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A comparison between adsorption mechanism of tricyclic antidepressants on silver nanoparticles and binding modes on receptors. Surface-enhanced Raman spectroscopy studies

Aleksandra Jaworska, Kamilla Malek *

Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

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ABSTRACT

A series of the tricyclic antidepressants known as a surface-active drugs, has been used as a model for an evaluation of their adsorption mechanism on the metal substrate and its relationship to pharmacological action of the chosen drugs. In these studies, six antidepressants were adsorbed on the metal substrate in a form of silver nanoparticles (ca. 30 nm in diameter) and afterwards their interactions have been examined in terms of surface-enhanced Raman spectroscopy (SERS). An analysis of SERS spectra has revealed that the dibenzopine moiety is a primary site of the adsorption with some differences in the orientation with respect to the metal among the studied molecules. The spectral changes due to the interactions with the silver particles also appear in the region typical for vibrations of the side chain. These observations are consistent with a model, in which the tricyclic ring is docked in the outer vestibule of biogenic amine transporters whereas the dimethyl-aminopropyl side chain is pointed to the substrate binding site. This work sheds a light on a potential of SERS technique in predicting a key functional group responsible for drug action.

1. Introduction

Tricyclic antidepressants (TCAs) are the drugs commonly used to treat depression. Chemically, they are cationic, amphiphilic, small-size secondary or tertiary amines with a common core consisting of two flanking aromatic rings attached to a seven-membered ring. The latter can be a N- or O-heterocyclic ring (see Fig. 1). The most common TCAs are imipramine (Imi), desipramine (Des), clomipramine (Clo), amitriptyline (Ami), nortriptyline (Nor), and doxepine (Dox). They are categorized as selective serotonin, norepinephrine and dopamine reuptake inhibitors [1]. This type of the antidepressants still remains in clinical use, especially for treatment-resistant depression [2]. In toxic doses, however, they can lead to hypotension and cardiovascular disease and their use is associated with poisoning causing death. Thus, numerous analytical techniques such as fluorimetry, chemiluminometry, chromatography, enzyme and fluorescence polarization immunoassay and others [3 and therein] have been developed to identify and detect TCAs in biological matrix. The most often, their use is proceeded by an extraction the drugs from biological materials through liquid–liquid, solid phase, and microwave-assisted extraction techniques [4,5]. A potential application of surface-enhanced Raman spectroscopy (SERS) without a need of the extraction process has been also proposed [4].

Although tricyclic antidepressants have been used for years, their orientation within a primary target of their action has been yet unresolved. Sarker et al. have proposed a model, in which the tricyclic ring of the antidepressant is docked into the outer vestibule of serotonin transporter (SERT) whereas the drug's dimethyl-aminopropyl side chain points to the substrate binding site. Consequently, such binding can create a structural change in the inner and outer vestibule, which precludes docking of the tricyclic ring [6]. In addition, studies on simultaneous binding of more than one antidepressant have indicated the presence of a second binding site in the inner vestibule, which can be the pseudo-symmetric fold of monoamine transporters [1]. Imipramine is a ligand that stabilizes SERT in the outward-facing conformation. Despite the fact that the flexibility of the methyl-aminopropyl side chain is restricted by a double bond in Ami, Nor and Dox, they adopt a docking pose similar to imipramine [6–8]. In studies of Sinning and co-workers on a series of structural analogues of imipramine, a salt bridge between the tertiary aliphatic amine of TCAs and Asp^{98} of the human serotonin transporter (hSERT) was identified while the 7-position of the imipramine ring was found vicinal to

* Corresponding author. Fax: +48 12 634 0515.
E-mail address: malek@chemia.uj.edu.pl (K. Malek).

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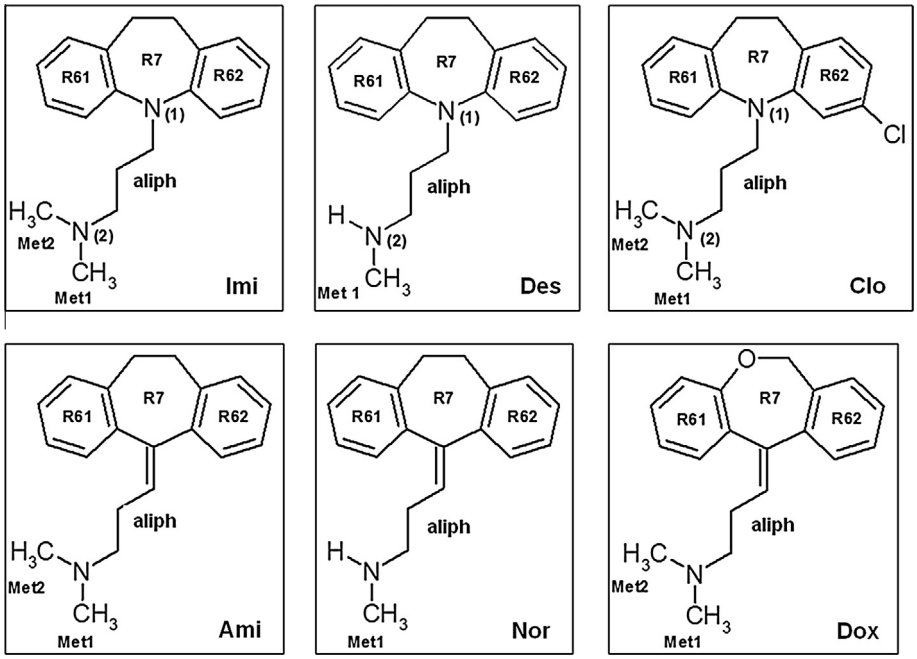

Fig. 1. Structures of the studied tricyclic antidepressants.

Phe^{335} [2]. A similar docking fashion of clomipramine into a transporter has been resolved in crystal structure of a complex between Clo and a biogenic amine transporter Leu-BAT [9]. In turn, crystal structure of the *Drosophila melanogaster* dopamine transporter (DAT) in complex with nortriptyline showed that the TCA along with cholesterol molecules stabilize the open conformation of the receptor through ionic interaction with one chloride and two sodium ions [8].

TCAs are also known as agents inducing lipidosis and intracellular accumulation of lipids. However, these interactions strongly depend on the type of the drug as well as a lipid structure and lipid phase transitions [10 and therein]. Optical-trapping confocal Raman spectroscopy has been used in the evaluation of the interaction of amitriptyline and nortriptyline with various phospholipid membranes [10]. Changes in an acyl chain conformation of the membranes and their intra- and intermolecular order due to the drugs action have been determined by monitoring peak intensities and positions of Raman signal originating from phospholipid vesicles. These studies have proposed that the TCAs interact via their cyclic rings with the acyl chains of the membranes whereas the tertiary aliphatic amine group is located near the lipid head groups. These results were found in agreement with pharmacological studies [10 and therein] and they have shown that Raman spectroscopy is an effective technique to elucidate drug–membrane interactions using micromolar concentrations of both lipids and drugs.

The modern modification of Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), utilizes generation of very strong electromagnetic field resulting from exciting of the localized surface plasmons in the metallic nanoparticles. SERS spectrum is observed if a molecule is in a close contact with a SERS-active support. Nowadays, SERS has been widely used in detection, identification and monitoring various biochemical processes since this technique has fast, label-free and non-invasive nature together with its high molecular specificity and sensitivity [11,12]. First of all, SERS provides valuable information on the adsorption mechanism of a (bio)molecule on a metallic surface pointing what functional groups or atoms participate in metal–adsorbate interactions.

In this study, we present SERS studies on six antidepressants: imipramine, desipramine, clomipramine amitriptyline, nortriptyline, and doxepine, whose structures are depicted in Fig. 1. We analyze the two groups of the tricyclic antidepressants: the first group contains the nitrogen atom inserted into the tricyclic ring (Imi, Des, Clo) and the second group represents the molecules with the double CC bond attached to the ring system (Ami, Nor, Dox). To record surface-enhanced Raman signal of the molecules, colloidal silver particles were used and spectra were recorded with a laser excitation in the near-infrared region. The SERS spectra are analyzed in order to get insight into the adsorption behavior on the metal surface. Finally, we compare the proposed models of the surface adsorption of the chosen molecules with their docking profile into receptors.

2. Experimental

2.1. Chemicals

All chemicals were purchased from Sigma, Germany and were of analytical grade. The TCAs were in a form of hydrochloride salt. Aqueous solutions of the antidepressants with the concentrations of 0.2 M (for normal Raman spectra) and 1×10^{-2} M (for SERS) were prepared by dilution of an appropriate amount of the analytes in the 4-fold distilled water. Silver colloid was prepared according to procedure described previously [13]. In this synthesis, Ag ions are reduced in the alkaline solution of hydroxylamine. UV–Vis spectrum of the silver colloid shows the presence of a resonant absorption band at ca. 412 nm (Fig. S1, Supporting Information). This position of the absorption maximum indicates that the size of silver nanoparticles varies in the range of 30–40 nm. Next, for SERS measurements, 10 μL of a sample solution was mixed with 500 μL of a colloid. The final concentration of the analytes in the mixture was 2×10^{-4} M.

2.2. Instrumentation

The absorption spectra were recorded with a UV–Vis–NIR Nicolet spectrophotometer (model Evolution 60) in the range of