

Demonstration of structure-selective binding of organic cations to anionic polyelectrolytes

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Abstract

The binding of diprotonated *trans*-4,4'-diaminostilbene (DASH₂²⁺) to poly (styrenesulfonate) (PSS) and poly(vinylsulfonate) (PVS) in aqueous solution have been studied by following the changes in the absorption, fluorescence and polarization emission spectra of the complexed chromophors. It was found that in the DASH₂²⁺–PSS complex most of the DASH₂²⁺ molecules are bound in their *trans*-isomer form, while in the DASH₂²⁺–PVS complex, which is less stable and lack π – π interactions, the DASH₂²⁺ molecules are bound in the electrostatically preferred *cis* conformation.

The higher stability of the DASH₂²⁺–PSS complex is due to structural fitting between *trans*-DASH₂²⁺ and *p*-styrene sulfonic units. This structural fitting allows formation of π – π bonds between aromatic moieties in combination with electrostatic attraction between the protonated amine and sulfonate charged groups. Our findings demonstrate the delicate interplay between electrostatic and non-polar interactions which controls the binding of relatively large molecules to high-molecular weight receptors, and may be used to model a binding site in biological systems. These findings are corroborated by molecular mechanics simulation of the complexes. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: UV and Fluorescence spectra; Molecular mechanics simulation; Complex structure; *Trans*-4,4'-diaminostilbene (DASH₂²⁺); 1,5-Diaminonaphthalene (DANH₂²⁺); Polyelectrolytes; Energy transfer; Excimer; Monomer

1. Introduction

Polyelectrolytes are polymers derived from monomers which consist of an ionizable group covalently linked to a polymerizable hydrophobic moiety. For instance, *p*-styrene sulfonic acid upon polymerization forms polystyrene sulfonic acid which consists of a hydrophobic poly-phenyl ethane chain and ionizable sulfonic acid groups. In water the sulfonic groups are dissociated and form a polyvalent macroion surrounded by a large number of counterions. The high charge density of the macroion produces a strong electric field which induces strong long range interactions. While considerable effort has been expended in understanding the nature of the Coulombic interactions between polyions and other charged species in the solution [1–6] somewhat less attention has been paid to the hydrophobic interactions. Spectroscopic studies have shown that interactions between polyelectrolytes and charged organic probe

molecules do not depend solely on electrostatic interactions, and that the hydrophobicities of the polyelectrolyte and of the probe moiety play an important role. It was suggested that enhancement of excimer formation is the results of co-operative hydrophobic interactions between the probes and the macroion [7]. In addition, it was shown that the conformation of some specific flexible charged probes may be controlled by the hydrophobic interaction of their carbonic chain with the macroion [8,9]. Likewise it was shown that the interaction between ruthenium-tris (bipyridine) and oppositely charged macroion is both electrostatic and, hydrophobic through the formation of hydrophobic domains [10,11]. Measurements by absorption and fluorescence spectroscopy of several polyelectrolytes bound to various oppositely charged probes have demonstrated incorporation of these probes into the polyion domain and the dependence of the binding constant on the length of the aliphatic side chain [12]. The existence of several types of interactions between macroions and small molecules in addition to structural constraints may define specific domains on the polymer to which the probe molecule is attracted and bind.

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Interaction between well defined domains on the macroion with small molecules is an important problem in biology and medicine [13,14], as for example, the development of specific blockers to catalytic or regulatory units or domains on the enzyme. In order to study small molecules binding to polyelectrolytes which involves both electrostatic and hydrophobic π – π interactions, we have investigated the binding of doubly-charged aromatic amino derivatives to aromatic and non-aromatic anionic polymers. *Trans*-4,4'-diaminostilbene (DAS) and 1,5-diaminonaphthalene (DAN) have been used as probe molecules. The DAS molecule has several advantages for investigating the binding phenomena. The diprotonated form of the probe (DASH_2^{2+}) has two positive charges located on the two ends of the molecule. The DASH_2^{2+} structure backbone was found by computer simulation to correlate well with the segment structure of PSS. This results in a strong binding between the probe molecule and the polyion and, to a formation of a stable complex. DAS possess high sensitivity to changes in the properties of the surrounding environment due to its *trans-cis* photoisomerization. The spectroscopic behavior of DAS is thus sensitive to its binding state (bound or free). We have used UV and fluorescence spectroscopy to monitor the association between these cationic probes and polyions in aqueous solutions. In addition, likely probe-polyelectrolyte complex structures were generated by molecular mechanics simulation methods (vacuum conditions). We have found these simulation helpful in visualizing the geometric aspects and in exploring the nature of the interaction between the positively charged probes and the anionic polymers.

2. Material and methods

2.1. Materials

The polyelectrolytes used in our experiments were fully sulfonated sodium hydroxide neutralized poly(styrenesulfonate) (Pressure Chemical Co MW177,000 kD.) and poly(vinylsulfonate) (Aldrich, 25 W% water solution, $d=1.276$ and $n_D^{20}=1.3890$). The stock polyion solution was purified by using exchange resin (BIO RAD, AG501-X8 mixed bed resin) in order to remove the inorganic electrolyte and ionizable organic impurity from the polyion solution. The *trans*-4,4'-diaminostilbene (dihydrochloride salt, 95% Aldrich) was in its uncharged form and was further purified by recrystallization. The 1,5-diaminonaphthalene (97%, Aldrich) was used without further purification. DAS and DAN were dissolved in 2×10^{-3} M HCl solution to a concentration of 2.5×10^{-4} M (stock solution). Acetate buffer at 10 mM concentration was used to reach the final conditions, namely pH=5 and $\mu=0.01$, where μ is the ionic strength. Other chemical reagents were of analytical grade.

Doubly distilled water was further filtered through a Milipore cleaning device (pore size 220 nm) until 18 M Ω re-

sistivity was reached. In the experiment, the probe concentration was maintained constant at 1.0×10^{-5} M. When the macroion concentration was varied, the ratio between the polyelectrolyte concentration in monomeric units and the probe concentration P/D was used to describe the conditions of the experiment. $P/D=100$ was taken as a state in which the probes are fully bound to polyions.

2.2. Methods

UV absorption spectra were recorded on a Hewlett-Packard UV b4527 diode array spectrophotometer. A 1 cm quartz cuvette was used. Emission spectra were measured on a SLM 4800 spectrophotometer. The molecular mechanics simulations were performed by the Insight and Discover packages of BIOSYM Technology, INC., San Diego on a Silicon Graphics workstation. The intramolecular and intermolecular energies were calculated from the force field, which involves terms of stretching, bending, torsion, out of plane, van der Waals and electrostatic interactions. The co-ordinates of the favored structure of the PSS segment consisting of eight monomer units and the probes were used as the starting points for energy minimization of the complex. The energy minimized conformations of the probe molecules were docked to the optimal conformations of the different stereomers of the polymer. All the earlier mention calculations were restricted to vacuum conditions and no solvation and entropic effects were considered.

2.2.1. The segment structure of PSS

In order to elucidate the possible modes of interaction between the organic ion and the charged macromolecule, preliminary investigation was required to establish the segment structure along the polyelectrolyte chain. This study was performed by using the cylindrical model of a single polyelectrolyte chain. Although the organic ions are much bigger than metal ions, they are still very small in comparison to the length of the polymer chain. Thus it was reasonable to assume that tight binding of organic ions to polyions is mainly related to relatively short segment structure of the polyelectrolyte of several monomeric units length rather than to a large scale chain configurations [11]. In this study, eight monomers of PSS were used to generate the segment structure of PSS. The backbone configuration of PSS is defined as S–R–S–R. Five segment structures of PSS are presented in Fig. 1. The results of minimization show that the total potential energy in group(I) which contains the structures $g(-)g(+)$ and $g(+)g(-)$ is higher than that in group(II) containing $g(+)g(+)$, $g(+)t$ and $tg(+)$. The difference is larger than 20 kcal/mol. The energy difference between the structures in group(II) is less than 3 kcal/mol. Among these five segment structures of PSS, the $g(-)g(+)$ and $g(+)g(-)$ are not suitable for binding of either DASH_2^{2+} or DANH_2^{2+} . Binding the probe to the $g(+)t$ and $tg(+)$ induces the configuration to changes to $g(+)g(+)$. This leads us to

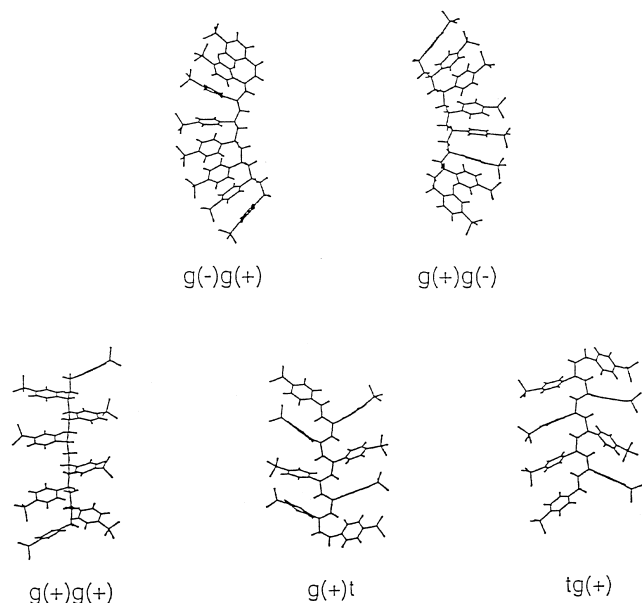


Fig. 1. Five possible fragment structure of PSS. g and t are *gauche* and *trans* orientation of the torsion angle of polymer backbone chain.

conclude that g(+g(+)) is the most suitable structure for binding protonated DAS and DAN molecules. Thus we used the g(+g(+)) segment structure to study the organic ion/polyion complex.

3. Results

3.1. Binding DASH_2^{2+} to PSS and PVS at pH=5

The pronounced differences in the effect of binding of DASH_2^{2+} to PSS as compared to the binding of DASH_2^{2+} to PVS are presented in Figs. 2 and 3. At pH 5 (in 10 mM NaAc buffer solution), the solution of DAS contains a mixture of

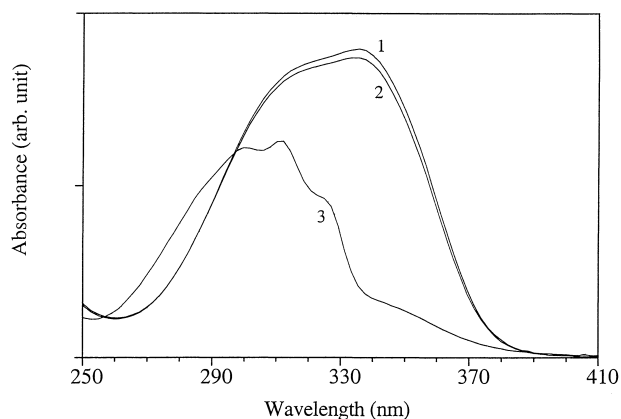


Fig. 2. Absorption spectra of DAS in water at pH=5 in the absence and presence of polyions. Curve 1, $P=0$ in water; curve 2, with PVS ($P/D=100$); curve 3, with PSS ($P/D=100$). P and D are the polymer and DAS concentrations, respectively.

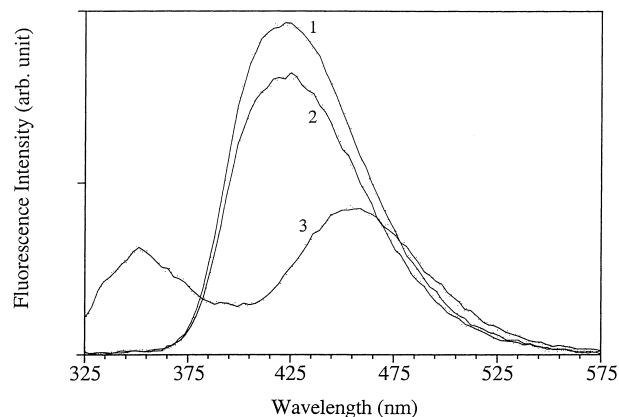
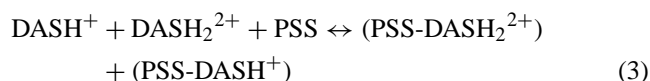
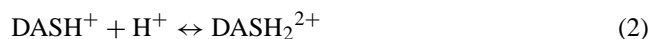


Fig. 3. Emission spectra of DAS in water at pH=5 in the absence and presence of polyions. Curve 1, $P=0$ in water; curve 2, with PVS ($P/D=100$); curve 3, with PSS ($P/D=100$). P and D are the polymer and DAS concentrations, respectively.

its three acid–base forms as follows: 4% DASH_2^{2+} , 55% DASH^+ and 41% DAS ($pK_{a1}=3.87$, $pK_{a2}=5.12$) [15]. The presence of PVS did not lead to changes in the absorption spectra of DAS component mixture (Fig. 2), so that the relative population of the three species was not altered by the presence of PVS. Under the same conditions, the fraction of the diprotonated form of DAS was considerably enhanced in presence of PSS, due to a significant change in the acid–base equilibrium of DAS towards DASH_2^{2+} following its binding to PSS (Figs. 2 and 3).

In the presence of PSS two new fluorescence bands appeared. The one centered at 350 nm belongs to DASH_2^{2+} and the one centered at 460 nm belongs to DASH^+ . These observations show that the dication form of DAS is stabilized by its interaction with PSS and that the spectra of the bound DASH_2^{2+} is that of the *trans*-isomer. The data in Fig. 3 also show that the wavelengths of the emission maxima of this mixture in PVS remains the same as it is in pure water solution, except for a 20% decrease in intensity which is due to the environment of the polyelectrolyte. Under the same conditions, the fraction of the diprotonated species of DAS is greatly enhanced by the presence of PSS as indicated by the new emission band at 350 nm. The large shifts in the first and the second protonation constants are the result of the consecutive equilibrium processes induced by the presence of PSS, Eqs. (1) and (3):



The apparent pK_a shift of DASH_2^{2+} was 2.5 units toward more basic values at ionic strength of 10^{-3} M. The shift was reduced to 1.2 unit at 10^{-2} M.

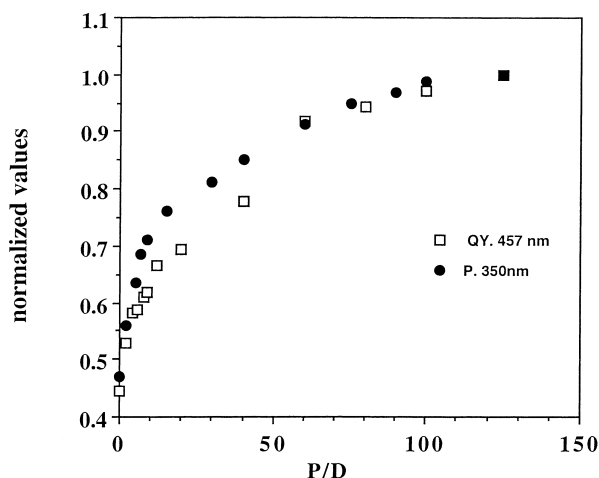


Fig. 4. The normalized values of quantum yield at 457 nm and polarization fluorescence at 350 nm of DASH_2^{2+} (pH=2) vs. the P/D ratio. P and D are the polymer and DAS concentrations, respectively.

3.2. Binding DASH_2^{2+} to PSS and PVS at pH=2

At pH=2 the DAS molecule is fully diprotonated in the solution. Fig. 4 shows the normalized values of polarization and quantum yield of trans-DASH_2^{2+} as a function of the P/D (the ratio between the monomeric concentration of the polymer P and the concentration of the probe D , where the value of D was 1×10^{-5} mol/l). A typical binding isotherm of DASH_2^{2+} to PSS was found following the quantum yield (QY) and the fluorescence polarization changes (P) at 350 nm as a function of P/D . At saturation concentrations of P/D the quantum yield and polarization of DASH_2^{2+} increase by as much as 2.7-fold. The large effect of the binding on the chromophore fluorescence allowed us to follow the dependence of the binding process versus ionic strength. Polystyrene sulfonic acid is a moderately strong acid and almost fully dissociated in aqueous solution [19]. Therefore, the changes of the emission spectra of bound DASH_2^{2+} induced by increasing the proton concentration (additional HCl) can be simply attributed to the change in the ionic strength of the solution. For solution of lower ionic strength such as 0.001 mol/l the quantum yield and polarization were much more affected by binding, by as much as a factor of 5. The ionic strength effect on the binding as was found by our group and by others [3,4], shows that the ionic strength acts to decrease the bound fraction of the probe molecules. We have found that at a ionic concentration of 0.04 M HCl DANH_2^{2+} became totally free of the polymer chain, while DASH_2^{2+} became free at higher ionic concentrations (0.2 M). This difference shows that the DASH_2^{2+} -PSS complex is more stable than the DANH_2^{2+} -PSS complex. Fig. 5 shows the long-wavelength absorption band of DASH_2^{2+} in the absence and in the presence of PSS in 10 mM HCl water solution. The band of free DASH_2^{2+} is characterized by three distinguishable features at wavelength of 296, 308 and 322 nm. This struc-

ture is similar to that of the unsubstituted *trans*-stilbene and is mainly due to the $^1A \rightarrow ^1B$ transition of p -electrons [16]. Increasing the ratio (P/D) between the monomeric concentration of the polymer (P) and the concentration of DAS (D) have led to changes in the absorption spectra. The absorption spectra of free and bound DANH_2^{2+} to PSS are presented in the insert of Fig. 5. The absorption band of free DANH_2^{2+} in the range of 250–300 nm is also characterized by three features at wavelength of 270, 278, 288 nm. This spectrum resembles that of naphthalene and is due to the $^1A_g \rightarrow ^1L_a$ transition [17]. The experimental results show the differences in absorption spectra between free ($P/D=0$) and bound ($P/D=100$) DASH_2^{2+} and DANH_2^{2+} . The 2 nm red shift and 25% reduction in the absorbance are the two common features of both bound diprotonated probes. Similar changes in the absorption spectra can also be found in the binding of (arylmethyl) ammonium cations by PSS [7,8].

The two spectral features of the complexed probes mentioned earlier can serve as an additional indication for the association between our probes and polyions. Indeed it is well documented that small (2–3 nm) reproducible spectral shifts are characteristic of host–guest complexes of aromatic molecules. In these cases dynamic and kinetic factors such as the rotational correlation time, the isomerization and the proton transfer rate of the chromophore molecule are much more effected by coplexation than the steady state spectral properties [18].

The absorption spectra in Fig. 5 also provides information on the configuration of the bound DASH_2^{2+} . The absorption spectra of *cis*-stilbene has no fine vibrational structure and its spectra appear at a considerably shorter wavelength as compared to that of the *trans*-isomer [20]. The *trans-DASH}_2^{2+} has the same spectral features as *trans*-stilbene due to the diminished effect of the two lone-pairs of electrons on the amino-groups. The fine vibrational structure of DASH_2^{2+} is*

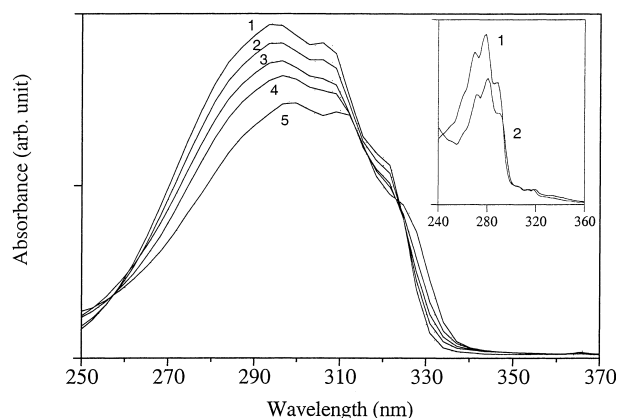


Fig. 5. The influence of PSS on the absorption spectra of DASH_2^{2+} in 0.01 M HCl. The ratio of P/D for each curve are as follows: curve 1, $P/D=0$; curve 2, $P/D=2$; curve 3, $P/D=5$; curve 4, $P/D=10$; curve 5, $P/D=100$. The plot in the insert of Fig. 5 is the absorption spectra of DANH_2^{2+} . The ratio of P/D for each curve are as follows: curve 1, $P/D=0$ in 0.1 M HCl for fully protonation of DAN; curve 2, $P/D=100$ in 0.01 M HCl. P and D are the polymer and DAS concentrations, respectively.

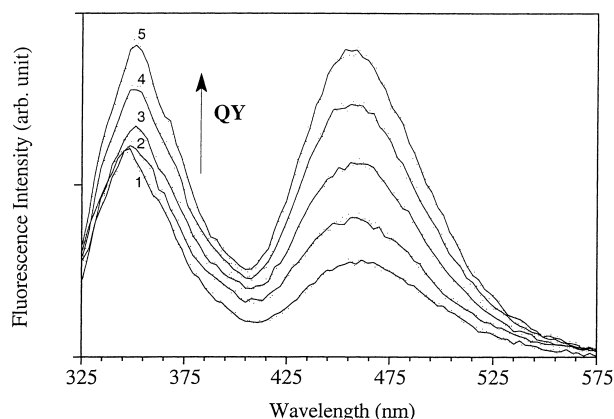


Fig. 6. The influence of PSS on the emission spectra of DASH_2^{2+} in 0.01 M HCl. Excitation wavelength, 310 nm; curve 1, $P/D=0$; curve 2, $P/D=2$; curve 3, $P/D=5$; curve 4, $P/D=10$; curve 5, $P/D=100$. P and D are the polymer and DAS concentrations, respectively. The intensity ratios (I_{460}/I_{350}) are summarized in Table 3.

retained when it is in its bound state (Fig. 5). This suggests that DASH_2^{2+} at pH=2 keeps the *trans*-isomer configuration in its PSS complex.

The fluorescence emission spectra of DASH_2^{2+} in the absence and in the presence of PSS in the solution are shown in Fig. 6. The emission bands between 330–390 nm and 400–550 nm correspond to the emission from DASH_2^{2+} and DASH^+ , respectively. The existence of the mono protonated band of DAS, DASH^+ at pH 2 is due to the excited state proton transfer reaction of DASH_2^{2+} which is a strong photoacid and dissociates in the excited state ($pK_a^* \approx -2$ according to our excited-state titration experiment, not shown). The fluorescence intensity is increased by a factor of 2.5 upon addition of the PSS owing to an enhancement in the fluorescence quantum yield. This suggests that the rate of the *trans* to *cis* photoisomerization, the major non radiative decay process of DASH_2^{2+} and DASH^+ in the excited state [15,21], is reduced by the association of the probe with the hydrophobic region of PSS.

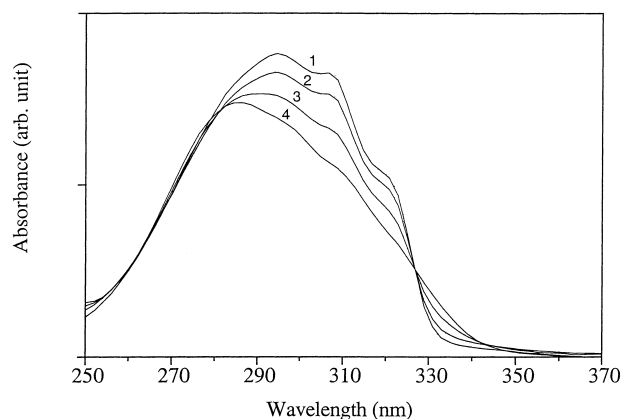


Fig. 7. The influence of PVS on the absorption spectra of DASH_2^{2+} in 0.01 M HCl. The ratio of P/D for each curve are as follows; curve 1, $P/D=0$; curve 2, $P/D=20$; curve 3, $P/D=40$; curve 4, $P/D=100$. P and D are the polymer and DAS concentration, respectively.

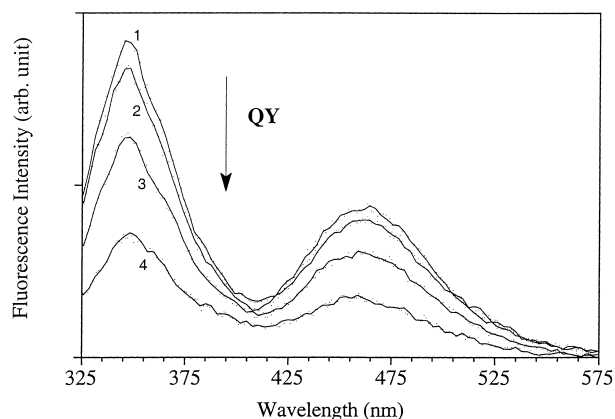


Fig. 8. The influence of added PVS on the emission spectra of DASH_2^{2+} in 0.01 M HCl. Excitation wavelength, 310 nm; curve 1, $P/D=0$; curve 2, $P/D=20$; curve 3, $P/D=40$; curve 4, $P/D=100$; curve 5, $P/D=100$. P and D are the polymer and DAS concentrations, respectively. The intensity ratios (I_{460}/I_{350}) are summarized in Table 3.

The effect of binding of DASH_2^{2+} to PVS on the absorption and fluorescence spectra of DASH_2^{2+} are presented in Figs. 7 and 8, respectively. When PVS is added to the solution spectra of DASH_2^{2+} shifts towards the *cis*-isomer (short wavelength side). (Fig. 7) while the quantum yield of DASH_2^{2+} is reduced considerably indicating the formation of the none fluorescence *cis*-isomer (Fig. 8).

4. Discussion

The interaction of 4,4'-diaminostilbene (DAS) with negatively charged polyions affects the ground state protonation equilibrium of the two amine residues of DAS, by stabilizing the diprotonated DASH_2^{2+} form (Figs. 2 and 3), thus making the complexed DAS a stronger base (and equivalently making DASH_2^{2+} a weaker acid). At pH=2 DAS is fully diprotonated in solution, so only the interaction of the DASH_2^{2+} form is needed to be considered. The DASH_2^{2+} molecule has two stable *cis*- and *trans*-isomers. Similarly to its parent stilbene molecule, the *trans*-isomer is preferred over the *cis*-isomer in solution. Two processes affect the dynamic aspects of DASH_2^{2+} in solution. The first is *trans* to *cis* (and *cis* to *trans*) conversion and the second is the acid base equilibrium $\text{DASH}_2^{2+} \leftrightarrow \text{DASH}^+ + \text{H}^+$. Both processes were found in this study to be affected by complexation with negatively charged polyions. Another issue of this study was to explore the interplay between the electrostatic interaction and the hydrophobic interactions and their combined and separated effect on the binding between the chromophore and the polyions. We have found out that the strong binding affinity of DASH_2^{2+} to PSS is due to the hydrophobic interaction caused by the aromatic rings which act in co-operation with the short range electrostatic attraction. In contrast with PSS, lack of hydrophobic π - π interaction causes organic ions to bind to PVS mainly by

Table 1

Calculated energy parameters (kcal/mol) of the complexes between doubly charged probes and PSS

Energies	<i>trans</i> -DASH ₂ ²⁺ -PSS	<i>cis</i> -DASH ₂ ²⁺ -PSS	DANH ₂ ²⁺ -PSS
Total system energy	608.4	626.8	648.3
Stretch bond energy	107.4	107.9	113.2
Bend angle energy	48.9	61.3	56.9
Torsion energy	108.7	112.4	109.4
Out of plane energy	0.5	0.3	0.4
van der Waals energy	284.2	280.5	292.4
Electrostatic energy	58.7	64.4	76.0

electrostatic interactions which are weaker than the combined hydrophobic–hydrophilic interactions in the DAS/PSS complex. The partial disappearance of the fine vibrational structure of PVS-bound DASH₂²⁺ (Fig. 7) shows that the binding of DASH₂²⁺ to PVS is mainly in the *cis* configuration and is less favored than the *trans*-DASH₂²⁺-PSS complex. The gradual decrease in the fluorescence intensity due to DASH₂²⁺-PVS binding as shown in Fig. 8 (the opposite behavior was found in the DASH₂²⁺ PSS complex, see Fig. 6) also indicates that the dark *cis*-isomer is formed upon binding to PVS so that the residual fluorescence originates from the unbound *trans*-isomer. In agreement with this observation, no spectral shift in the emission band was found in these experiments indicating that the observed fluorescence originates from the residual unbound *trans*-isomer form. These findings are corroborated by the molecular mechanics simulation of the various DASH₂²⁺ complexes (*cis*- and *trans*-DASH₂²⁺ complexed with PVS and PSS). Tables 1 and 2 and Figs. 9 and 10 show the results of our calculations done in vacuum. In case of the coplexation with PSS, the *trans*-isomer was found to be preferred over the *cis*-isomer by 18 kcal/mol (Table 1) and in the case of the DASH₂²⁺-PVS complex the *cis*-isomer was found to be preferred over the *trans*-isomer by 39 kcal/mol. Figs. 9 and 10 clearly demonstrate the polyelectrolyte selection of the DASH₂²⁺ isomer. Fig. 9 depicts the energy minimized structures of *trans*-DASH₂²⁺-PSS. Due to the flexibility of the polymer chain, the relevant segments of PSS arrange their configuration so to fit the structure of the organic ions. The structure in Fig. 9 suggests a remarkable structure fit between the *trans*-DASH₂²⁺ and the segment arrangement of g(+)g(+) configuration of PSS, the elec-

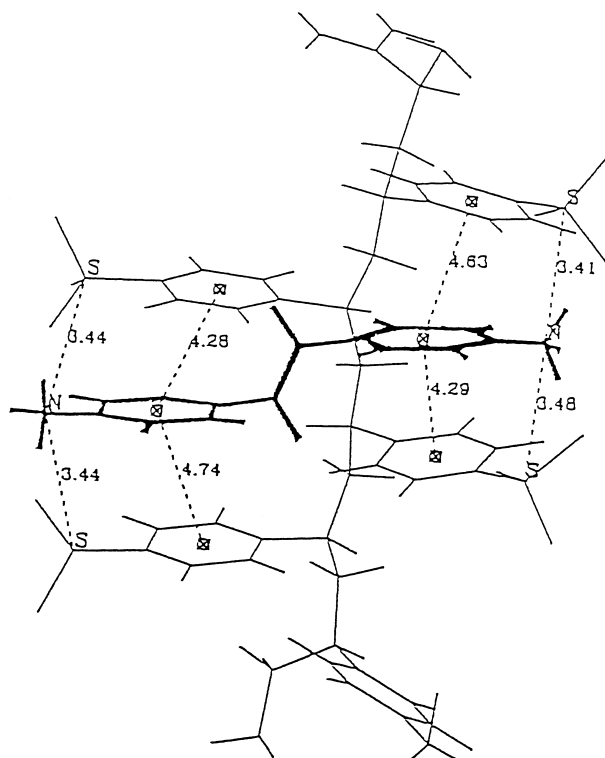
trostatic and the π - π aromatic interactions are being almost optimized. Table 1 also include the energy minimization of the DANH₂²⁺-PSS complex. The comparison with *trans*-DASH₂²⁺ shows that the DANH₂²⁺ complex is less stable than the *trans*-DASH₂²⁺ complex in both the electrostatic and π - π interactions by 17 and 8 kcal/mol, respectively. This demonstrates again to the importance of a structural backbone fit between the host and guest molecule.

The computer generated DASH₂²⁺-PVS complex shown in Fig. 10 is for a complex formed with *cis*-DASH₂²⁺. The calculated energy parameters show that the *cis*-DASH₂²⁺-PVS complex has stronger electrostatic interactions than that of the *trans*-DASH₂²⁺-PVS complex due to the better fit between the location of the positive charges of the probe molecule and the position of the negatively charged sulfonate groups. The difference in the electrostatic interaction being about 29 kcal/mol. This suggests that the *cis*-isomer

Table 2

Calculated energy parameters (kcal/mol) of the complexes between doubly charged probes and PVS

Energies	<i>trans</i> -DASH ₂ ²⁺ -PVS	<i>cis</i> -DASH ₂ ²⁺ -PVS
Total system energy	694.1	655.0
Stretch bond energy	31.9	31.0
Bend angle energy	52.2	48.0
Torsion energy	62.6	51.7
Out of plane energy	62.1	62.1
van der Waals energy	215.9	221.3
Electrostatic energy	269.4	240.9

Fig. 9. Computer generated structure of *trans*-DASH₂²⁺-PSS complex in vacuum. The numbers are given in Angstroms.

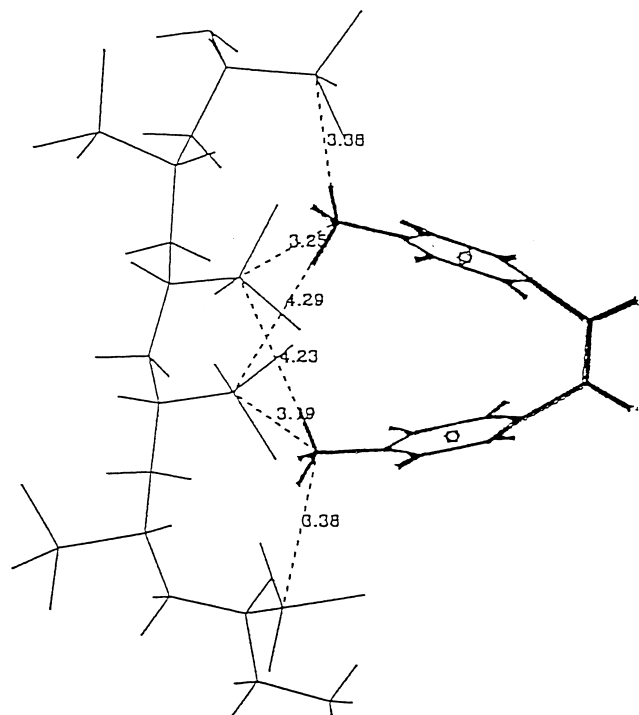


Fig. 10. Computer generated structure of the *cis*-DASH₂²⁺-PVS complex in vacuum. The numbers are given in Angstroms.

is stabilized mainly by Coulombic attraction (Table 3). The above vacuum calculation together with our ‘real-life’ solution measurements point to the major importance of a structural fit. In the case of the DASH₂²⁺-PSS complex there is an harmony between the location of the coulombic ‘hot spots’ which are at the two ends of the molecule and the general structural fit (i.e. in dimension and in contour) between the chromophore and the macroion. This situation reassemble the general description of a typical host-guest interactions such as encountered in many biological systems and as such may serve as a simple model for biological host-guest interactions. The *trans* configuration of DASH₂²⁺ in the complex is thus determined by both the location of the coulombic interactions and the general structural overlap within the host-guest pair. In comparison, PVS does not fit well with the structure of *trans*-DASH₂²⁺ and also lack π - π interactions. The best ‘solution’ for a stable complex in this case is to gain as much coulombic interac-

tion as possible. This is achieved by ‘forcing’ the molecule to complex in its less favorable *cis*-conformation thus bringing the two charged ends of DASH₂²⁺ closer together (Fig. 10). An additional aspect of the complexation reaction is its effect on the excited-state proton transfer reaction of DASH₂²⁺, i.e. DASH₂²⁺ \leftrightarrow DASH⁺ + H⁺. The fluorescence intensity of the monocation (at 460 nm) and the dication (at 350 nm) of DAS in PSS and PVS solutions and the observed emission are compared in Figs. 6 and 8. The ratio of the fluorescence intensity (I_{460}/I_{350}) represents the effect of the polyanions on the relative proportions of DASH₂²⁺ and DASH⁺ in the excited state due to the excited-state proton transfer reaction. The results collected in Table 3 show that the ratio is increased with increasing *P/D* in PSS solutions, while the presence of PVS does not affect this ratio. The change in the quantum yield of DASH₂²⁺ and DASH⁺ may be found directly from the change in the area of their respective fluorescence bands. In the case of the *trans*-DASH₂²⁺-PSS complex the total quantum yield increased with the *P/D* ratio by more than 2-fold (Fig. 6) while the quantum yield of the *trans*-DASH₂²⁺-PVS complex decreased by almost 3-fold. In combination with the ratio of the fluorescence intensity data (I_{460}/I_{350}) these observation point out, once more, that the DASH₂²⁺ is complexed to PVS mainly in the form of the non fluorescent *cis*-isomer, so the residual fluorescence of the *trans*-isomer which is observed even in *P/D* of 100:1 is due to either uncomplexed *trans*-DASH₂²⁺ or to *trans*-DASH₂²⁺ which is complexed through only one end -NH₃⁺ terminal via a purely electrostatic interaction. Such a complexation has much less effect on the photophysical properties of DASH₂²⁺ then a two end complexation which combine both electrostatic and van der Waals interactions. Similar behavior was found by us in the complexation of the analog molecule *trans*-4, trimethyl-ammonium-4'-aminostilbene (TAAS) to PVS [22]. The DASH₂²⁺ PSS complex shows a very different behavior. Both the total quantum yield, the relative quantum yield of the monoprotonated form of DASH⁺ and the polarization of DASH₂²⁺ increase upon the complexation of DASH₂²⁺ with PSS (Figs. 4 and 6). This indicates a rigid binding of the *trans*-isomer using the coulombic interaction of the two NH₃⁺ terminals as docking anchors (Fig. 9). Analogously, we have found out a 3-fold increase in both the lifetime and the quantum yield of bound *trans*-TAAS molecule to PSS [22]. The main reason for the increase in the total quantum yield of the complexed *trans*-DASH₂²⁺ is the slow down in its *trans*-*cis* photoisomerization rate which is the main non-radiative decay route for both excited DASH₂²⁺ and DASH⁺. Finally the rate of the proton transfer may also be affected by the complexation of DASH₂²⁺ to PSS, due to a change in the pK_a^* . However, this effect is expected to be very small because of the large free-energy gain and the exothermic nature of this proton-transfer reaction which make the reaction rate insensitive to small changes in pK_a^* [23].

Table 3

Emission intensity ratio (I_{460}/I_{350}) of DASH⁺ and DASH₂²⁺ in the presence of PSS and PVS in 0.01 M HCl solution

Medium	I_{460}/I_{350} (in PSS)	I_{460}/I_{350} (in PVS)
Water	0.49	0.48
<i>P/D</i> =2	0.68	—
<i>P/D</i> =5	0.87	—
<i>P/D</i> =10	0.94	—
<i>P/D</i> =20	—	0.49
<i>P/D</i> =40	—	0.48
<i>P/D</i> =100	0.97	0.51

5. Conclusion

The interaction between diprotonated diamine molecules namely: DASH_2^{2+} and DANH_2^{2+} to both aromatic (PSS) and non-aromatic (PVS) polyelectrolytes was investigated by using spectroscopic and molecular mechanics simulation techniques. Binding of amino derivatives to PSS is a co-operative binding process which involves electrostatic, hydrophobic and/or π – π interactions. As demonstrated by the UV absorption results, most of the DASH_2^{2+} that were complexed with PSS were in the *trans*-isomer form. A good correlation between structural calculations in vacuum and experimental results were obtained. The photochemical and photophysical properties of DAS and its protonated species is affected significantly by the interaction with the polyions in the solution. In particular, the rate of *trans*–*cis* photoisomerization is slowed down relatively to the uncomplexed dication. The binding of DAS and DAN to PSS shifts significantly the acid–base equilibrium toward the diprotonated species. The binding of the diprotonated DAS molecules to PVS was in their *cis*-isomer form, suggesting selective binding due to enhanced coulombic and lack of π – π interactions of the *cis*-isomer with the polyelectrolyte. This experimental finding is backed by our generated optimized structure of DASH_2^{2+} –PVS complex in vacuum.

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