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Ionization and binding equilibria of papaverine in ionic micelles studied by ^{1}H NMR and optical absorption spectroscopy

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Abstract

The binding of the vasodilator drug papaverine (PAV) to micelles of zwitterionic N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (HPS), cationic cetyltrimethylammonium chloride (CTAC) and anionic sodium dodecylsulfate (SDS) in aqueous solution was studied by ^{1}H NMR and electronic absorption spectroscopy. In the presence of HPS or CTAC, the apparent pK_a of PAV decreased by about 2 units, while it increased by about 2 units upon binding to SDS. However, the chemical shift patterns of both protonated ($PAVH^+$) and deprotonated (PAV^0) forms of PAV are not sensitive to the type of surfactant. The association constants were estimated as $5 \pm 2\ M^{-1}$ for $PAVH^+$-CTAC, $8 \pm 3\ M^{-1}$ for $PAVH^+$-HPS, $(7 \pm 2) \times 10^5\ M^{-1}$ for $PAVH^+$-SDS, and 1.5×10^3 to $3.0 \times 10^3\ M^{-1}$ for the complexes of PAV^0 with all three types of micelles. Using these data, an electrostatic potential difference on the micelle-water interface was calculated as 150 ± 10 mV for CTAC, 140 ± 10 mV for HPS and -140 ± 10 mV for SDS. The results suggest that PAV aromatic rings are located in the hydrophobic part of the micelle. The electrostatic attraction or repulsion of the protonated quinoline nitrogen and surfactant headgroups changes the affinity of PAV to micelles and, thus, shifts the ionization equilibrium of PAV. The electrostatic potential of HPS micellar surface is determined by the cationic dimethylammonium headgroup fragment, whereas the anionic sulfate fragment attenuates the effective charge of HPS headgroup.

Keywords: Papaverine; Vasodilator drug; Micelle; Interaction; Surfactant; NMR

1. Introduction

Papaverine, 6,7-dimethoxy-1-veratryl-isoquinoline (PAV, Fig. 1), possesses a wide range of biological effects, being commonly known as a cerebral vasodilator and smooth muscle relaxant [1]. It is also used in clinics as a cardiac vasodilator, and its direct action upon coronary blood vessels with a reduction in muscular tonus is

Abbreviations: CMC, critical micelle concentration; CTAC, cetyltrimethylammonium chloride; HPS, N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; PAV, papaverine; PAV^0, deprotonated form of papaverine; $PAVH^+$, protonated form of papaverine; and SDS, sodium dodecylsulfate.

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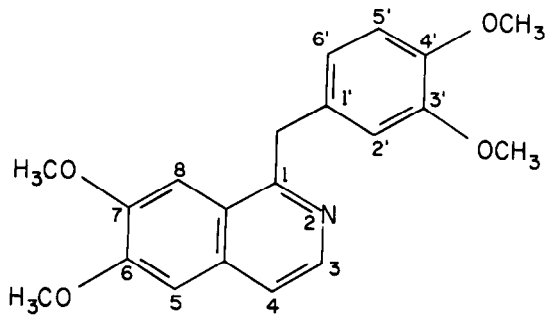

Fig. 1. Chemical structure of papaverine.

associated with the response to stimulation of β-adrenergic receptors [2]. It has been classified more recently as a nonspecific vasodilator [3]. Its biological effects imply interactions of PAV with cellular components such as membranes and biopolymers. In fact, it has been recently reported that PAV affects the hemoglobin oxygen affinity both in isolated protein and intact red blood cells [4,5]. Some PAV effects involve interaction with cellular membranes [6–11].

One of the most important events in the interaction of drugs with biological tissues at the molecular level is the interaction of small molecules, existing in charged and noncharged forms, with membranes [12–14]. Recently we have studied the interaction of the cationic form of PAV with micelles of surfactants with different headgroup charge. It has been shown that at constant pD value of 4.9 ± 0.1 the positively charged fragment of the polar head of the zwitterionic N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (HPS) defines the character of interaction of PAV with HPS micelles [15]. The pD and surfactant concentration dependences of PAV chemical shifts allowed us to assume that a change in PAV ionization state could take place as a result of interaction of this drug with different types of micelles.

A change in pK_a suggests complex formation between ionizable molecules. The interaction of nitrogen heterocyclic molecule of histamine with heparin, a negatively charged polymer, is an example of this phenomenon. The displacement of the chemical shift titration curves to lower pD for heparin and to higher pD for histamine, which accompanied the interaction, was demonstrated by ^1H NMR [16,17].

Lee developed a formalism for membrane binding studies of compounds that undergo ionization equilibria [12]. This formalism was applied to data on drug-induced decreases in phase transition temperatures of lipids. It was shown that a shift in pK_a on binding can occur as the result of the preferential binding of either the charged or the uncharged form of the drug. The proposed approach was used to analyze the tetracaine and procaine binding to phospholipid bilayers [13,14] and dibucaine binding to micelles [18].

We report herein the study of binding of PAV to micelles of zwitterionic HPS, cationic cetyltrimethylammonium chloride (CTAC) and anionic sodium dodecylsulfate (SDS) in aqueous solution in a wide pH range. Polar lipids of biological membranes are predominantly zwitterionic phosphatidylcholine and phosphatidylethanolamine. Therefore, micelles of HPS may be considered as a model of lipid aggregates, whereas CTAC and SDS may be suitable for studying the interaction of PAV with different parts of the polar HPS headgroup separately. HPS, CTAC and SDS micelles are commonly accepted model systems for studying various aspects of membrane interactions [19,20]. Proton NMR allowed us to discriminate between different chemical groups of interacting molecules in dynamic equilibrium, while optical absorption spectroscopy allowed more accurate measurements of pK_a values and equilibrium parameters.

2. Materials and methods

Materials and experimental techniques. PAV hydrochloride and HPS (Sigma), SDS (Bio-Rad), sodium acetate and potassium phosphate (Quimis-Mallinckrodt), sodium borate (Carlo Erba) and D_2O (Aldrich) were used as purchased. CTAC (Herga) was purified by acetone–methanol extraction. H_2O was distilled and deionized. The solutions for NMR were prepared in a 20 mM acetate buffer in D_2O as solvent. For optical measurements of pK_a and of binding be-

low the pK_a of PAV, a 20 mM acetate buffer in H_2O was used. Studies above the pK_a of PAV were done in 20 mM phosphate–borate buffer in H_2O. The pH and pD values were measured and adjusted using a Corning-130 pH meter. The pD values were calculated by adding 0.4 to the pH meter readings [21].

Proton NMR spectra were run on a Bruker AC-200 (resonant frequency 200.13 MHz) at ambient probe temperature (23°C) in 5 mm sample tubes (Wilmad). Residual water suppression in proton spectra was achieved using a presaturation by gated irradiation during the delay of 2 s between scans. The 30° pulses of 2 μs duration were repeated every 5.7 s with a bandwidth of 2 kHz. The free induction decay signals were digitized using 16 K data points and processed by an exponential filter with a line broadening factor of 0.2 Hz prior to Fourier transformation.

Electronic absorption spectra were measured on Hitachi U-2000 and Shimadzu UV-180 spectrophotometers. The wavelengths ensuring maximum effect were chosen after subtraction of PAV absorption spectra in the presence and absence of surfactants at different pH values. The pK_a measurements were done at 241 and 256 nm. Wavelengths of 228, 234 and 248 nm were used for binding studies at pH 8–11. Binding studies at pH 1.5 to 4.5 were carried out at 242 and 248 nm for CTAC and HPS, and at 248 and 256 nm for SDS.

Determination of binding parameters. For NMR measurements, the concentration of PAV was 1 mM, and the concentration of surfactant varied in the range of 2 to 130 mM. For electronic absorption measurements, the concentration of PAV was 10 μM, and the concentration of surfactant varied in the range of 0.2 to 80 mM. Assuming that complex formation between PAV and surfactant is a second order process and that the equilibrium obeys the law of mass action, we can write an equation

$$1/\alpha = 1 + 1/K_A[S_f], \quad (1)$$

where α is a fraction of PAV bound to micelles, K_A is an association constant for the complex, and $[S_f]$ is the concentration of the 'free' surfactant. The α value is given by $(A - A_f)/(A_b - A_f)$, where A, A_f and A_b are the observed optical absorption and its values for free and bound PAV, respectively. Similarly, taking into account that chemical exchange is fast in the NMR timescale (a single resonance was observed for each PAV proton in all cases), we can define α as $(\delta - \delta_f)/(\delta_b - \delta_f)$, where δ, δ_f and δ_b are the observed chemical shifts of a particular PAV resonance and its values for free and bound PAV, respectively. In conditions of large excess of surfactant $[S_f] \approx [S_0]$ ($[S_0]$ is the total concentration of the surfactant), we can determine K_A in any linearizing frame (e.g. Scatchard or Hughes and Klotz representations) [22,23] or by computer fitting.

Binding constants were averaged over 2–3 wavelengths in case of optical measurements or over 6–7 resonances when NMR data were used.

3. Results

Chemical shift values of the quinoline ring protons of PAV in D_2O solution are pD dependent, changing over the pD range where the nitrogen-bonded proton is titrated. The H4 proton is the most sensitive to pD changes due to the peculiarities of electron density distribution in the PAV molecule. At pD values above the pK_a, PAV precipitates, thus leaving only a very low concentration of solubilized fraction. In the presence of micelles precipitation did not occur. The chemical shift of the H4 resonance of 1 mM PAV in aqueous solution and in the presence of different surfactants is plotted as a function of pD in Fig. 2. A PAV concentration of 1 mM was used, since self-aggregation of PAV becomes negligible at this concentration [15]. In the presence of HPS or CTAC, the chemical shift titration curves for all protons of PAV are displaced to lower pD, while those in the presence of SDS are displaced to higher pD. The chemical shifts at pD values where PAV is present predominantly in protonated ($PAVH^+$) and deprotonated (PAV^0) forms are listed in Table 1.

The data of Fig. 2 and corresponding data for other PAV protons enabled an estimation of the pK_a value of PAV in solution and when it is

Table 1
Proton chemical shifts (ppm) of 1 mM papaverine in the absence and presence of 128 mM surfactant [a]

Surfactant	pD	H3	H4	H8	H5	H2′	H5′	H6′	Methoxy			
none	4.31	8.18	8.07	7.66	7.58	7.04	7.02	6.89	4.09	3.98	3.83	3.80
none	8.87	8.23	7.70	7.51	7.38	6.98	6.96	6.91	4.01	3.92	3.81	3.76
HPS	2.62	8.33	8.19	7.75	7.70	7.18	6.91	6.91	4.11	4.02	3.83	3.79
HPS	8.41	8.35	7.65	7.51	7.33	7.15	6.80	6.86	4.01	3.96	3.84	3.80
CTAC	1.47	8.28	8.16	7.73	7.67	7.19	6.90	6.90	4.10	4.01	3.82	3.79
CTAC	8.48	8.33	7.64	7.49	7.34	7.13	6.79	6.85	4.01	3.95	3.82	3.80
SDS	5.03	8.33	8.21	7.73	7.69	7.17	6.87	6.84	4.14	–	3.88	3.83
SDS	11.44	8.26	7.61	7.48	7.26	7.03	6.86	6.86	≈ 4.1	–	3.81	3.81

[a] In D_2O containing 20 mM acetate.

incorporated into different micellar systems. A more precise evaluation of the pK_a could be made by optical measurements, since a more detailed curve can be obtained at a lower concentration of PAV (allowing one to avoid self-aggregation and precipitation at high pH values) and at larger excess of surfactant over PAV. The results of measurements using both techniques given in Table 2 are fairly compatible.

Unlike the interaction of histamine and heparin [16,17], the binding of PAV to micelles is accompanied not only by the change in pK_a, but also by changes in chemical shifts both at low and high extremes of pD range (Table 1). This phenomenon could serve for estimation of the association constant K_A of the complexes of $PAVH^+$ and PAV^0 with surfactants. However, K_A determination for the complexes of PAV^0 with all types of micelles and $PAVH^+$ with SDS encounters the problem of non-micellar aggregates of surfactant below the critical micelle concentration (CMC) [24] involving PAV molecules. The aggregation leads to a dramatic decrease in the intensity of both PAV and surfactant NMR signals, and the remaining signals in solution present sharp changes in chemical shifts and lineshapes. Therefore, NMR estimations of K_A appeared to be reliable only for CTAC and HPS micelles at low pD. For all binding equilibria, the K_A values were calculated based on optical data taken above the CMC. Data for the binding of PAV to CTAC and SDS as well as the best fits which yield K_A values are presented in Fig. 3.

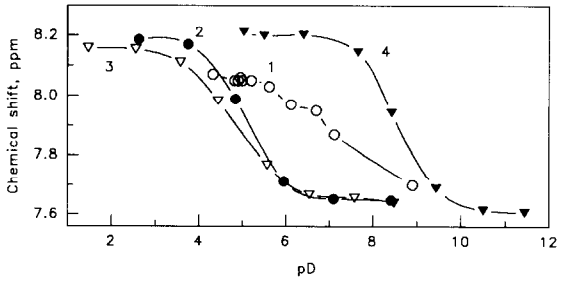

Fig. 2. Chemical shift of the H4 proton of 1 mM papaverine in the absence or presence of 128 mM surfactant in D_2O containing 20 mM acetate: (1) without surfactants, (2) HPS, (3) CTAC, and (4) SDS.

Table 2
The pK_a values of papaverine in the absence and presence of surfactants and energy equivalent of the pK_a changes

	Surfactant			
	none	CTAC	HPS	SDS
pK_a^{NMR} [a]	6.7	4.8	5.5	8.6
pK_a^{SP} [b]	6.46	4.29	5.66	8.27
ΔpK_a [c]	–	-2.6 ± 0.2	-2.3 ± 0.2	2.4 ± 0.2
E (meV) [d]	–	150 ± 10	140 ± 10	-140 ± 10

[a] NMR measurements. The pD values (pH meter readings corrected for isotopic effect) were used for pK_a calculations. Concentrations of PAV and surfactant are 1 and 128 mM, respectively, in D_2O containing 20 mM acetate.

[b] Spectrophotometric measurements. The pH values were used for pK_a calculations. Concentration of PAV is 10 μM. Concentrations of surfactants are 40, 80 and 100 mM for HPS, CTAC and SDS, respectively, in H_2O containing 20 mM acetate.

[c] The difference of pK_a in the micelle-bound state and in aqueous phase calculated using spectrophotometrically determined binding constants.

[d] Derived as $-2.303\ kT\ \Delta pK_a$ using ΔpK_a estimated from binding studies.

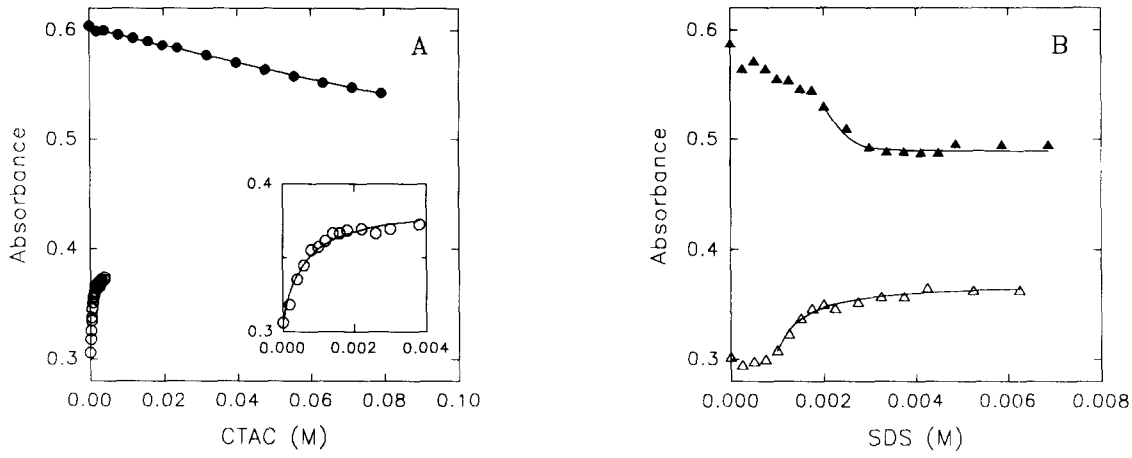

Fig. 3. Absorbance of a 10 μM solution of papaverine as a function of surfactant concentration monitored at 248 nm against a surfactant solution without papaverine: (A) CTAC, pH 1.5 (filled circles) and 8.0 (open circles and insert); (B) SDS, pH 4.5 (filled triangles) and 10.9 (open triangles). Solid lines are best fits to the data taken above CMC. All solutions were in 20 mM acetate (filled symbols) or phosphate–borate (open symbols) buffer in H_2O.

The results in Table 3 display a reasonable agreement between NMR and optical data.

According to Lee [12,25], the difference of pK_a of the drug in the micelle bound state and in aqueous phase ΔpK_a is connected with the association constants for PAV^0 and $PAVH^+$ by the equation

$$K_A^{PAVH^+}/K_A^{PAV^0} = \exp(2.303\ \Delta pK_a). \quad (2)$$

The experimental results fit this equation satisfactorily (Table 2). Since the concentrations of surfactants were not fully saturating, the results of direct pH and pD titrations are somewhat lower. An energy equivalent E of ΔpK_a is, evidently,

$$E = -2.303\ kT\Delta pK_a. \quad (3)$$

The calculated E values for all studied systems are also given in Table 2.

4. Discussion

Taken together, the chemical shift data in Fig. 2 and Tables 1 and 2 provide evidence that all types of micelles bind both $PAVH^+$ and PAV^0.

Table 3
Association constants K_A (M^{-1}) for binding of protonated and neutral papaverine to surfactants

	Surfactant				
	CTAC		HPS		SDS
	SP	NMR	SP	NMR	SP
$PAVH^+$ [a]	5 ± 2	5 ± 2	8 ± 3	–	$(7 \pm 2) \times 10^5$
$PAVH^+$ and PAV^0 [b]	–	68 ± 9	150 ± 40	40 ± 8	–
PAV^0 [c]	1800 ± 200	–	1560 ± 60	–	2900 ± 200

Designations: SP, spectrophotometric measurements; NMR, NMR measurements. Errors are reported as ±SEM.
[a] Spectrophotometric data were obtained at pH 1.5, 2.1 and 4.5 for CTAC, HPS and SDS, respectively. NMR data were obtained at pD 1.4.
[b] A mixture of protonated and neutral forms; pH 4.6 for spectrophotometric data, pD 4.9 for NMR data.
[c] pH 8.0, 9.1 and 10.9 for CTAC, HPS and SDS, respectively.

This binding results in changes in chemical shifts of most PAV protons and in the apparent pK_a value of PAV. Alternations in pK_a usually indicate complex formation between charged molecules [16,17]. The analysis of the changes of pK_a of PAV (Fig. 2 and Table 2) shows that HPS interacts with PAV in the same manner as CTAC rather than SDS. However, the chemical shift patterns of both $PAVH^+$ and PAV^0 are not sensitive to the type of surfactant (Table 1). For the protons of the small cycle, this pattern is practically independent of pD.

Therefore, it is reasonable to assume that the role of interactions of charged groups is mainly in modulating the hydrophobic interaction by changing the affinity of PAV to micelles. The electrostatic attraction or repulsion of the protonated quinoline nitrogen and surfactant headgroups defines a shift of ionization equilibrium of PAV (i.e. pK_a) and K_A. In agreement with this assumption, K_A has the lowest value for the complexes of $PAVH^+$ with CTAC and HPS, highest value for $PAVH^+$-SDS, and approximately equal intermediate values for the complexes of PAV^0 with all three types of micelles (Table 3).

Affinity of charged drugs to ionic membranes is determined by two mechanisms: a gradient of standard chemical potential and electrostatic field gradient [12,25,26]. The pK_a shifts and, accordingly, their energy equivalents E have opposite signs and approximately the same absolute values for CTAC and SDS. It, seemingly, implies that the contribution of the standard chemical potential term is relatively small, and the changes in pK_a are defined mainly by electrostatic forces. Therefore, the value E/e, where e is the elementary charge, can be considered as the difference of electrostatic potentials on the micelle–water interface.

In a previous study, we have shown that the modes of PAV binding to HPS and CTAC at pD = 4.9 are similar, being quite different from that for SDS [15]. In the present work, we again observed similar behavior of HPS and CTAC different from that of SDS with respect to PAV binding in a wide pD range. It apparently suggests that the positively charged dimethylammonium headgroup fragment of HPS defines the mode of binding of PAV to HPS. The sulfate anionic fragment attenuates the effective charge of HPS headgroup, leading to the less significant displacement of pK_a value due to the lower surface potential. It was shown recently that this charge attenuation may result in more weak solvent structure perturbation by HPS as compared with CTAC due to weaker overall hydration and electrostatic field effects [20]. This was explained by the significant intra and intermolecular electrostatic interaction between the surfactant head-ions [19]. The pK_a value of another cationic drug, tetracaine, also decreases on binding to bilayers of zwitterionic phospholipid, egg phosphatidylcholine [14], although the cationic and anionic fragments in HPS and phosphatidylcholine are arranged in a different order.

In summary, we have demonstrated that PAV in both charged and noncharged forms interacts with micelles formed by amphiphilic molecules with different headgroup charge. The chemical nature of these complexes is not sensitive to the type of surfactant. The aromatic heterocycle of PAV in its protonated state can facilitate or hinder this interaction by electrostatic forces. The electrostatic potential of HPS micellar surface is determined mainly by the cationic dimethylammonium headgroup fragment, whereas the negatively charged sulfate fragment attenuates the effective charge of HPS headgroup.

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