NH}$ angle demonstrates decreasing by 4.1$^\circ$ in the diacid species (Fig. 1).

Fig. 2 shows that formation of porphyrin diacid results in essential transformation of the Raman spectrum of $\text{H}_2\text{P}$ in the range of $1000 \div 1650$ cm$^{-1}$. Two major effects responsible for this are (i) modification of porphyrin ring dynamics upon diacid formation, and (ii) changes in the electronic structure of the diacid form, providing completely different conditions for excitation of resonance Raman scattering. These changes lead to the enhancement of the types of vibrational
modes, which are effectively coupled with different electronic transitions in the absorption of H₂P and H₄P²⁺.

The excitation of the Raman spectrum of H₂P with the wavelength of 514.5 nm leads to the resonance with the S₀-S₂ electronic transition [39] and – as a result – to the enhancement of the modes of B₁g symmetry type. The intensive lines with the frequencies of 805, 1138, 1176, 1353, 1490, and 1607 cm⁻¹ (B₁g type of symmetry) have been detected in the Raman spectrum of H₂P [13]. Actually, spectral manifestations of lines with the participation of B₁g modes are most diagnostic for the presence of vibronic coupling in the RRS of H₂P. Earlier on, similar effects of intensity enhancement of B₁g modes as a result of vibronic coupling with the S₀-S₂ electronic transition have been discussed for fine-line absorption spectrum of H₂P embedded in solid rare gas matrices at low temperatures [57]. The activities of lines of different type of symmetry in the RRS spectra of porphyrin have been interpreted in dependence on the type of excited electronic transitions [21, 58].

As was previously shown [23, 24, 26, 28], two lowest electronic transitions of H₄P²⁺ have nearly degenerate character and the excitation of Raman spectra in this case (degenerate electronic states) leads to an additional complication in the interpretation of the Raman intensity. For example, the lines belonging to B₂ symmetry, analogs of lines of B₁g symmetry type in the case of H₂P show very low activity in the spectrum of H₄P²⁺ (Fig.2) and the lines with frequencies of 806, 1176, 1354, 1481, and 1614 cm⁻¹ are practically absent in the spectrum. At the same time, relatively weak lines with frequencies of 1379 and 1543 cm⁻¹ in H₂P correspond to the most intensive lines in the Raman spectrum of H₄P²⁺ with frequencies of 1379 and 1532 cm⁻¹.

In our opinion, the enhancement of these modes may be explained by vibronic coupling with the Soret band. Similar effect of the enhancement of lines with frequencies near 1370 and 1562 cm⁻¹ have been observed for Cu metallocomplex of porphyrin [58] with a similar degenerate character of the two lowest electronic states as described above upon formation of the porphyrin diacid. For example, for excitation of the Raman spectra in the spectral range near the Soret band (441.6 nm), the enhancement of lines with frequencies near 1370 and 1562 cm⁻¹ was detected, in contrast to the situation with the most intensive lines with frequencies of 1322, 1498, and 1587 cm⁻¹ observed for excitation near the S₀-S₂ electronic transition with 556.5 nm wavelength. A similar effect of the enhancement of lines with frequencies of 1382 and 1516 cm⁻¹ in the RR spectrum of Ni-OEP has been reported [21] upon excitation at 406.7 nm (near the Soret band), which lead to corresponding changes in the RR spectral features.
Conclusions

1. Raman microscopy on a crystal powder have been effectively used for recording the Raman spectra of the diacid of porphyrin, \( \text{H}_4\text{P}^{2+} \). Based on the experimental data and quantum chemical calculations, spectral assignments of the vibrational modes have been proposed.

2. Spectral manifestation of additional modes in the Raman spectra of the porphyrin diacid (spectral range below 1000 cm\(^{-1}\)) is the result of the *saddle* type distortion of the macrocycle of the diprotonated species due to the appearance of two additional inner protons. The noticeable structural changes of molecule after the protonation and as a result changes in the spectral features were observed and elucidated. The changes of molecular symmetry from \( \text{D}_{2h} \) to \( \text{D}_{2d} \) upon diprotonation lead to the appearance in the Raman spectrum of a new set of lines corresponding to out-of-plane vibrational modes.

3. Significant discrepancies between the Raman spectra of \( \text{H}_2\text{P} \) and \( \text{H}_4\text{P}^{2+} \) in the spectral range 1000 – 1650 cm\(^{-1}\) are provided by changes in electronic structure after protonation and consequently completely different conditions of excitation of resonance Raman scattering for normal and diprotonated species.

4. The data of Raman study of the diprotonated form of porphyrin combined with results of quantum chemical calculations provide an effective methodology for quantifying of macrocyclic deformations of biological photoreceptors as well as of the synthetic analogues in acidic environments. Recorded Raman features become important for understanding of processes occurring in the three-dimensional structures of photosynthetic antenna systems and reaction centers, heme proteins, and related pigment-protein complexes routinely exhibit nonplanar distortions of the tetrapyrrole moiety. The findings may have implications for the understanding of structure/function properties of tetrapyrrole moieties in biological processes.

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