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SURFACE-ENHANCED RAMAN SPECTROSCOPY EMPLOYED IN ANTIMALARIAL MECHANISM OF CHLOROQUINE DRUG UPON HEMATIN

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Abstract. Near infrared surface-enhanced Raman spectroscopy (SERS) was employed in the investigations of the chloroquine antimalarial drug upon hematin. Surface-enhanced Raman spectra of the two compounds were analyzed in order to drag conclusion about their functional groups involved in adsorption. The 1:1 molar ratio of the hematin/chloroquine system was than investigated and based on the surface selection rules, a possible orientation geometry and binding site involved into the antimalarial mechanism was proposed.

Key words: chloroquine diphosphate, hematin, SERS.

INTRODUCTION

Due to its non-destructive character Raman spectroscopy became during time an invaluable tool in the study of the structure of biologically active molecules [1]. However, the application of conventional Raman spectroscopy is limited by the weak intensity of the Raman scattered light and the appearance of fluorescence. One way to overcome these disadvantages is the use of surface-enhanced Raman spectroscopy (SERS) [3, 6, 7]. Although the theoretical understanding of the mechanism of surface enhancement is not definite and still evolving, the experimental data accumulated in the last years has demonstrated SERS to be a sufficiently sensitive spectroscopic method for nano-science, surface science, analytical applications and biophysics [2].

New technologies and improvements in existing instrumentation push the envelope of detection limits. Technologies for investigating living cells hold promise for non-invasive dynamic analyses of living processes. Nanoparticles and

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other new probes and labels, homogeneous assay designs, and direct detection of compounds or specific binding events are having an impact on applied and basic research, diagnostics, drug discovery and development, and screening for contaminants and pathogens in the field.

Chloroquine phosphate ($C_{18}H_{26}ClN_3$ $2H_3PO_4$, 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline diorthophosphate) (CQ, Fig. 1, right) and related quinoline compounds have been used extensively throughout the world as prophylactics to prevent the development of malaria [6]. Although the mechanism of action of CQ on the parasite is not completely understood, it is thought to interfere with the function of the food vacuole in the mature stages of the erythrocytic parasite [10]. CQ is a weak base and accumulates to high concentrations within the acidic food vacuole. Within these vacuoles, hemoglobin is degraded by proteases to provide a supply of amino acids for the parasite, but also producing toxic heme moieties as a by-product (hematin; Fig. 1, left). The parasite normally detoxifies hematin by polymerizing the by-product to hemozoin, although the details of this process are unclear. Because CQ is known to bind to hematin, it was thought to exert its antimalarial activity by forming a complex with hematin, which inhibits its sequestration into hemozoin [9]. CQ complexation with hematin was thought to be toxic to the parasite.

Here we report our recent SERS investigations regarding the antimalarial mechanisms of the blood containing schizonticide, namely CQ. Recently, the structure of malaria pigment was reported [8]. Combining the most recent resonance Raman (RR) data [4, 12] of hematin (Fig. 1) and even the hematin-chloroquine complex [4], we employed SERS in order to get more insight into the vibrational behavior of hematin in the presence of CQ antimalarial drug. The adsorption behavior to a given nanostructureted surface, the orientation geometry, the functional groups involved in adsorption and the surface mechanisms are subject of surface enhanced Raman analysis. Therefore, the CQ was monitored upon hematin adsorption on the gold colloidal surface in order to investigate their potential complexation upon adsorption.

MATERIALS AND METHODS

CQ and hematin were purchased from Aldrich and Roche respectively, and used as received. Au colloidal nanoparticles of controlled size, employed as SERS substrates were freshly prepared according to the method reported by Sutherland and Winefordner [11]. 40 ml distilled water with 0.1 ml of 4% (w/v) $HAuCl_4$ was stirred, then 1 ml of 1% (w/v) 3natriumcitrate-2hydrate solution was added drop by drop, and the resulting mixture was boiled for 5 minutes without stirring. After 10 minutes from citrate adding, the colour of mixture developed into intensive blue, and at the end of boiling the colour was intensive magenta-red. 2 μl CQ and hematin aqueous solution were separately added to 2 ml gold colloid and the final concentrations of the SERS samples were: $3{,}9\cdot10^{-5}$ mol l^{-1} for chloroquine, $2\cdot10^{-5}$ mol l^{-1} for hematin, $1.5\cdot10^{-5}$ mol l^{-1} for hematin-chloroquine (1:1 molar ratio).

SERS spectra were recorded with a Dilor Labram spectrometer using the 785 nm excitation lines from a diode laser. The spectra were collected in the back-scattering geometry using a microscope equipped with an Olympus LMPlanFL 50x objective with a spectral resolution of 2 cm^{-1}. The detection of Raman signal was carried out with a CCD camera (Photometric model 9000), and for the signal acquisition was employed the analyzing software package LabSpec. The laser power of the NIR diode laser was 10 mW.

RESULTS AND DISCUSSIONS

The chloroquine molecule is a dication with a hydrogen atom from each of the phosphate moieties residing on the quinoline and the terminal chain nitrogen atoms. Neighbouring phosphate chains are bridged by chloroquine molecules via hydrogen bonding. Each hydrogen atom on each nitrogen atom, on each phosphate oxygen atom, and in each water molecule participates in hydrogen bonding [5]. The helical manner in which the side chains of the chloroquine molecules wrap around phosphate chains and the stacking interval of the quinoline rings between the phosphate groups may be indicative of the interaction of chloroquine molecules with cellular constituents important to antimalarial action. The modulator effect of CQ on hematin inhibition is consistent with related binding sites for both compounds, and the CQ/ hematin adduct, on the surface.

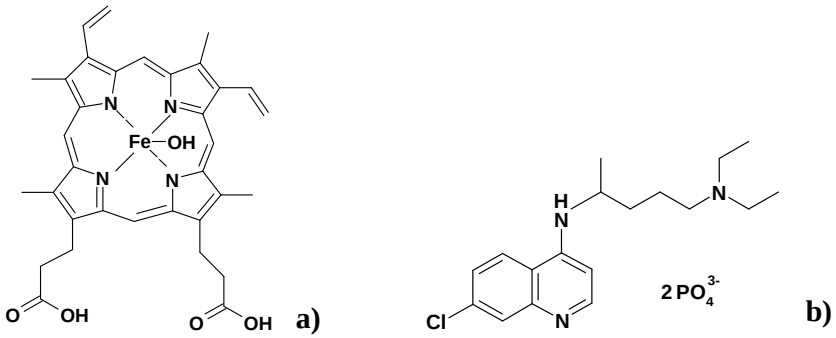


Fig. 1. Molecular structure of hematin (a) and CQ (b).

The proximity of the CQ binding site to the surface and/or hematin is both important for its activity and its unique structure which implies that the inclusion of quinoline moieties in composite compounds capable of bridging these regions might lead to dramatic additive increases in both selectivity and inhibitory activity. A dominant feature in the electron density for the quinoline is the chlorine atom.

The bulk of this predominantly hydrophobic atom may be an important factor in orienting the quinoline for binding.

NIR-SERS spectra of the CQ, hematin, and the hematin in the presence of the CQ drug were analyzed (Fig. 2). The surface enhanced Raman modes of CQ adsorbed on Au are located at 1533 cm^{-1} (the strongest band), 1583, 1450, 1373, 1246, 1097, 1002, 760, 591, 527, 489, and 390 cm^{-1}. Taking into account the information from the electronic spectra (not given here), one can conclude the formation of a CQ-Au SERS complex, where the charge transfer (CT) between the electron density of the surface plasmon and of the molecule takes place. The 785 nm line employed for excitation of the SERS spectra matches exactly the resonance condition for this CT band. Theoretically, the CQ species could be chemisorbed through the lone pairs of each N atom, Cl atom or through the π ring system. According to the surface selection rules [3], the vibrational modes that have the polarizability tensor component perpendicular with the respect of the surface will be predominantly enhanced whereas those having large tensor components parallel to the surface will result in weak SERS bands. Moreover, for molecules with a planar structure, the orientation geometry at the surface could be predicted from the relative intensity of the in-plane and out-of plane C-H modes. In the case of adsorption through the π ring system, a characteristic red shift of the SERS bands together with their broadening on passing from Raman to SERS is usually observed.

Analysing the NIR-SERS spectrum of CQ the strongest band at 1533 cm^{-1} has the corresponding Raman band at 1555 cm^{-1}, being assigned to the C=N stretching of the ring. Significant red shift from the Raman position was observed for the most representative quinoline ring stretching modes present in the SERS spectrum. Supposing the N-adsorption, the expected blue shift of the enhanced vibrational modes was not observed, therefore a perpendicular staking of the skeletal molecular plane with respect of the surface would be less probable. One significant band at 1373 cm^{-1} (ring stretching mode) was blue shifted with 3 cm^{-1}, when comparing with the correspondent band from the normal Raman spectrum. Adsorption through one or both the N atoms from the dialkylamine substitute was not consistent since the corresponding modes are less representative in the SERS spectrum.

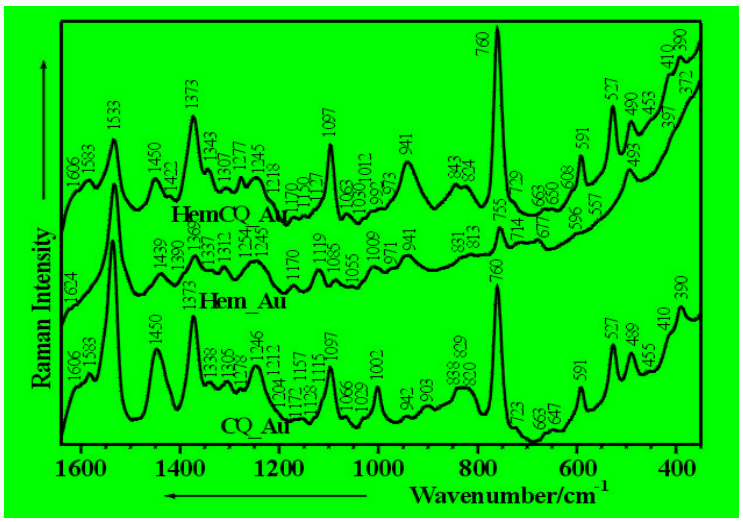
paraphrasing

In the case of the NIR-SERS spectrum of hematin, a characteristic spectral feature is observed. The dominant band is located at 1532 cm^{-1}, whereas the totally symmetric stretching modes of porphyrine ring are less enhanced. Appreciable enhancement of non-totally symmetric modes occurs. The out of plane bending modes of hematin are dominantly represented in this spectrum.

The dramatic changes exhibited in the spin state region (1650–1500 cm^{-1}) on passing from resonance Raman data to NIR-SERS of hematin could also reflect the dynamics of the porphyrine perturbation under different excitation energies of the laser lines, together with the change in the hematin orientation on the surfaces.

Several differences in the band positions or relative intensities of the NIR-SERS bands of the CQ-hematin complex, compared with those from the NIR-SERS of the pure hematin or pure CQ drug, allowed the presumption of an interaction evidence between the drug and the hematin.

The dominant characteristic is the presence of the intense and broad band at 941 cm^{-1}. This band shape modification reflects perturbations in the stretching modes of the adsorbed hematin in the presence of the drug. The same characteristic was observed in the 1300–1450 cm^{-1} spectral range.



This Figure is identical with Figure 4 of ***Panzaru2006***

Fig. 2. NIR-SERS spectra of the CQ, hematin, and CQ-hematin system, as indicated.

The NIR-SERS spectrum of hematin-CQ system is more similar to those of the CQ (Fig. 2), excepting the dominance of the band at 760 cm^{-1}, which became stronger. This fact could suggest as well a preference of CQ-adsorption instead of hematin on the Au particles. Such coverage effect of the metal nanoparticles only with the CQ species would not allow to observe contributions from hematin raised above. The increase in the relative intensity of the band at 1097 cm^{-1} (Cl-quinoline ring stretching) and the quenched band at 1002 cm^{-1} of adsorbed CQ, together with the decreased mode at 1450 cm^{-1} indicate that the two species interact upon adsorption on the surface. The contribution of the CQ aliphatic chain was not observed in the spectrum of the hematin-CQ system, suggesting that this moiety is not involved into the interaction of both species. The involvement into this possible interaction is more predictable for the quinoline ring. These band shape modifications reflect perturbations in the stretching modes of the adsorbed hematin-CQ system.

CONCLUSIONS

NIR-SERS spectra of CQ, hematin and their 1:1 molar ratio mixture were recorded and discussed. Based on the high sensitivity of the SERS technique, a possible interaction between hematin-CQ complex upon adsorption on Au nanoparticles was investigated. Their possible interaction as antimalarial mechanism was discussed.

The CQ-hematin co-adsorption on the Au colloidal surfaces was evidenced. The adsorption sites of both compounds were proposed. Further investigations of the surface structure and physical chemistry of hematin and malaria pigment would be of crucial interest in getting more insight into their antimalaria mechanism.

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