Complexation of Photochromic Crown Ether Styryl Dyes with Mg^{2+} As Probed by Surface-Enhanced Raman Scattering Spectroscopy

Alexei Feofanov,[†] **Anatoli Ianoul,**[†] **Sergey Gromov,**[‡] **Olga Fedorova,**[‡] **Michail Alfimov,**[‡] **and Igor Nabiev***[,§]

Optical Spectroscopy Division, Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117871 Moscow, Russia, Semenov Institute of Chemical Physics, Russian Academy of Sciences, 117913 Moscow, Russia, and Laboratoire de Spectroscopie Biomoléculaire, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51096 Reims Cedex, France

Received: December 16, 1996; In Final Form: March 10, 1997[⊗]

Complexation with magnesium cations of 2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin (**1**), as well as derivatives of crown ether styryl dyes perchlorate 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-ethylbenzothiazolium (**2**), betaine 2-[2-(2,3,5,6,8,9,-11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-(3-sulfopropyl)-benzothiazolium (**3**), betaine 2-[2-(2,3,5,6,8,9,11,12-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-(3-sulfoethyl)benzothiazolium (**4**) was studied by surface-enhanced Raman scattering (SERS) spectroscopy. Comparative studies of isomerization and complexation of photochromic ionophores **2**−**4** with Mg^{2+} in solution and when adsorbed on the silver surface were performed. The aim was to characterize parameters of these processes depending on the nature (alkyl versus sulfo-alkyl) as well as the length (sulfoethyl versus sulfopropyl) of the N substituent in the benzothiazolium residue. The data obtained indicate stabilization of the *trans* conformation and prevention of the *trans*−*cis* isomerization for the adsorbed molecules as well as for their complexes with Mg^{2+} due to fast nonradiative energy transfer from the dye excited state to metal surface. Adsorption of a dye having initially *cis* conformation in solution is accompanied by surface-induced *cis*−*trans* relaxation. Adsorption constants calculated from the Langmuir isotherms revealed slightly preferential adsorption of **2**−**4** complexed with Mg^{2+} as compared to the free dyes. Desorption of the dyes was found to occur on the hours time scale, whereas reversible capture/release of metal ions by adsorbed molecules is quite fast and may serve as an indicator of a cation level and/or complexation processes in bulk solution. The SERS titration procedure was developed permitting the complexation of the ionophores with metal cations to be followed and equilibrium constants of these processes to be measured in a wide range (down to 10^{-7} M) of ligand concentrations. By the measurement of the equilibrium constants, adsorption on the silver surface was concluded not to affect the complexation of **1** and *trans*-**2** with Mg^{2+}. Association of the Mg^{2+} cation with the sulfonate group was found to be more favorable than binding with the crown ether moiety for the adsorbed (*trans*-**3**)Mg^{2+} and (*trans*-**4**)Mg^{2+} complexes. Appearance of the (*trans*-**3**)(Mg^{2+})$_2$ and (*trans*-**4**)(Mg^{2+})$_2$ species was detected in the solution at increased metal/ligand molar ratios. These complexes were found to be unperturbed if absorbed on the silver surface. Since the measurement of equilibrium constants are intractable by other techniques, measuring equilibrium constants for (*trans*-**3**)-(Mg^{2+})$_2$ and (*trans*-**4**)(Mg^{2+})$_2$ complexe formation is readily acquired by SERS spectroscopy.

Introduction

A wide family of photo- and ion-responsive crown ethers including stilbene derivatives,[1,2] azobenzene derivatives,[3−5] and styryl dyes containing heteroaromatic residues[6−10] have been synthesized recently, and they are currently under intense investigation. The primary aim of these studies is to achieve high selectivity of different metal cation binding accompanied by considerable changes in ligand absorption and, therefore, color. The selectivity of the binding is controlled by changing the size of the crown ether cavity and introducing the various combinations of the O, N, and S heteroatoms in the crown ether cycle. By the coupling of different chromophoric groups to the crown ether moiety, the ion-induced color response is achieved.[6−10]

In addition, considerable efforts were applied to get reversible photoresponsive stabilization of ligand−metal complexes, and control of cation binding was accomplished in this way.[4,6,11] It can be accomplished by introducing an anionic molecular group that is able to participate in the cooperative binding of the cation. If the proximity of the anionic group to the crown ring can be varied by means of photoisomerization, the photoreversible capture/release of cations occurs.

Type of cation sensitive color indication and quantification of the cation level in a solution, photoresponsive ion extraction, light-driven ion transport across membranes, and photocontrolled regulation of the cation concentration are generally considered as the fields of the most probable applications of chromoinophoric crown ethers.[1−3,12]

Compounds **2**−**4** (Figure 1) are typical styryl dyes containing the crown ether group and heteroaromatic residue. They selectively bind with alkaline earth metal cations and are

---
[†]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry.
[‡]Semenov Institute of Chemical Physics.
[§] Université de Reims Champagne-Ardenne.
* To whom correspondence should be addressed (address Laboratoire de Spectroscopie Biomoléculaire, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq Jay, 51096 Reims Cedex, France; telephone +33-3-26053554; fax +33-3-26053550; e-mail igor.nabiev@univ-reims.fr).
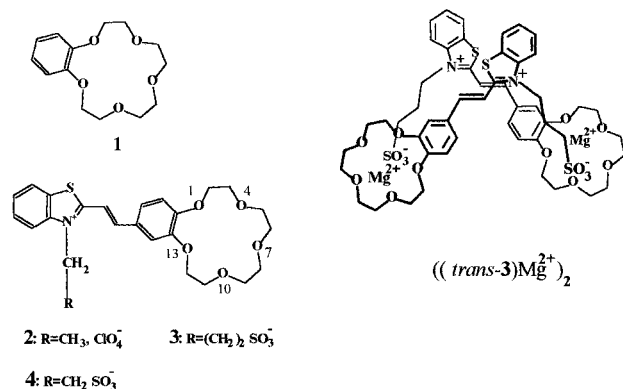[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1997.

Figure 1. Structures of compounds **1**−**4** and ((*trans*-**3**)Mg^{2+})$_2$ dimers.

characterized by high quantum yields (ranging from 0.4 to 0.6) for *trans*-to-*cis* and *cis*-to-*trans* photoisomerization.[11] Having the strong electronic transition in the blue region, *trans* isomers of **2**−**4** show significant (up to 42 nm) hypsochromic shifts upon complexation with cations and a disappearance of visible absorption upon *trans*-to-*cis* photoisomerization of the complexes.[11] These dyes are considered as chromoinophores with a wide range of applications.

Aside from application aspects, the knowledge of structure- and conformation-dependent properties of crown ether styryl dyes (CESD) requires detailed data on inter- and intramolecular interactions for the dyes both free and complexed to metal cations. UV−vis and fluorescence spectroscopy, being successfully used for such studies, have revealed photo- and cation-dependent changes in the conformation, intermolecular interactions, and stoichiometry of metal−ligand complexes.[11,13,20] Introducing a sulfopropyl N substituent was found to give rise to some new properties of **3** in relation to **2** and **4**. The sulfopropyl substituent induces more than 2 orders of enhanced stability of the (*cis*-**3**)Mg^{2+} complex versus (*trans*-**3**)Mg^{2+}, whereas the sulfoethyl N substituent of **4** is too short to participate in the cooperative binding of the cation in *cis* conformation.[13] The equilibrium constant of the (*trans*-**3**)Mg^{2+} complex formation (K_1) is considerably higher than that of (*trans*-**2**)Mg^{2+} or even (*trans*-**4**)Mg^{2+}. At the same time the presence of the sulfoethyl group does not change K_1 for the (*trans*-**4**)Mg^{2+} complex compared to the (*trans*-**2**)Mg^{2+} complex.[13,14] UV−vis absorption and time-resolved fluorescence experiments[11] led to the hypothesis of (*trans*-**3**)Mg^{2+} dimerization in solutions. Formation of head-to-tail dimers containing olefinic bonds with fixed mutual arrangement (Figure 1) has been confirmed by the occurrence of the photochemical stereo- and regioselective reaction of cycloaddition.[15]

Although the UV−vis absorption studies were able to provide general characterization of the dyes, more specific techniques are required to understand the additional details of the structure, complexation, and molecular interactions of CESDs.

Recently, we have developed a new approach to the study of CESD based on surface-enhanced Raman scattering (SERS) spectroscopy.[16,17] It has been demonstrated that these photochromic ionophores can be traced by the SERS technique in the 10^{-5}−10^{-8} M range of concentrations not achievable with other spectroscopic (UV−vis, fluorescence, NMR) techniques. By combination of Langmuir−Blodgett and SERS techniques, the extreme sensitivity of the method was utilized in the course of the study of structural organization and aggregation properties of a monolayer of amphiphilic CESD.[18,19] The vibrational spectra of the dyes bring a wealth of information regarding the state and interaction of functionally important molecular groups.

Here, we employ a potential of SERS spectroscopy as a quantitative analytical tool. The SERS titration procedure tailored to follow the complexation of CESDs is reported. By use of the SERS titration, the features of complexation with Mg^{2+} cations were investigated for the photochromic ionophores **2**−**4** differing in the nature (alkyl versus sulfoalkyl) as well as the length (sulfoethyl versus sulfopropyl) of a N substituent in the benzothiazolium residue. Widely varying the dye concentration, which influences both the dimerization of the molecules in a solution and probable intermolecular interactions on the complexation, was considered. The features of adsorption and isomerization of ionophores **2**−**4** as well as their complexes on metal surfaces (used as SERS-active substrate) were characterized.

Experimental Section

Compounds **2**−**4** (Figure 1), namely, perchlorate 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-ethylbenzothiazolium (**2**), betaine 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-(3-sulfopropyl)benzothiazolium (**3**), and betaine 2-[2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,-10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-(3-sulfoethyl)benzothiazolium (**4**) were synthesized as described elsewhere.[7] The 2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin (**1**, Figure 1) was purchased from Merck. Acetonitrile (HPLC grade) was doubly distilled from P$_2$O$_5$ and CaH$_2$ to remove traces of water. Magnesium perchlorate (high grade) was dried under vacuum at 180 °C. All experiments with dyes were performed in the acetonitrile solution under red light at room temperature.

SERS spectra were recorded with a single-channel Ramanor HG-2S spectrometer (Jobin Yvon, France) using 5 mW of 457.9 nm Ar$^+$ laser line (Spectra-Physics, Model 164-03).

The spectra were generally recorded without accumulation in the 150−1700 cm^{-1} range at 1 cm^{-1} increments with a 1 s integration time. The 1400−1650 cm^{-1} spectral region was recorded in the course of the titration procedure.

SERS spectra were obtained for the molecules adsorbed on electrochemically roughened silver electrode. The oxidation−reduction cycle was performed in the electrochemical cell in an aqueous electrolytic solution following the standard procedure described previously.[20] The roughened electrode was washed with triply distilled water, dried, and washed again with acetonitrile to remove the electrolyte and traces of water. For SERS measurements the roughened electrode was immersed in a small quartz cell with 150 μL of analyte. Stability and reproducibility of the SERS signal of CESDs in this experimental system were proved as described previously.[16]

The concentrations of **2**−**4**, their isomerization state, and the metal ion complexation were monitored by UV−vis spectroscopy with a Cary 209 spectrophotometer (Varian). The molar absorption coefficients of compounds **2**−**4** are the same and equal to 39 000 M^{-1} cm^{-1} (at ca. 434 nm) for the *trans* isomer of a free dye and 36 000 M^{-1} cm^{-1} (at ca. 393 nm) for the *trans* isomer of the dye complexed with Mg^{2+}.[11,13] The concentration of **1** was determined using the molar absorption coefficient of 3500 M^{-1} cm^{-1} (at 280 nm).

SERS titration of the ionophores **1**, *trans*-**2**, *trans*-**3**, and *trans*-**4** by Mg(ClO$_4$)$_2$ was performed for 1 × 10^{-3} M solution of **1** and 1 × 10^{-5}, 1 × 10^{-6}, and the 1 × 10^{-7} M solutions of **2**, **3**, and **4**, respectively, under the same experimental conditions of laser power, procedure of electrode roughening, and time of the spectrum registration. All the titrations were performed by mixing equimolar acetonitrile solutions of a pure ionophore and

SERS of Crown Ether Dyes

J. Phys. Chem. B, Vol. 101, No. 20, 1997 **4079**

TABLE 1: SERS Frequencies (cm^{-1}) and Assignments^a for Compounds 1−4

1	2−4	assignments
1608^b sh^c	1608 sh	$\nu_{8b}{}^d$
1592 vs	1589 vs	ν_{8a}
1507 s (+4)^e	*1508 s* (+4)	ν_{19b}
1488 sh	1488 sh	δ(H−C−H)
1461 w	1461 w	δ(H−C−H)
1439 s	1439 s	ν_{19a}
1361 m	1361 m	ν_{14}
1321 s (+4)	1319 s (+6)	CH$_2$ wag & δ(C−C−H)
1264 s	1264 s	ν_3
1225 w (−4)	1225 w (−4)	ν_{7a}
1177 w (−8)	1176 w (−3)	ν_{9a}
1142 w	1140 w	ν_{18a} & ν(C−O−C), asym
1075 vw	1075 vw	ν(C−C)
1036 vw	1036 vw	ν(C−C−O), sym
1023 vw	1023 vw	ν_{18b}
851 vw	851 vw	ν_{17a} & ν_{12}
792 vw	796 vw	ν_{11}
635 vw (+2)	633 vw (+4)	ν_4
613 vw (+5)	611 vw (+7)	ν_{6a}
510 vw	510 vw	ν_{6b}
454 vw	454 vw	δ(C−O−C), sym

^{a} Assignments from refs 16 and 17. ^{b} The bands with intensities sensitive to the complexation with Mg^{2+} are marked by italic. ^{c} Relative intensity of bands: vw, very weak; w, weak; m, medium; s, strong; vs, very strong; sh, shoulder. ^{d} Wilson notation of vibrational modes of benzene. ^{e} Shifts on complexation with Mg^{2+} are given in parentheses.

a complex of the ionophore with Mg^{2+} (metal/ligand molar ratio of 200/1) in order to have the prescribed metal/ligand molar ratio at the constant ligand concentration. Each titration was reproduced three times with different sample preparations. The SERS titrations of **1**, *trans*-**2**, and *trans*-**4** were accompanied by the UV−vis titrations according to the procedure described previously.[11] All quartz cuvettes and glass tubes used for manipulations with samples were treated by trimethylchlorosilane to minimize dye adsorption on the cuvette walls.

The computational procedure based on the Levenberg−Marquart method of curve fitting[21] was used to deconvolve overlapped bands of the ν_{8a} and ν_{8b} modes and to define a correct value of the band intensity.

Results and Discussion

General Characteristics of the SERS Spectra of the Ionophores. The SERS spectra of compounds **1**, *trans*-**2**, and *trans*-**3** as well as complexes **1**Mg^{2+}, (*trans*-**2**)Mg^{2+}, and (*trans*-**3**)Mg^{2+} were presented and analyzed previously.[16,17] Vibrational assignments of the major SERS active modes of these chromoinophores are listed in Table 1. The SERS spectra of *trans*-**4** and (*trans*-**4**)Mg^{2+} are shown in Figure 2. Compound **1** does not absorb light in the visible region, and its SERS spectrum can be measured at a concentration of 10^{-4} M or higher. The dyes **2**−**4** have an intense electronic transition with a maximum located at ca. 434 nm (Figure 2II) ascribed to the *trans* isomer.[11,13] Coupling resonance Raman with surface-induced enhancement (resonance SERS effect) increases sensitivity of the method and enables the spectra of *trans*-**2** and *trans*-**3** adsorbed on the electrode to be recorded at concentrations from 10^{-4} to 10^{-8} M.[16] The upper boundary of the detection limit (10^{-4} M) is determined by interference with fluorescence emission of the dyes (λ_{max} = 540 nm). It is close to the solubility limit of **3** in acetonitrile (ca. 4 × 10^{-4} M).

A detailed comparison of the Raman and SERS spectra of **1**−**3** and some model compounds[16,22] as well as the data of normal mode analysis of substituted benzene derivatives[23]
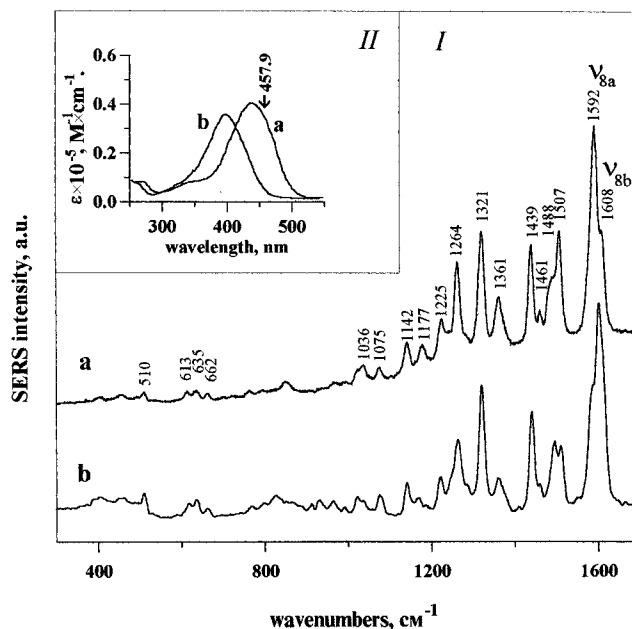

Figure 2. SERS (I) and UV−vis (II) spectra of *trans*-**4** (a) and (*trans*-**4**)Mg^{2+} (b) in acetonitrile solution. For SERS spectra, [**4**] = 2 × 10^{-5} M, [Mg^{2+}] = 10^{-4} M, and the excitation wavelength is 457.9 nm. The spectrum of the solvent was subtracted. For UV−vis spectra, [**4**] = 2 × 10^{-5} M, [Mg^{2+}] = 10^{-4} M.

allowed a model of adsorption of these ionophores on the SERS-active surface to be proposed. According to this model, the molecules are adsorbed through the crown ether part so that the plane of the conjugated benzene ring is nearly perpendicular to the surface. The ethylene group, benzothiazolium moiety, the sulfonate group of **3**, and the alkyl radical of **2** are not positioned beside the SERS-active surface. A distant position of these chromophores relative to the SERS-active surface was found to induce a selective enhancement of benzene vibrational modes of benzo-15-crown-5 moiety of the molecules **2** and **3** (Table 1). As a result, very similar, except for the intensity, SERS spectra were observed for **1**, *trans*-**2**, and *trans*-**3** despite the prominent difference in the electronic structure of **1** compared with those of *trans*-**2** and *trans*-**3**. Moreover, in contrast to **1**, the SERS of *trans*-**2** and *trans*-**3** is resonance-originated.[16] The SERS spectrum of *trans*-**4** (Figure 2I) is essentially identical with the spectrum of *trans*-**3** and can be detected in the same range (10^{-4}−10^{-8} M) of concentrations.

Interaction with the silver surface occurring upon adsorption of **1**, *trans*-**2**, *trans*-**3**, and *trans*-**4** on a SERS-active electrode does not preclude these molecules from complexing with Mg^{2+}. The pattern of the spectral changes induced by complexation with Mg^{2+} is almost the same for **1**, *trans*-**2**, *trans*-**3**,[16] and *trans*-**4** (Figure 2I).

The identity of the SERS spectra of *trans*-**4** and *trans*-**3**, together with the identity of the spectra of the complexes (*trans*-**4**)Mg^{2+} and (*trans*-**3**)Mg^{2+}, and the similarity of the chemical structures of the compounds enable the results of the analysis made for **3** previously[16,17] to be used directly to characterize SERS properties of **4**, in particular, for the geometry of adsorption, assignments, and spectral changes induced by complex formation (Table 1).

Formation of the complex with Mg^{2+} affects the frequencies of the SERS bands of **1**, *trans*-**2**, *trans*-**3**, and *trans*-**4** at 1507 cm^{-1} (ν_{19b}), 1321 cm^{-1} (δ(C−C−H) and CH$_2$ wagging), 1225 and 1177 cm^{-1} (ν_{7a}, ν_{9a}), and 635 and 613 cm^{-1} (ν_4, ν_{6a}), (Figure 2I, Table 1). In addition, the complexation with Mg^{2+} is accompanied by changes in the relative intensity of the ν_3, δ-(H−C−H), ν_{19b}, ν_{8a}, and ν_{8b} modes compared with the intensity

TABLE 2: Ratios of SERS Cross Sections, Adsorption Constants, and Equilibrium Constants for Complex Formation with Mg^{2+} for Compounds 1−4

						log K_1		
compound	σ_{1c}/σ_f	σ_{2c}/σ_{1c}	log B_f	log B_{1c}	log B_{2c}	SERS^a	absorption^b	log K_2
1	0.4					≥6.2	≥6.2	
2	0.26		5.6 ± 0.3	5.9 ± 0.4		4.3 ± 0.4	4.5 ± 0.3	
3		0.24	5.7 ± 0.3	5.8 ± 0.4	6.0 ± 0.3		6.8 ± 0.3^d	4.6 ± 0.4^c
4		0.51	5.8 ± 0.3	5.9 ± 0.4	6.0 ± 0.3		4.5 ± 0.3	3.6 ± 0.4

^{a} According to SERS titration at [1] = 10^{-3} M, [2] = 10^{-5} M. ^{b} According to UV−vis titration at [1] = 10^{-3} M, [2] = [3] = [4] = 10^{-5} M. ^{c} Measured at [3] = 10^{-7} M. ^{d} Apparent equilibrium constant not corrected for the dimerization process.

of the δ(C−C−H) and CH$_2$ wagging and ν_{19a} vibrations (Figure 2I, Table 1). Some alterations of the relative intensities of the bands are also detected in the region of 1100−150 cm^{-1}, but they are less pronounced because of the weaker intensities of the low-frequency modes. The intensities of ν_{8b} and ν_{8a} vibrational modes of benzene ring conjugated to the crown ether group, as well as their ratio, were found to be the most characteristic of the complexation of **1, trans-2,** and **3** with Mg^{2+}.[16] The same conclusion is valid for *trans-4* as follows from the data of this work. Furthermore, in preliminary studies the ratios of intensities $I(\nu_{8a})/I(\nu_{19a})$, $I(\nu_{8b})/I(\nu_{19a})$, and $I(\nu_{8b})/I(\nu_{8a})$ were pointed as a possible measure of equilibrium constants for complexation of **1** and *trans-2* with Mg^{2+} ions.[24]

Features of Complexation of Compounds 1−4 with Mg^{2+} Cations in Solution. Complexation of the crown ether part of **1**, *trans-2*, *trans-3*, and *trans-4* molecules with Mg^{2+} is described by

$$L + Mg^{2+} \overset{K_1}{\rightleftharpoons} LMg^{2+} \qquad (1)$$
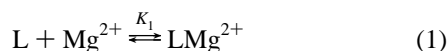
where K_1 is an equilibrium constant of this process and L is a ligand.

Complexation of a sulfonate group with a second Mg^{2+} ion is possible for *trans-3* and *trans-4* according to the following equation:

$$LMg^{2+} + Mg^{2+} \overset{K_2}{\rightleftharpoons} L(Mg^{2+})_2 \qquad (2)$$

where K_2 is an equilibrium constant of this process. The association of the sulfonate group with a metal cation cannot be followed by UV−vis, since it does not change the absorption spectra of the dyes. Nevertheless, occurrence of this process was proved by means of time-resolved fluorescence spectroscopy for the *trans-3* compound.[11] SERS spectroscopy permits the K_2 equilibrium constant to be measured for the *trans-3* and *trans-4* compounds, as will be discussed below.

As was found earlier, the (*trans-3*)Mg^{2+} is subjected to dimerization in acetonitrile,[7,11,13] which can be described by equation

$$LMg^{2+} + LMg^{2+} \overset{K_d}{\rightleftharpoons} (LMg^{2+})_2 \qquad (3)$$
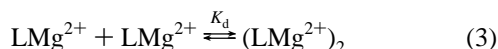
where K_d is an equilibrium constant of dimerization. Correspondingly, when the complexation of *trans-3* with Mg^{2+} is studied, eqs 1−3 should be taken into account.

It is supposed that dimeric structure of (*trans-3*)Mg^{2+} is stabilized by interaction of the sulfonate group of one molecule with the Mg^{2+} cation located in the cavity of crown ether moiety of another.[7,11,13] It should be noted that the sulfoalkyl group of *trans-4* is too short to provide formation and stabilization of (*trans-4*)Mg^{2+} dimers.[13] Therefore, the complexation of *trans-4* with Mg^{2+} occurs according to eqs 1 and 2.

Thus, despite the similarity in chemical structure, different schemes of Mg^{2+} complexation are realized for *trans-2*, *trans-*
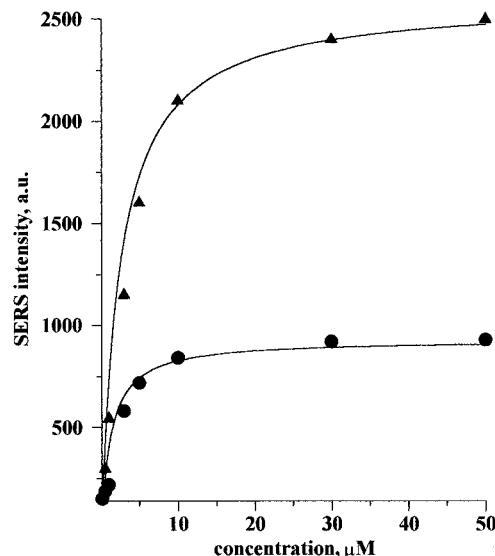

Figure 3. Dependence of SERS intensity on the concentration of **2** (▲) and **2**Mg^{2+} (●) in solution. Solid lines are Langmuir isotherms calculated from the Langmuir adsorption theory by using the experimental data. See text for details. Excitation wavelength is 457.9 nm.

3, and *trans-4* in the acetonitrile solution. According to the UV−vis data,[13] the appearance of the sulfopropyl group in *trans-3* increases the K_1 constant compared to that for *trans-2* and *trans-4* (Table 2).

Properties of the Adsorbed Dye Molecules. As shown in our previous experiments, SERS spectra of the *trans* isomer of CESDs and their complexes with Mg^{2+} are the only observed in mixtures of *cis* and *trans* isomers.[16,17] We failed to find any spectral features attributable to the *cis* isomer in SERS spectra of *cis/trans* mixtures recorded at different excitation wavelengths providing various relative concentrations of *cis/trans* conformers. The dominance of a *trans* isomer in SERS spectra was explained in terms of prominent differences between the SERS cross sections of the vibrational modes of the *trans* and *cis* isomers in the visible region. A new set of data combined with the previous one suggest the existence of an additional strong reason for this selectivity to appear in the course of CESD adsorption. The data can be summarized as follows.

The intensity of the SERS signal of the *trans* isomer of the dye is independent of the photostationary state of the solution and was found to be a function of the total concentration of the dye (Figure 3) rather than a concentration of *trans* or *cis* isomer. A very intense SERS signal of the *trans* isomer was observed even for the solution of (*cis-3*)Mg^{2+} at 10^{-8} M where the relative amount of (*trans-3*)Mg^{2+} was less than 0.01% (i.e., less than 10^{-12} M), and the *cis* conformation would persist in the solution by irradiation with 457 nm laser light.[16]

Attempts to measure SERS spectra from the excited state of the CESDs using a picosecond time-resolved technique were unsuccessful.[25] This suggests that the lifetime of the excited state of the adsorbed molecules is very short compared to those
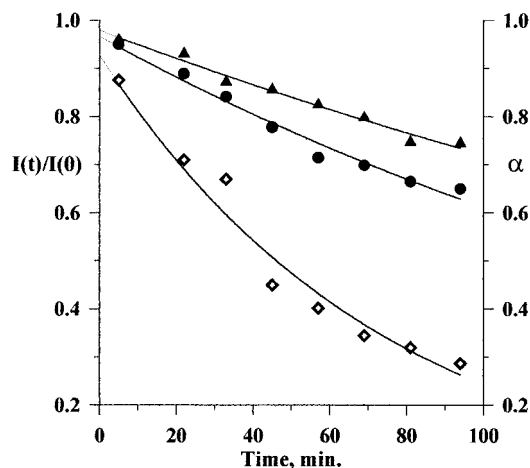
SERS of Crown Ether Dyes

J. Phys. Chem. B, Vol. 101, No. 20, 1997 **4081**

Figure 4. Desorption kinetics of **2** (▲) and **2**Mg^{2+} (●) from silver surface to pure acetonitrile and to acetonitrile solution of Mg(ClO$_4$)$_2$, respectively, measured as a change in relative SERS intensity $I(t)/I(0)$ versus time. Desorption kinetics of Mg^{2+} ions from **2**Mg^{2+} complexes (◇) from the silver surface to pure acetonitrile was calculated from the changes of the SERS spectrum versus time. See text for details.

in the solution. Both fluorescence quenching[17] and a reduced excited-state lifetime favor the fast nonradiative energy transfer from the adsorbed molecules to the metal surface.

When silver electrode with adsorbed molecules was transferred into pure acetonitrile, the SERS signal did not disappear as might be expected if there was depletion of the *trans* isomer because of laser-induced *trans−cis* photoisomerization of the adsorbed molecules.

All these facts taken as a whole provide strong evidence that the *trans* conformation is stabilized and that *trans−cis* isomerization is prevented for the adsorbed molecules and their complexes because of the fast nonradiative energy transfer from the excited state of the dye to the metal surface. The data obtained also suggest adsorption of the dye having initially the *cis* conformation in solution to be accompanied by a surface-induced relaxation of the molecule to the lower energy state corresponding to the *trans* conformation.

Adsorption of CESDs and their complexes with Mg^{2+} on the silver surface is described by Langmuir isotherms similar to that shown for **2** and **2**Mg^{2+} (Figure 3). As it follows from Langmuir adsorption theory, the SERS intensity I_i of the ν_i mode depends on SERS cross section σ_i of this mode, the number of adsorption sites Z, the concentration C of molecules in a solution, and the adsorption constant B:

$$I_i = Z\sigma_i BC/(1 + BC) \qquad (4)$$

As expected for the Langmuir adsorption, a linear relationship is observed in the plot of $I_i/(I_i(C_{sat}) − I_i)$ ratio versus concentration C, where $I_i(C_{sat})$ is the SERS intensity corresponding to a saturation level of the Langmuir isotherm. The slope of the curve gives a value of the adsorption constant (Table 2). The adsorption constants indicate slightly preferential adsorption for the CESDs complexed with Mg^{2+} in relation to the free dyes.

Considering an interrelation between the molecules adsorbed and those in a bulk solution, the desorption rate of the dye **2** was measured by placing an electrode with adsorbed molecules in pure acetonitrile (Figure 4). A similar experiment was performed to evaluate desorption kinetics of the complexes by placing an electrode with adsorbed **2**Mg^{2+} complexes in an acetonitrile solution of magnesium perchlorate (Figure 4). In both cases very slow kinetics of desorption from the electrode was observed, being more remarkable for **2**Mg^{2+} complexes.

Slow desorption was found to be a general property of all of the studied CESDs and their complexes with Mg^{2+} (data not shown). This suggests that an exchange of the molecules between the surface and a bulk solution of the dye occurs on an hours time-scale.

It should be mentioned that the exchange of the molecules is practically negligible during the time of a spectrum recording (5 min). Then assuming that the electrode is immersed into an equilibrated solution of the *trans* isomer of a dye and its complexes with Mg^{2+} cations, one can suggest that the state of the adsorbed molecules and complexes is defined by an initial (before irradiation by laser) equilibrium state of the solution. The state of the adsorbed molecules and complexes is practically independent of a further photoinduced *trans−cis* isomerization in the solution during the spectrum measurement, and the SERS-active silver surface can be considered as a system having a long-time "memory".

Exchange of metal ions between the adsorbed molecules and bulk solution was estimated by placing an electrode with adsorbed **2**Mg^{2+} complexes in pure acetonitrile. The SERS spectrum changes in time from one of **2**Mg^{2+} to one of **2**. Recording the SERS spectra as a function of time (one after another), we calculated the fraction of the dye molecules in a complex with Mg^{2+} (α) (according to the method described below). Thus, we estimated the exchange of metal ions between the adsorbed molecules and bulk solution to be very prominent (Figure 4). Therefore, the state of the adsorbed molecules is quite sensitive to the cation level in a solution, and reversible capture/release of metal ions by the adsorbed molecules may serve as an indicator of complexation in the bulk solution.

At the same time SERS spectroscopy is quite restricted as for the study of a *cis* isomer or mixed *trans−cis* solutions of the dyes, since *cis−trans* relaxation occurs upon adsorption of the CESDs on the silver surface.

SERS Titration of 1 and *trans*-2 with Mg^{2+}. The ratio R_1 of intensities $I(\nu_{8b})/I(\nu_{8a})$ was used for the SERS titration procedure. This ratio being equal to the value of $R_f = 0.5$ for free **1** and *trans*-**2** increases when titrating the ligand with Mg^{2+} cations. R is equal to $R_{1c} = 1.9$ when the titration curve flattens out at high Mg^{2+}/L molar ratios. At a given Mg^{2+}/L molar ratio the intensity of a SERS band $I(\nu_i)$ is defined by

$$I(\nu_i) = \delta_f I_f(\nu_i) + \delta_{1c} I_{1c}(\nu_i) \qquad (5)$$

where ν_i is the ν_{8b} or ν_{8a} mode and $I_f(\nu_i)$ and $I_{1c}(\nu_i)$ are the intensities of the ν_i band in an equimolar solution of a free ligand and LMg^{2+} complex (at complete ligand complexation), respectively. δ_f and δ_{1c} are the relative amounts of a free ligand and LMg^{2+} complex in a total amount of the ligand adsorbed on the SERS-active surface.

Based on eq 5 written for the both ν_{8b} and ν_{8a} modes, a δ_{1c}/δ_f ratio can be calculated as

$$\delta_{1c}/\delta_f = (R_f − R_1)R_{1a}^{-1}(R_1 − R_{1c})^{-1} \qquad (6)$$

where R_{1a} is the ratio of the ν_{8a} band intensity of the LMg^{2+} complex (at complete ligand complexation) to the intensity of the ν_{8a} band of a free ligand. The R_{1a} was determined from SERS spectra of a free ligand and its complex with Mg^{2+} recorded under fixed experimental conditions and the constant concentration of the ligand as an average of three independent pairs of measurements.

The fact that the geometry of the ligand adsorption is unchanged upon complexation with Mg^{2+} enables us to assume that the very adsorption sites as well as their quantity are the same for the free ligand and the LMg^{2+} complex. Then
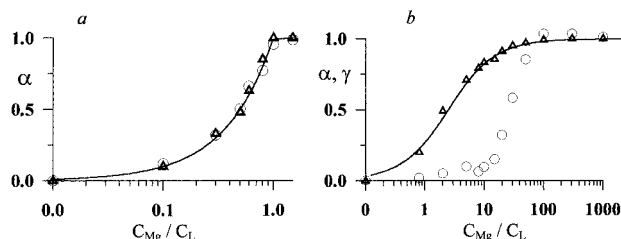
Figure 5. (a) Dependence of relative amount of $1Mg^{2+}$ complexes, α, on the Mg^{2+}/ligand molar ratio in solution ($[1] = 10^{-3}$ M) according to data of UV–vis ($\triangle$) and SERS ($\bigcirc$) titration; (b) dependence of relative amount of (*trans*-**4**)Mg^{2+} complexes, α ($\triangle$ and solid line), and (*trans*-**4**)($Mg^{2+})_2$ complexes, γ ($\bigcirc$), on the Mg^{2+}/ligand molar ratio in solution at [*trans*-**4**] = 10^{-5} M according to the data of UV–vis (α) and SERS (γ) titrations. See text for details.

according to the Langmuir adsorption theory, δ_{1c}/δ_f is defined as

$$\delta_{1c}/\delta_f = \theta_1 \alpha (1 - \alpha)^{-1} \qquad (7)$$

where θ_1 is the B_{1c}/B_f ratio of the adsorption coefficient of a LMg^{2+} complex to that of a free ligand and α is the relative amount of the complexes in the solution at a given Mg^{2+}/L molar ratio.

Then α can be defined from eqs 6 and 7 as

$$\alpha = (1 + \theta_1 R_{1a}(R_{1c} - R_1)/(R_1 - R_f))^{-1} \qquad (8)$$

It should be noted that there is a more straightforward way to estimate θ_1 other than measuring Langmuir isotherms. At the concentration C_{sat} corresponding to the saturation of adsorption sites on the SERS-active surface, $R_{1a}(C_{sat})$-simplified eq 4 can be presented as

$$R_{1a}(C_{sat}) = \sigma_{1c}/\sigma_f \qquad (9)$$

where σ_{1c} and σ_f are the SERS cross sections of the ν_{8a} mode of a free ligand and LMg^{2+} complex, respectively.

At very low concentrations of the ligand C_{low}, the $R_{1a}(C_{low})$ eq 4 can be simplified to

$$R_{1a}(C_{low}) = \theta_1 \sigma_{1c}/\sigma_f \qquad (10)$$

By measurement of R_{1a} at C_{low} and C_{sat}, the θ_1 value can be determined by combining eqs 9 and 10.

Dependencies of α on the Mg^{2+}/L molar ratio measured by means of both SERS and spectrophotometric titrations coincide with those for **1** (Figure 5a). The similar overlapping of the SERS and spectrophotometric titration curves was observed for *trans*-**2** (data not shown). The coincidence of SERS and spectrophotometric titration data proves the applicability of SERS spectroscopy for determining the equilibrium constants for $1Mg^{2+}$ and (*trans*-**2**)Mg^{2+} complex formation. When experimental data are fitted by the least-squares procedure, the K_1 equilibrium constant can be estimated as

$$K_1 = \frac{\alpha}{(1 - \alpha)([M] - \alpha[L])} \qquad (11)$$

Here, [M] and [L] are the concentrations of metal cations and the ligand in solution, respectively. The equilibrium constants measured in the course of the titration procedure are presented in Table 2.

Though the SERS spectrum of **1** can be detected at 10^{-4} M concentration, a solution of 10^{-3} M or even higher concentration is only suitable for SERS titration to provide both a relevant signal-to-noise ratio in SERS spectra and validity of calculations.

Consequently, θ_1 cannot be determined for compound **1** using SERS spectroscopy. θ_1 was supposed to be equal to ca. 1 as estimated by fitting the SERS titration to the spectrophotometric titration curve by means of the least-squares procedure. It should be noted that the K_1 of $1Mg^{2+}$ complex formation is very high and cannot be accurately determined using 10^{-3} M concentration of the ligand. The lower concentration of **1** (10^{-5} M), which is required for the correct measurement of K_1, is not suitable for the SERS titration. Despite these limitations, a rather good correlation between K_1 measured both by SERS and spectrophotometry should be mentioned.

The equilibrium constants of (*trans*-**2**)Mg^{2+} complex formation calculated by SERS and spectrophotometric titrations (Table 2) are in a good agreement at ligand concentrations of 10^{-5} and 10^{-6} M (10^{-7} M is not applicable for spectrophotometric measurements). K_1 measured by SERS is not changed on going from 10^{-5} to 10^{-7} M solutions of *trans*-**2**. Therefore, interactions between neighboring adsorbed molecules as well as the influence of the ionic strength of the solution are weak or even negligible and do not affect Mg^{2+} complexation.

SERS Titration of *trans*-3 and *trans*-4 with Mg^{2+}. Though the ratio of intensities $I(\nu_{8b})/I(\nu_{8a})$ is sensitive to the titration of *trans*-**3** and *trans*-**4** with Mg^{2+}, the dependence of $I(\nu_{8b})/I(\nu_{8a})$ on the Mg^{2+}/L molar ratio was found to be prominently different from that obtained for **1** and *trans*-**2**. Briefly, an increase in the $I(\nu_{8b})/I(\nu_{8a})$ ratio was observed at high Mg^{2+}/L molar ratios when formation of the LMg^{2+} complexes was already completed according to the data of UV–vis titration. Therefore, before application of SERS titration to the study of complexation of *trans*-**3** and *trans*-**4** with Mg^{2+}, two interrelated questions should be addressed. Why are SERS spectra of *trans*-**3** and *trans*-**4** not affected by the formation of (*trans*-**3**)Mg^{2+} and (*trans*-**4**)-Mg^{2+} complexes in a solution? What process would result in an increase of the $I(\nu_{8b})/I(\nu_{8a})$ ratio (R_2) as a function of Mg^{2+}/L ratio if it is not LMg^{2+} complex formation?

No changes in the SERS spectra of **3** and **4** were observed when a LMg^{2+} complex formed in the solution, and it cannot be ascribed to either intermolecular interactions of the crown ether moieties of adsorbed molecules preventing complexation on the SERS-active surface or preferential adsorption of free molecules. The first conclusion is evident from the absence of any adsorption-induced intermolecular interactions disturbing complexation with Mg^{2+} for *trans*-**2** or even for **1** (the compound that may first of all be expected to show some stacking interactions of neighboring molecules). The second conclusion stems from the fact that the intensities of the SERS spectra recorded from the solutions of free *trans*-**3** and *trans*-**4** are comparable to those recorded for (*trans*-**3**)Mg^{2+} and (*trans*-**4**)Mg^{2+} complexes. The relative content of the free ligand is less than 1% in the solution of complexes, and therefore, a decrease in the SERS signal should be remarkable if (*trans*-**3**)-Mg^{2+} and (*trans*-**4**)Mg^{2+} complexes are poorly adsorbed on the SERS-active surface.

The structures of *trans*-**2** and *trans*-**4** differ only in the sulfonate group attached to the alkyl chain. The compound *trans*-**3** contains also a sulfoalkyl fragment. Therefore, it is reasonable to suggest that the presence of the sulfonate group would be responsible for the peculiarities observed in SERS titration both for *trans*-**3** and *trans*-**4**. We assume that displacement of a Mg^{2+} cation from the crown ether cavity toward the sulfonate anion occurs for the adsorbed molecules. Induced by the cation displacement, a rupture of coordination bonds between a Mg^{2+} cation and the O1, O13 oxygen atoms conjugated with the benzene ring (Figure 1) may lead to insensitivity of the SERS spectra to the presence of a cation

SERS of Crown Ether Dyes

J. Phys. Chem. B, Vol. 101, No. 20, 1997 **4083**

near the sulfonate group. These O1 and O13 atoms are directly coupled with the Ph$-$N$^+$$=C-C=C-$Ph conjugated π-electron system in which the long-wavelength electronic transition is localized for the *trans* isomer of compounds **2**$-$**4**. An appearance of characteristic changes in ν_{8b}, ν_{8a} as well as some other vibrational modes of the benzene ring of benzo-15-crown-5 moiety of the dyes (Table 1) directly depends on whether these two oxygen atoms are involved in Mg^{2+} binding or not. The vibrations of the sulfoalkyl fragment being out of resonance are scarcely enhanced and cannot be used to detect interactions with Mg^{2+} cations in SERS spectra.

The increase of the Mg^{2+}/L molar ratio in a solution of *trans*-**3** and *trans*-**4** promotes the complexation in accordance with eq 2. The molecule's sulfonate group associating with the second Mg^{2+} cation prevents the Mg^{2+} cation located within a crown ether cavity from displacement toward the sulfonate group upon adsorption of the molecules on the SERS-active surface. Therefore, an increase in the R ratio in the SERS spectra of **3** and **4** is concerned with the accumulation of (*trans*-**3**)(Mg^{2+})$_2$ and (*trans*-**4**)(Mg^{2+})$_2$ complexes as a function of Mg^{2+}/L molar ratio in the solution. Accordingly, the SERS titration of *trans*-**3** and *trans*-**4** with Mg^{2+} cations enables the formation of L(Mg^{2+})$_2$ complexes from LMg^{2+} ones to be followed.

Since the L(Mg^{2+})$_2$ complexes are formed at Mg^{2+}/L molar ratios such that the formation of LMg^{2+} complexes is already completed, eq 1 can be omitted. Then the complexation process detectable in the SERS spectra of **4** can be described by eq 2 (Figure 5b). It is valid also for **3** at a low concentration of the ligand, when the amount of dimers [(*trans*-**3**)Mg^{2+}]$_2$ is negligible.

In this approximation a relative amount γ of the L(Mg^{2+})$_2$ complexes in the solution can be calculated using the general scheme described above to determine α. Accordingly, γ can be calculated as

$$\gamma = (1 + \theta_2 R_{2a}(R_{2c} - R_2)/(R_2 - R_{1c}))^{-1} \qquad (12)$$

where θ_2 is the B_{2c}/B_{1c} ratio of the adsorption constant of a L(Mg^{2+})$_2$ complex to that of a LMg^{2+} complex and R_{2a} is the intensity ratio of the ν_{8a} band of a L(Mg^{2+})$_2$ complex to the ν_{8a} band of a LMg^{2+} complex. The R_2 ratio being equal to the value of $R_{2c} = 0.5$ for the LMg^{2+} complexes of compounds **3** and **4** increases up to $R_{2c} = 1.7$ when L(Mg^{2+})$_2$ complexes are formed. The K_2 equilibrium constant was estimated from

$$K_2 = \frac{\gamma}{(1 - \gamma)([\mathrm{M}] - (1 + \gamma)[\mathrm{L}])} \qquad (13)$$

by fitting the set of γ values (determined as a function of the Mg^{2+}/L molar ratio) by the least-squares procedure. Here, [M] and [L] are the concentrations of metal cations and the ligand in solution, respectively. The K_2 equilibrium constants measured by the SERS titration of *trans*-**3** and *trans*-**4** with Mg^{2+} cations are shown in Table 2.

The K_2 constant of *trans*-**4** was found to be independent of the concentration of the ligand over the range $10^{-5}$$-$$10^{-7}$ M. In contrast, a change in the K_2 constant was observed for *trans*-**3** as a function of ligand concentration (data not shown) and assigned to the concentration-dependent accumulation of [(*trans*-**3**)(Mg^{2+})]$_2$ dimers (Figure 1) affecting the apparent value of K_2. It is currently under investigation with the aim to estimate a K_d constant from this dependence.

Conclusion

The SERS titration procedure was elaborated for measuring the equilibrium constants of complex formation between the CESDs and metal cations in solution. The four main factors provide applicability of the procedure for measuring these constants.

(1) The first factor is the absence of photoinduced *trans*$-$*cis* isomerization of the adsorbed molecules and their complexes. The stabilization of the *trans* isomers seems to occur due to the fast nonradiative energy transfer from the excited state of a *trans* isomer to the metal surface, preventing in this way a *trans*$-$*cis* isomerization on the surface.

(2) Adsorption of the CESDs and their complexes with Mg^{2+} on the SERS-active surface can be characterized by the Langmuir adsorption theory. The amount and type of adsorption sites and the geometry of adsorption are the same both for free molecules and for their complexes with metal cations.

(3) The third factor is a slow kinetics of dissociation of the adsorbed molecules and complexes. As a result, changes in the relative amount of the complexes and/or dimers in the bulk solution appeared for some dyes due to photoinduced changes of *trans*$-$*cis* equilibrium that do not affect the initial state of the adsorbed molecules. The "memory" of the adsorbed molecules makes the SERS spectrum nearly independent of the current state of the bulk solution for the time to measure a spectrum.

(4) High sensitivity of the SERS technique allows the dye spectra to be measured in a wide range of concentrations ($10^{-4}$$-$$10^{-7}$ M) and, thereby, is beneficial in correcting SERS titration data for adsorption constants of the free molecules and complexes.

A comparison of the results obtained by SERS and UV$-$vis titration for **1** and **2**$-$**4** revealed that the SERS-active surface does not disturb the formation of **1**Mg^{2+} and (*trans*-**2**)Mg^{2+} complexes and that corresponding equilibrium constants can be evaluated by means of the SERS technique. The value of the apparent equilibrium constant for (*trans*-**2**)Mg^{2+} complex formation was found to be independent of the concentration of the ligand in the $10^{-5}$$-$$10^{-7}$ M range, clearly indicating the absence of self-aggregation of the molecules and their complexes in solution, being adsorbed on the silver surface of a SERS-active substrate. The presence of the sulfoalkyl N substituent in **3** and **4** affects the structure of the complexes for the molecules adsorbed on the surface compared to the molecules in the bulk. A displacement of a Mg^{2+} cation from the crown ether cavity toward the sulfonate anion occurs for the adsorbed molecules. An association of a Mg^{2+} cation with a sulfonate group can be concluded as more favorable than binding with the crown ether moiety for the adsorbed ionophores. A preferential complexation of Mg^{2+} with the sulfonate group does not preclude adsorption, and the persistence of (*trans*-**3**)(Mg^{2+})$_2$ and (*trans*-**4**)(Mg^{2+})$_2$ complexes on the surface at an increased metal/ligand molar ratio leads to the formation of such complexes in the solution. The displacement of a cation from the crown ether cavity causes SERS spectroscopy to be insensitive to the formation of the (*trans*-**3**)Mg^{2+} and (*trans*-**4**)Mg^{2+} complexes in solution. However, SERS spectroscopy is beneficial for the study of (*trans*-**3**)(Mg^{2+})$_2$ and (*trans*-**4**)-(Mg^{2+})$_2$ complex formation and for measurement of the corresponding K_2 equilibrium constants. The K_2 constants are not accessible via spectrophotometric titration. The measurements using the SERS technique resulted in the dependence of the value of the apparent equilibrium constant of (*trans*-**3**)-(Mg^{2+})$_2$ complex formation on the concentration of the ligand, whereas K_2 for (*trans*-**4**)(Mg^{2+})$_2$ complex was not changed in the $10^{-5}$$-$$10^{-7}$ M range of the dye concentration. The dependence observed is suggested to be related to the existence of the [(*trans*-**3**)Mg^{2+}]$_2$ dimers in solution, which affect the

(*trans-***3**)(Mg^{2+})$_2$ complex formation at high concentrations of the dye. The data obtained confirms a previous conclusion[13,14] that the self-aggregation ability of the crown ether styryl dyes is strongly geared to the length of the sulfoalkyl N substituent. The (*trans-***4**)Mg^{2+} complexes do not form dimers either in solution or from being adsorbed on the SERS-active surface.

Author-Supplied Registry Numbers: 2,3,5,6,8,9,11,12-Octahydro-1,4, 7,10,13-benzopentaoxacyclopentadecin, 69271-98-3; Ag, 7440-22-4.

Abbreviations

SERS = surface-enhanced Raman scattering

1 = 2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin

2 = perchlorate 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,-13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-ethylbenzothiazolium

3 = betaine 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-16-yl)ethenyl]-3-(3-sulfopropyl) benzothiazolium

4 = betaine 2-[2-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclo-pentadecin-16-yl)ethenyl]-3-(3-sulfoethyl) benzothiazolium.

References and Notes

(1) Dix, J. P.; Vogtle, F. *Chem. Ber.* **1980**, *113*, 457.

(2) Dix, J. P.; Vogtle, F. *Chem. Ber.* **1981**, *114*, 638.

(3) Lohr, H. G.; Vogtle, F. *Acc. Chem. Res* **1985**, *18*, 65.

(4) Shinkai, S. In *Cation Binding by Mycrocycles: Complexation of Cationic Species by Crown Ethers*; Inoue, Y., Gokel, G. W., Eds.; Marcel Dekker: New York, 1990; Chapter 9.

(5) Shinkai, S.; Nakamura, S.; Nakashima, M.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2340.

(6) Gromov, S. P.; Fomina, M. V.; Ushakov, E. N.; Lednev, I. K.; Alfimov, M. V. *Dokl. Akad. Nauk SSSR* **1990**, *314*, 1135−1139.

(7) Alfimov, M. V.; Lednev, I. K.; Gromov, S. P. *Chem. Phys. Lett.* **1991**, *185*, 455−458.

(8) Gromov, S. P.; Fedorova, O. A.; Alfimov, M. V.; Tkachev, V. V.; Atovmyan, L. R. *Dokl. Akad. Nauk SSSR* **1991**, *319*, 1141.

(9) Alfimov, M. V.; Buevich, O. E.; Gromov, S. P.; Ushakov, E. N.; Lifanov, A. P.; Fedorova, O. A. *Dokl. Akad. Nauk SSSR* **1991**, *319*, 1149−1158.

(10) Gromov, S. P.; Fedorova, O. A.; Ushakov, E. N.; Stanislavsky, O. B.; Alfimov, M. V. *Dokl. Akad. Nauk SSSR* **1991**, *321*, 104.

(11) Barzykin, A. V.; Fox, M. A.; Ushakov, E. N.; Stanislavsky, O. B.; Gromov, S. P.; Fedorova, O. A.; Alfimov, M. V. *J. Am. Chem. Soc.* **1992**, *114*, 6381−6385.

(12) Shinkai, S.; Manabe, O. *Host Guest Complex Chemistry;* Vogtle, F., Weber, E., Eds.; Springer-Verlag: Berlin, 1984; Vol. III, p 67.

(13) Ushakov E. N. Ph.D. Thesis, Institute of Chemical Physics, Chernogolovka, Russia, 1995.

(14) Stanislavsky, O. B.; Ushakov, E. N.; Gromov, S. P.; Fedorova, O. A.; Alfimov, M. V. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1996**, *45*, 605−613.

(15) Gromov, S. P.; Fedorova, O. A.; Ushakov, E. N.; Buyevich, O. E.; Alfimov, M. V. *Russ. Chem. Bull.* **1995**, *44*, 2131−2136.

(16) Feofanov, A.; Ianoul, A.; Oleinikov, V.; Gromov, S.; Fedorova, O.; Alfimov, M.; Nabiev, I. *J. Phys. Chem.,* **1996**, *100*, 2154−2160.

(17) Feofanov, A.; Ianoul, A.; Oleinikov, V.; Nabiev, I.; Gromov, S.; Fedorova, O.; Alfimov, M. *Russ. Chem. Bull.* **1995**, *44*, 2323−2330.

(18) Maskevich, S.; Sveklo, I.; Feofanov, A.; Ianoul, A.; Oleinikov, V.; Fedorova, O.; Gromov, S.; Alfimov, M.; Nabiev, I.; Kivach, L. *Opt. Spektrosk.* **1996**, *81*, 95−102.

(19) Kryukov, E.; Feofanov, A.; Oleinikov, V.; Vereschetin, V.; Zaitsev, S.; Gromov, S.; Fedorova, O.; Maskevich, S.; Kivach, L.; Zubov, V. P.; Nabiev, I.; Alfimov, M. *Russ. Chem. Bull.,* in press.

(20) Nabiev, I. R.; Efremov, R. G.; Chumanov, G. D. *Sov. Phys. Usp.* **1988**, *31*, 241−262.

(21) Feofanov, A.; Sharonov, S.; Valisa, P.; Da Silva, E.; Nabiev, I.; Manfait, M. *Rev. Sci. Instrum.* **1995**, *66*, 3146−3158.

(22) Lednev, I. K.; Gromov, S. P.; Ushakov, E. N.; Alfimov, M. V.; Moore, J. N.; Hester, R. E. *Spectrochim. Acta* **1992**, *48a* , 799−809.

(23) Varsanyi, G. *Assignments for Vibrational Spectra of Seven Hundred Benzene Derivatives*; Akademiai Kiado: Budapest, 1974; Vol.1.

(24) Feofanov, A.; Ianoul, A.; Kryukov, E.; Oleinikov, V.; Zaitsev, S.; Gromov, S.; Fedorova, O.; Alfimov, M.; Nabiev, I. *Metal Ions in Biology and Medicine;* Collery, Ph., Domingo, J. L., Etienne, J. C., Llobet, J. M., Eds.; John Libbey Eurotext: Paris, 1996; Vol. IV, pp 6−8.

(25) Hester, R. E. Personal communications.