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Raman spectroscopic analysis of isomers of biliverdin dimethyl ester[1]

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Abstract

The constitutional isomers of biliverdin dimethyl ester, IXα and XIIIα, were studied by resonance Raman spectroscopy. The far-reaching spectral similarities suggest that despite the different substitution patterns, the compositions of the normal modes are closely related. This conclusion does not hold only for the parent state (*ZZZ*, *sss* configuration) but also for the configurational isomers which were obtained upon double-bond photoisomerization. Based on a comparison of the resonance Raman spectra, a *EZZ* configuration is proposed for one of the two photoisomers of biliverdin dimethyl ester IXα, while a *ZZE*, *ssa* configuration has been assigned previously to the second isomer. © 1997 Elsevier Science B.V.

Keywords: Biliverdin; Tetrapyrroles; Photoisomer; Raman spectroscopy; Vibrational analysis

1. Introduction

Tetrapyrroles are ubiquitous prosthetic groups which are involved in a variety of physiological processes [1,2]. The bile pigment biliverdin which is an intermediate formed during the degradation of the heme group in hemoglobin to bilirubin is composed of four methine-bridged pyrrole rings. Furthermore, related tetrapyrroles play a crucial role in various plant photoreceptors such as phytochrome and antenna pigments of photosynthetic systems [1–3]. The biological action of phytochrome, for instance, is initiated by light-induced structural changes of the tetrapyrrole chromophore involving a double bond ($Z \rightarrow E$) isomerization at one of the methine bridges followed by a series of yet unknown conformational changes at the methine single bonds. Hence, a better understanding of the physiological processes associated with these prosthetic groups requires the determination of their molecular structures and structural changes.

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In principle, vibrational spectroscopic techniques are the most appropriate tools to elucidate the configuration and conformation of the tetrapyrrole chromophore, although presently a comprehensive extraction of the structural information from the spectra is not yet possible [4–6]. However, a recent normal mode analysis of biliverdin dimethyl ester IXα (BVE IXα) as well as an increasing body of experimental data have brought about considerable progress in the interpretation of the vibrational spectra of tetrapyrroles [7–9]. In continuation of our previous studies, we have now focused on the Raman spectroscopic characterization of two tetrapyrroles, BVE IXα and BVE XIIIα and as well as their photoisomers (Fig. 1).

2. Materials and methods

Biliverdin was prepared from bilirubin, esterified and separated with respect to the constitutional isomers BVE IXα according to Lehner et al. [10]. Photoisomers of BVE IXα and BVE XIII were obtained following the method described by Falk et al. [11] and separated by thin-layer chromatography (Al_2O_3) using $CHCl_3$ with 2% (v/v) CH_3OH. Tetrapyrroles deuterated at the pyrrole nitrogens were readily obtained upon addition of D_2O to the dry compounds.

Fourier-transform (FT) resonance Raman (RR) spectra were measured by using a BioRad FT-Raman spectrometer equipped with a Spectra Physics Nd-YAG laser (1064 nm) [6]. The spectra of the stable parent species were measured as microcrystalline solids using melting-point tubes

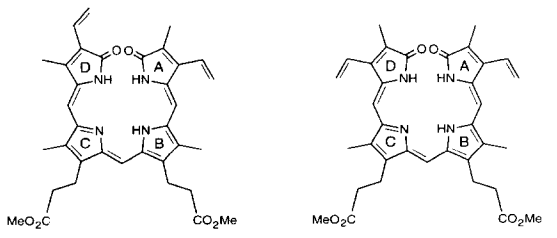

Fig. 1. Structural formulas of BVE IXα and BVE XIIIα.

while those of the photoisomers were obtained directly from thin-layer chromatographic plates. FT infrared spectra (Bruker) were measured from solutions in CCl_4. Further details of the experimental procedures including the spectra analysis are given elsewhere [6–8,12].

3. Results and discussion

In our previous work [7] we have carried out a normal mode analysis for BVE IXα based on a force field, calculated by the semi-empirical AM1 method and scaled according to Pulay's method [13]. On the basis of the calculated frequencies and IR and RR intensities, a plausible assignment for most of the observed vibrational bands was obtained which was, in addition, consistent with the spectra of BVE IXα deuterated at the pyrrole nitrogens [7].

BVE XIIIα is a constitutional isomer of BVE IXα. The methyl and vinyl groups of ring D are interchanged, resulting in a symmetrical substitution pattern with respect to the two dipyrrole fragments A–B and C–D (Fig. 1).

3.1. The parent states

The RR spectra of both compounds reveal far-reaching similarities in the band pattern (Fig. 2), suggesting that, to a certain extent, the vibrational analysis and the assignments of BVE IXα can be extended to BVE XIIIα. A striking difference, however, is noted in the so-called C=C stretching region which is displayed in an expanded view in Fig. 3. Apparently, the 1607 cm^{-1} band of BVE IXα is missing in BVE XIIIα while all other bands in this region reveal an excellent one-to-one correspondence both with respect to the relative intensities and, except for the 6 cm^{-1} downshift of the 1583 cm^{-1} band, also to the frequencies. The bands in this region are due to modes mainly involving stretching vibrations of the chromophoric skeleton (C=C/C–C, C=N/C–N). They are regarded as marker bands for the conformational and configurational state of the tetrapyrrole [8]. According to the normal mode calculations, these modes have a localized charac-

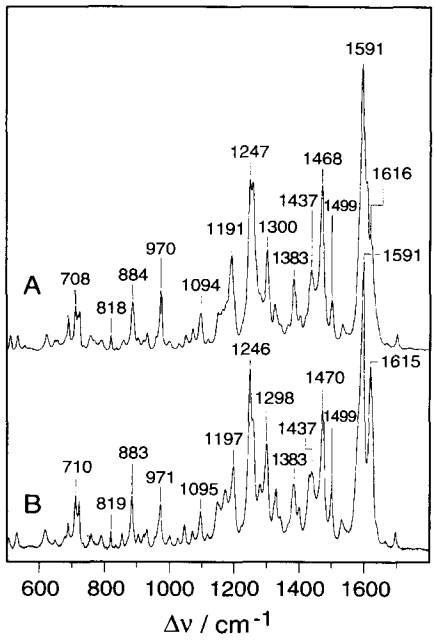

Fig. 2. RR spectra of BVE IXα (A) and BVE XIIIα (B).

ter inasmuch as their dominant contributions come from internal coordinates of individual rings and the adjacent methine bridges. The agreement between both spectra suggests that, in a first approximation, the normal mode compositions should largely be the same in BVE IXα and BVE XIIIα so that most of the RR bands of the latter can be assigned adhering to the same scheme. Apparently, the mode ν_{44} (mode numbering refers to [7]) which in BVE IXα was assigned to the 1607 cm^{-1} band represents the only exception in the C=C stretching region. However, it is highly unlikely that just for a single fundamental the RR intensity should vanish without causing a distinct intensity redistribution for the adjacent modes. Thus, it may be that in BVE XIIIα mode ν_{44} is upshifted and may contribute to the asymmetric peak centered at 1615 cm^{-1} which exhibits a somewhat higher RR intensity to its counterpart in BVE IXα. In the latter compound, two components of this peak were identified and attributed to the mode ν_{43} and the overtone $2\nu_{147}$ which gains RR intensity via Fermi resonance [7]. In BVE XIIIα, these two bands as well as that of the upshifted mode ν_{44} may strongly overlap so that they cannot be resolved.

While for the mode ν_{46}, which is predicted to include a significant contribution from ring D vibrations, the 6 cm^{-1} downshift can qualitatively be understood in view of the different substitution pattern of this ring, a similar explanation for the shift of ν_{44} is not possible within the framework of the present BVE IXα normal mode analysis. This mode (as well as the adjacent mode ν_{43}) should originate, on the one hand, from the invariant rings B and C. On the other hand, it should be noted that comparable frequency shifts are also found for phycocyanobilin [12], a tetrapyrrole which as well differs from BVE IXα with respect to the constitution of ring D. Presumably, these discrepancies reflect the limitations of the normal mode analysis of BVE IXα and/or its limited transferability to related tetrapyrroles.

A careful inspection of the RR spectrum in the entire frequency region reveals that the band widths are generally smaller in BVE XIIIα (12.1 cm^{-1}) as compared to BVE IXα (13.6 cm^{-1}). Since in the crystalline state BVE IXα forms hydrogen-bonded dimers, it is reasonable to as-

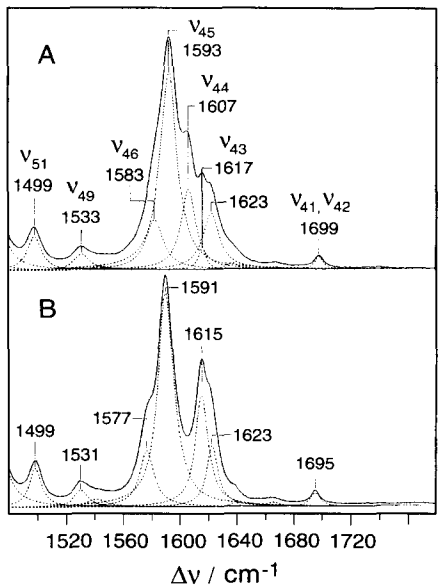

Fig. 3. RR spectra of BVE IXα (A) and BVE XIIIα (B) in the C=C stretching region. The dashed lines represent fitted Lorentzian line shapes.

sume that such dimers also exist for BVE XIIIα. However, due to the higher symmetry the number of distinguishable tautomers of these dimers is 3 for BVE XIIIα as compared to 10 for BVE IXα. Hence, it is tempting to relate these differences to the different band widths, since heterogeneous broadening may be caused by the coexistence of the various tautomers and potentially different proton transfer rate constants (for the effect of kinetic line broadening, compare [14]).

3.2. Photoisomers

Upon irradiation with white light, BVE IXα and BVE XIIIα undergo a double-bond ($Z \rightarrow E$) isomerization at the methine bridges. As the dipyrrole fragments A–B and C–D are identical in BVE XIIIα but different in BVE IXα, isomerization at the outer methine bridges (A–B, C–D) should yield two different photoisomers of BVE IXα but only one of BVE XIIIα. In fact, we have isolated only these three and no others. Apparently, no photoisomerization occurs at the central methine bridge (B–C). The RR spectra of these products, non-deuterated and deuterated at the pyrrole nitrogens, are shown in Figs. 4 and 5.

In our previous work we have attempted to determine the structures of the BVE IXα photoproducts based on a comparison of the measured RR and IR spectra and those calculated for 15 possible configurational and conformational isomers [8]. In this way it was possible to assign the *ZZE*, *ssa* configuration to one isomer (# 1), which is in line with previous NMR results by Falk et al. [11] as far as the configurational state is concerned. In the case of the second isomer (# 2), however, this approach did not provide an unambiguous conclusion.

The main spectral differences between the photoisomers of BVE IXα are found in three regions: (i) the high-frequency component of the dominant peak in the C=C stretching region at ca. 1625 cm^{-1} is much weaker in # 2, (ii) the poorly structured peak of # 1 at 1258 cm^{-1} has shifted to 1273 cm^{-1} in # 2, and (iii) the vibrational band pattern between 1400 and 1500 cm^{-1} has changed considerably including both frequency shifts and intensity alterations. Similar differences, albeit not so pronounced, are also observed for the deuterated isomers.

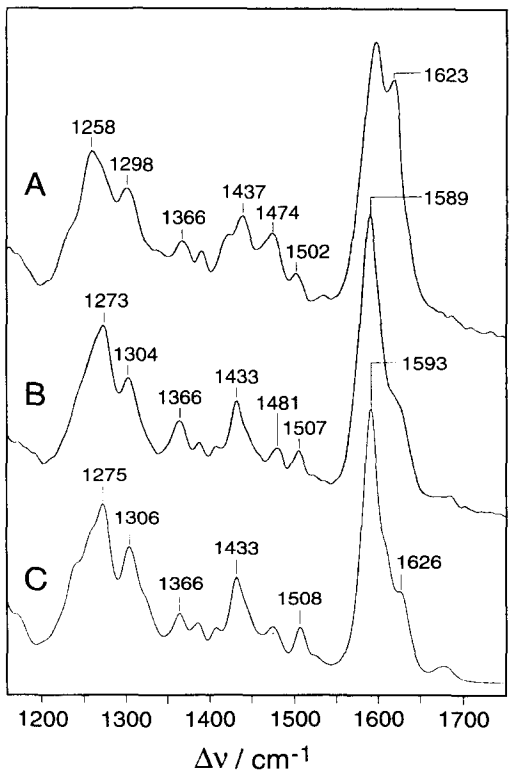

Fig. 4. RR spectra of the photoisomers # 1 (A) and # 2 (B) of BVE IXα and the photoisomer of BVE XIIIα (C). the numbering of the photoisomers of BVE IXα (# 1, # 2) follows the separation profile by thin layer chromatography with # 1 referring to the isomer of the lowest polarity (longest migration distance).

Upon comparison with the RR spectrum of the photoisomer of BVE XIIIα we note a striking agreement with photoisomer # 2 of BVE IXα in the entire frequency range. Consequently, we conclude that the configurational state of photoisomer # 2 of BVE IXα and the photoisomer of BVE XIIIα must be the same. In BVE XIIIα, photoisomerization at either of the methine bridges A–B and C–D yields the same isomer, which with regard to the partial structure corresponds to the photoproducts formed upon isomerization at the A–B bridge of BVE IXα. These considerations lead to the assignment of photoisomer # 2 of BVE IXα to an *EZZ* configuration

which again is in agreement with previous NMR data [11]. The spectral analysis does not allow for a distinction between an *EZZ, ass* or *EZZ, sss* configuration. However, in view of steric repulsions between ring substituents in the latter configuration and in analogy to isomer # 1, the *EZZ, ass* appears to be the most likely configuration of the BVE IXα isomer # 2, implying that its formation involves a (simultaneous) rotation around the A–B double and single bonds.

The RR spectroscopic comparison of these photoisomers of BVE IXα and BVE XIIIα demonstrates an agreement which is even more pronounced than in the parent compounds. This finding suggests that the spectra are dominated by the RR bands localized in those pyrrole rings which are identical with respect to the substitution pattern in BVE IXα and BVE XIIIα. As far as the C=C stretching region is concerned, this conclusion is supported by the normal mode analysis carried out for various configurational and conformational isomers of BVE IXα [7,8]. These calculations indicate that in the region between 1500 and 1700 cm^{-1} the modes localized in the pyrrole ring B should exhibit the strongest RR intensities ([6]).

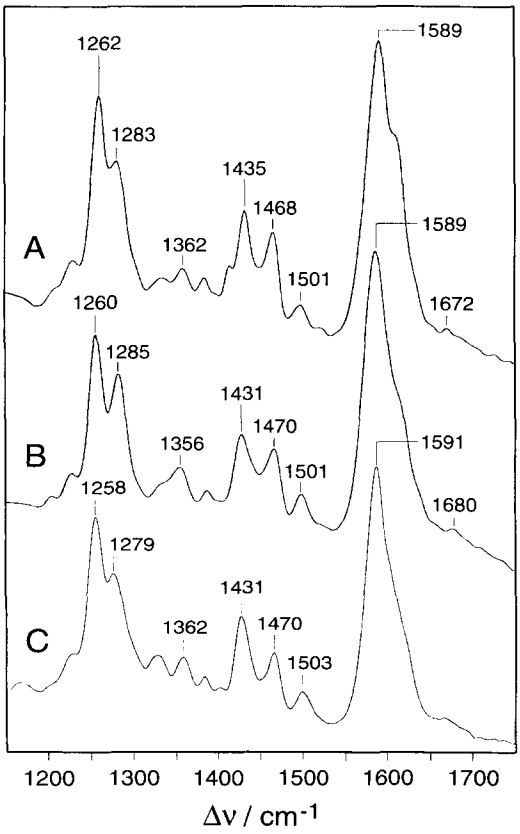

Fig. 5. RR spectra of the photoisomers # 1 (A) and # 2 (B) of BVE IXα and the photoisomer of BVE XIIIα, deuterated at the pyrrole nitrogens. For the notation '# 1, # 2' see Fig. 4.

4. Conclusions

Based on a comparative RR spectroscopic analysis it was possible to provide a plausible structural assignment for the photoisomers of BVE IXα and BVE XIIIα. These assignments are supported by semi-empirical force field calculations for BVE IXα indicating that, in a first approximation, this vibrational analysis can be also be applied to related tetrapyrroles which differ with respect to the constitution of the pyrrole rings A and D. These implications shall be of particular relevance with respect to the interpretation of the RR spectra of the phytochrome since the differences in the substitution pattern between phytochromobilin, the tetrapyrrole bound to the phytochrome protein, and BVE IXα are confined to the pyrrole ring A.

Acknowledgements

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