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Relationship between molecular structure and Raman spectra of quinolines

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ABSTRACT

DFT calculations were applied to investigate the relationship between the molecular structure and the Raman spectra of quinolines. A variety of different quinolines with increasing complexity was investigated and an aminoquinoline nucleus was found that describes the Raman spectrum of protonated chloroquine. It was discovered that the biological important, rigid C7-chloro group and C4-side chain of chloroquine significantly disturb certain molecular vibrations. The protonation at the N1 position causes dramatic changes of the Raman bands in the wavenumber region between 1500 cm^{-1} and 1650 cm^{-1}. These bands are putative marker bands of the aminoquinoline drugs for π–π interactions to the hematin targets in malaria infected cells. The calculation of the normal modes and the illustration of the associated atomic displacements are very valuable for a deeper understanding of the associated bands in the Raman spectra.

1. Introduction

Raman spectroscopy has great capabilities as analytical tool in life science [1–3], e.g. in Malaria research [4–13]. Malaria is a major world health problem and causes more than 1 million deaths every year [14,15]. An important reason for this disaster is the worldwide spread of resistances against key-drugs, e.g. chloroquine [15,16]. However, the development of new efficient drugs needs an understanding of the molecular mode of action of the antimalarials [16]. Chloroquine was one of the most successful drugs against an infectious disease ever and is related to quinine from cinchona bark [17,18]. These aminoquinoline anti-malaria drugs interfere with the detoxification process of haemoglobin digestion by-products in the red blood cell state of the malaria parasite *Plasmodium* [19–24]. It was discovered, that π–π interactions between the quinoline-ring of the aminoquinoline drugs and the porphyrin-system of the hematin target structures are very likely to exist [4,11,25–28]. Recently it was suggested that chloroquine may act as a tailor-made inhibitor of the growth of the crystals of the malaria pigment hemozoin [29,30]. In this scenario chloroquine would selectively bind to the small active growing faces of the hemozoin crystals [30].

Raman spectroscopy can be applied as a label-free molecular probe and help to localize the drugs and the biological target structures in the malaria parasite and to investigate their molecular interactions [8–11]. This is possible, because aminoquinolines and porphyrins have chromophoric and highly symmetric molecular structures and cause very intense Raman signals [4–11]. These Raman bands can strongly be enhanced by application of excitation wavelengths in the UV or visible which are resonant with the electronic absorptions of the aminoquinolines [5–10] or the hematin-targets [4,10–12], respectively. Hence it is possible to selectively localize and investigate small amounts of drugs even in a complex biological environment [7,8]. Nowadays Raman microscopic devices allow for an investigation of biological samples with a spatial resolution of less than 1 μm and also living cells can be investigated [10,12,31], because water shows only a week Raman signal. Very small wavenumber shifts that are caused by weak molecular interactions of the drugs and the targets can be measured with help of Raman difference spectroscopy [11].

A prerequisite for an interpretation of such changes in the Raman spectra of aminoquinolines, caused by the biological environment and the molecular interactions with the target structures, is a thorough assignment of the Raman bands of the pure molecules and a good understanding how certain normal modes will change due to differences in the molecular structure. The investigation of such relationships between the molecular structure and the Raman spectra can be assisted by density functional theory calculations (DFT) of the spectra. The DFT calculations do not only help to compute the Raman profiles, but also the atomic displacements of the associated molecular normal modes; which provide a much deeper understanding of the associated Raman bands.

2. Material and methods

2.1. Chemicals

Quinoline-*N*-oxide hydrate was purchased from Sigma–Aldrich and was used without further purification.

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2.2. Raman spectroscopy

The FT-Raman spectrum of quinoline-*N*-oxide was recorded with a Bruker FT Raman spectrometer (RFS 100/S) at the macroscopic mode with a spectral resolution of 2 cm^{-1}. The instrument was equipped with a Nd:YAG laser (λ_{exc} = 1064 nm, estimated laser power at the samples P = 50 mW) as excitation source and a liquid nitrogen cooled Germanium-detector.

2.3. Density functional theory calculation (DFT)

The density functional theory calculations (DFT) were performed with Gaussian03 [32]. The two hybrid exchange correlation functionals B3LYP [33–35] and B3PW91 [33,36] were applied in combination with the split valence basis set 6-31+G** [37–39]. These model chemistries are known to provide reliable estimates of experimental wavenumbers of organic molecules [40–44]. A finer integration grid was applied for all calculations. The calculated harmonic vibrational wavenumbers are overestimated compared with the experimentally observed ones due to the neglect of anharmonicity, incomplete incorporation of electron correlation, and the use of finite basis sets. Therefore scaling factors were applied to the calculated wavenumbers [45,46]. The values of the factors for B3LYP/6-31+G** and B3PW91/6-31+G** were 0.9781 and 0.965 for wavenumbers below 1800 cm^{-1} as well as 0.953 and 0.95 for wavenumbers above 1800 cm^{-1}. Raman intensities were calculated from the Raman scattering activities [47]. The Raman spectra with finite bandwidth were simulated by convoluting the theoretical stick spectra with a Gauss-Lorentz weighted profile (90% Gauss, FWHM: 6). Calculations with B3LYP/6-31+G** and B3PW91/6-31+G** yielded very similar results and both model chemistries have been applied.

3. Results and discussion

The investigation of structure–function relationships of quinoline antimalarials suggested that the C4-aminoquinoline nucleus is responsible for the binding to hematin [48] with possible stabilizing effects of the side chain [25]; and that chloroquine analogues without N1 (see Fig. 1A) do not show binding affinity anymore [49]. Analogues of chloroquine with shortened side chain may retain activity against chloroquine-resistant strains of *Plasmodium falciparum* [50]. The C7-chloro group (see Fig. 1A) is important for the hemozoin inhibitory activity [48] and electron-withdrawing groups at this position will lower the pK_a of the basic amino side chain [51] which in turn is required for drug accumulation in the acid food vacuole of the parasite [48,52–54]. Raman spectra of chloroquine solutions with the pH values of blood (pH 7.4) and the acid food vacuole (pH 5) can clearly be distinguished [5]. These spectral changes are caused by the protonation of chloroquine. The effect of the protonation onto the Raman spectra of chloroquine was discovered with help of DFT calculations [5]. The experimental spectrum of double protonated chloroquine was in very good agreement with the DFT calculation of chloroquine protonated at N1 and N16 (see Fig. 1A) while major parts of the experimental spectrum were not given by unprotonated chloroquine [5]. However the further protonation of chloroquine at N11 (see Fig. 1A and B), caused significant changes in the Raman spectrum [5]. Many new bands appear in the Raman spectrum of chloroquine that is protonated at N1, N16 and N11 (cq3p, Fig. 1B). It was found that the bond distances C4–N11 and N11–C12 increase from 136.6 pm and 145.9 pm up to 147.9 pm and 156.5 pm, respectively [5]. Hence the molecular structure of cq3p is more separated into the aminoquinoline nucleus and the side chain.

3.1. Comparison of the Raman spectrum of chloroquine with the spectra of different quinolines

Dramatic changes in the Raman spectrum of cq3p (Fig. 2A) are associated with these changes of the molecular structure. Dominant bands shift and a variety of new bands appear with significant intensity. These changes lead to many questions; e.g. whether the Raman spectrum of cq3p is a combination of modes which are now more localized in the quinoline nucleus and the side chain, respectively. It would also be of considerable interest to model the Raman spectrum of cq3p (Fig. 2A) with smaller substructures and to prove whether the major bands in the spectrum are well represented in the spectrum of a quinoline nucleus; whether Raman bands just shift or whether new bands appear; which Raman modes are still influenced by the side chain; the influence of a shortened side chain onto the spectrum, compared with the full side chain, etc. This thorough assignment and investigation of the relationship between molecular structure and Raman spectra is the basis for the future study of spectral changes in the spectra of the aminoquinolines, which are caused by molecular interactions with the target structures in malaria infected cells. Especially the DFT calculation of the atomic displacements of the molecular normal modes leads to a much deeper understanding of the associated Raman bands. Therefore a variety of quinoline structures (Fig. 1) has been calculated and the important atomic displacements are discussed in this contribution. The schematic structure of chloroquine with the atomic numbering that is used for the assignment is shown in Fig. 1A, alongside with the optimized structure of cq3p (Fig. 1B). The structures of the quinoline models which have been used for comparison with cq3p are displayed in the right column of Fig. 1. Starting with quinoline (q, Fig. 1E) and protonated quinoline (p-q, Fig. 1F), structures with increasing complexity were investigated: protonated aminoquinoline (aq-p, Fig. 1G); aminoquinoline with a 7-chloro group and a CH_3 side chain at N11 (aq-m, Fig. 1H); and the protonated form (aq-m-p, Fig. 1I).

In a first step the Raman spectrum of cq3p (Fig. 2A) was compared with the spectra of quinoline (q, Fig. 1E) and of quinoline that is protonated at N1 (q-p, Fig. 1F) (Fig. 2E), but the wavenumber positions of the major bands are very different. The calculation of the Raman spectrum of the protonated aminoquinoline nucleus of chloroquine (aq-p, Fig. 1G) (Fig. 2D) resulted in a better agreement. The addition of a short CH_3 side chain (aq-m, Fig. 1H) (Fig. 2C) is expected to have a strong influence on N11 and further improved the similarity with the Raman spectrum of cq3p (Fig. 2A). Finally the protonation of aq-m at N1 (aq-m-p, Fig. 1I) (Fig. 2B) leads to a very good agreement with the Raman spectra of cq3p (Fig. 2A). Almost all major bands of cq3p (Fig. 2A) are also resembled in the spectrum of aq-m-p (Fig. 2B) and the protonation remarkably improved the Raman bands in the important wavenumber region of the C=C stretching modes between 1500 cm^{-1} and 1650 cm^{-1}. This significant influence of a protonation at N1 onto Raman modes between 1500 cm^{-1} and 1650 cm^{-1} was also found for chloroquine [5]. The comparison of the Raman spectra in Fig. 2A and B also elucidates that many bands with smaller intensity (Fig. 2A) are caused by the side chain of chloroquine. It is of interest to compare the major bands in the Raman spectra in Fig. 2A and B in detail. The calculation of the atomic displacements will help to understand how certain normal modes will change due to the effect of the side chain of chloroquine.

The Raman band at 1619 cm^{-1} in the spectrum of cq3p (Fig. 2A) is resembled by the band at 1622 cm^{-1} in the spectrum of aq-m-p (Fig. 2B). Interestingly this band is shifted by 16 cm^{-1} in the Raman spectrum of chloroquine protonated at N1 and N16 (not protonated at N11) (cq2p) to 1635 cm^{-1} [5]. It was found that this Raman mode can be selectively enhanced with an excitation wavelength of λ_{exc} = 244 nm [5]. The atomic displacements of the normal