

MICROENVIRONMENT OF POLYMER CHAINS IN SOLUTION: pK_a OF INDICATORS BOUND TO THE POLYMER CHAIN

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Polymers bearing at the end of side chains acidobasic indicator moieties exhibiting a solvatochromic absorption band were prepared by a polymeranalogous reaction of copolymers N-(2-hydroxy propyl) methacrylamide-N-methacryloylated 4-nitrophenyl ester ω-aminoacids with 1-(2-aminoethyl)-4-(4-hydroxystyryl)pyridinium bromide. It was found that pK_a of reporters bound at a medium long side chain (six methylene groups) is close to pK_a of a low-molecular model compound, while pK_a of a reporter bound close to the polymer backbone is approximately by one pK_a unit higher. Changes in the pK_a value of the reporters are discussed in connection with a change in the polarity of microenvironment of polymer chains.

The utilization of synthetic polymers as carriers of biologically active compounds is determined by several basic factors, such as solubility of the polymer, its molar mass, polydispersity, size and character of groups bound to the polymer chain. The release of a biologically active compound from the polymer chain depends both on the length and on the structure of the side chain.

This study is an attempt to characterize the microenvironment of a solvatochromic reporter which is determined by the distance of the reporter from the polymer backbone. A shift of the sensitive solvatochromic UV-VIS absorption maximum of stilbazolium-betaine reporter with a change in the polarity of the medium along with a change in pK_a of the reporter bound on the polymer chain may help to elucidate the behaviour of variously long aliphatic side chains and to quantify properties of the domain of polymer chains.

EXPERIMENTAL

Preparation of Solvatochromic Compounds, Monomers and Copolymers

4-(4-Acetoxystryryl)pyridine. A mixture of 34.77 g (0.37 mol) 4-methylpyridine with 45.6 g (0.37 mol) 4-hydroxybenzaldehyde in 76 g (0.75 mol) acetanhydride was boiled for some 100 h. After that the excess of acetanhydride and acetic acid were removed by distillation from the reaction mixture. The residue was poured into 700 ml of water, and pH of the mixture was

adjusted to ~ 7 . The 4-(4-acetoxystyryl)pyridine separated from the mixture was removed by filtration and recrystallized from ethanol, m.p. 155°C. For $C_{15}H_{13}NO_2$ (239.3) calculated: 75.29% C, 5.48% H, 5.85% N; found 75.17% C, 5.60% H, 5.85% N.

4-(4-Hydroxystyryl)pyridine. A mixture of 750 ml of dilute hydrochloric acid (1 : 3 by vol.) and 30 g of 4-(4-acetoxystyryl)pyridine was boiled for 6 hrs and on cooling was neutralized with a 5% solution of sodium hydroxide to pH ~ 8 . The precipitated 4-(4-hydroxystyryl)pyridine was removed by filtration and recrystallized twice from ethanol, m.p. 230–235°C (in ref.¹ 214 to 215°C). For $C_{13}H_{11}NO$ (197.2) calculated: 79.17% C, 5.62% H, 7.10% N; found: 79.17% C, 5.79% H, 7.09% N.

1-Ethyl-4-(4-hydroxystyryl)pyridium iodide (Ia) was obtained² by the quaternization of 4-(4-hydroxystyryl)pyridine with ethyl iodide in ethanol at 100°C. The raw product was recrystallized from ethanol (yellow crystals), m.p. 195°C (ref.² 198°C). For $C_{15}H_{16}INO$ (256.2) calculated: 50.99% C, 4.53% H, 35.98% I, 3.97% N; found 50.76% C, 4.40% H, 36.15% I, 3.91% N.

1-(2-Aminoethyl)-4-(4-hydroxystyryl)pyridinium bromide hydrobromide (Ib) was obtained by the quaternization of 4-(4-hydroxystyryl)pyridine (0.985 g, 0.005 mol) with 2-bromoethylamine hydrobromide (1.005 g, 0.005 mol) in 5 ml of methanol (100°C, 60 h). The raw product was washed with methanol and recrystallized from ethanol. For $C_{15}H_{18}Br_2N_2O$ (402.1) calculated: 40.00% C, 4.29% H, 38.09% Br, 10.00% N; found: 40.12% C, 4.36% H, 38.32% Br, 10.11% N.

4-Nitrophenyl methacrylate (II) was obtained by a reaction of sodium nitrophenoxide with methacryloyl chloride³. The product was crystallized from an ethanol–hexane mixture, m.p. 94°C (in ref.³ 94°C).

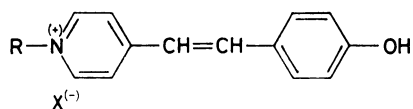
4-Nitrophenyl esters of methacryloylated ω -aminoacids (III). Methacryloylated ω -aminoacids were prepared under the conditions of Schotten–Baumann acylation^{4,5} and finally esters *III* were obtained by a reaction with 4-nitrophenol in the presence of dicyclohexylcarbodiimide, namely, N-methacryloylglycine (*IIIa*), β -(N-methacryloyl)alanine (*IIIb*), 4-(N-methacryloylamino)butanoic acid (*IIIc*), 5-(N-methacryloylamino)pentanoic acid (*IIId*), 6-(N-methacryloylamino)hexanoic acid (*IIIe*), 7-(N-methacryloylamino)heptanoic acid (*IIIf*), 8-(N-methacryloylamino)octanoic acid (*IIIg*), 11-(N-methacryloylamino)undecanoic acid (*IIIh*), and 12-(N-methacryloylamino)dodecanoic acid (*IIIi*) 4-nitrophenyl esters. The esters *IIIa–IIIi* thus obtained were homogeneous on TLC, the melting points agreed with the reported ones^{4,5}.

N-(2-Hydroxypropyl)methacrylamide (HPMA), provided by Dr J. Kopeček, Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, was crystallized from acetone. 2-Bromoethylamine hydrobromide was prepared by a reaction of ethanolamine with hydrobromic acid⁶; m.p. 169°C (in ref.⁶ 169°C).

Copolymerization

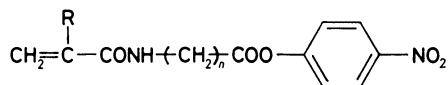
Copolymers of N-(2-hydroxypropyl)methacrylamide (HPMA) with polymerizable 4-nitrophenyl esters were prepared by the radical precipitation copolymerization in acetone. The starting mixture contained 15% of comonomers.

The polymerizations were carried out in glass sealed ampoules. Oxygen dissolved in the reaction mixture was removed prior to sealing by the usual “freeze–pump–thaw–purger with nitrogen” procedure. The reaction mixture was heated to 60°C for 7 h. The ampoule contents were transferred into 300 ml of acetone and the polymer was filtered off. The copolymers were reprecipitated twice from methanol into acetone. For characterization of the copolymers see Table I.



I a, R = CH₃CH₂; X = I

I b, R = HBr · H₂NCH₂CH₂; X = Br



III a, R = CH₃; n = 1

III f, R = CH₃; n = 6

III b, R = CH₃; n = 2

III g, R = CH₃; n = 7

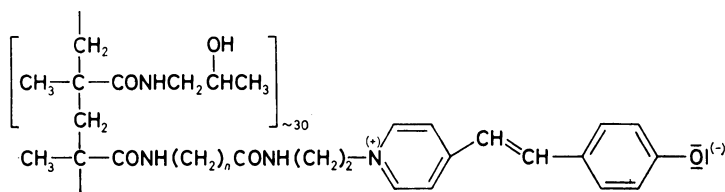
III c, R = H; n = 3

III h, R = CH₃; n = 11

III d, R = CH₃; n = 4

III i, R = CH₃; n = 12

III e, R = CH₃; n = 5



IV

Polymeranalogous Reaction

The reaction between the copolymers of HPMA and 1-(2-aminoethyl)-4-(4-hydroxystyryl)pyridinium bromide was carried out in dimethyl sulfoxide. The copolymer (0.5 g) was dissolved in 5 ml of dimethyl sulfoxide and 1 ml of a solution of 0.001 mol *Ib* with 0.001 mol triethylamine in dimethyl sulfoxide was added. The mixture thus obtained was heated in a sealed ampoule at 50°C for 7 h. The polymer solution was dialyzed against distilled water, and after that the polymer was isolated by lyophilization. The polymer (*IV*) thus isolated was used in pK_a measurements.

Determination of the Acidity Constants, pK_a

All pK_a were determined by the spectrometric titration while observing all principles valid for an exact determination of such constants⁷. Buffer solutions which do not absorb in the UV range of the spectrum were used. For the pK_a measurement the compound to be measured was dissolved in 0.01M-HCl, 0.01M-NaOH and in the buffer solution. The latter was chosen so that pK_a of the compound lay in its buffer region. The absorbancy of the basic form, A_∞ (at pH ~ 2), of the acid form, A_0 (at pH ~ 12), and the absorbancy of the compound under study, A , were read off from the absorption spectrum at a certain pH and at the analytical wavelength. The concentration of the compound to be measured was chosen so that at pH 12 and a wavelength

corresponding to the maximum of the basic form the absorbancy A is in the range 0.6–0.9. The absorption maximum of the basic form in an aqueous solution is at wavelength 457 nm (λ_∞), that of the acid form is at wavelength 375.5 nm (λ_0), with the isosbestic point it lies at wavelength 389.9 nm (λ_i).

The acidity constants were evaluated from these data by employing two procedures: (i) By calculation from a definition equation of the ionization constant according to

$$\text{p}K_a = \text{pH} + \log(A_\infty - A)/(A - A_0);$$

(ii) graphically, from the modified Henderson–Hasselbach equation⁸

$$\text{pH} = \text{p}K_a - a \log(1 - \alpha)/\alpha;$$

the $(1 - \alpha)/\alpha$ value was calculated from

$$(1 - \alpha)/\alpha = (A - A_\infty)/(A_0 - A),$$

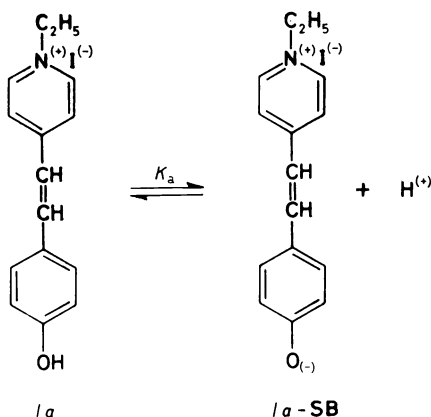
the acidity constant $\text{p}K_a$ was obtained from the plot of the dependence $\log(1 - \alpha)/\alpha$ on pH ; pH equals $\text{p}K_a$ at $\log(1 - \alpha)/\alpha = 0$, i.e. at $\alpha = 0.5$.

RESULTS AND DISCUSSION

$\text{p}K_a$ of 1-Ethyl-4-(4-hydroxystyryl) Pyridinium Iodide (Ia)

Iodide *Ia* is a weak acid and is ionized according to Scheme 1. The $\text{p}K_a$ value of the model *Ia* was determined in water and in two buffer solutions (0.01M “TRIS” and 0.01M potassium dihydrogenphosphate–0.01M ammonia). The $\text{p}K_a$ values, both calculated and obtained graphically, were approximately the same. The difference between the slopes of the $\log(1 - \alpha)/\alpha$ vs pH dependence for nonbuffer and buffer solutions was observed. The poorest reproducibility of the determination of $\text{p}K_a$ of *Ia* was found in the KH_2PO_4 – NH_4OH buffer solution. The $\log(1 - \alpha)/\alpha$ vs pH dependence was linear in all solutions and obeyed the modified Henderson–Hasselbach equation. The slope a varies between -0.7 and -1.5 , depending on the buffer solution used. The value by which the slope a deviates from -1 indicates the influence of electrostatic effects in the titration⁹. The value of a differs considerably from -1 just in the $\text{p}K_a$ measurement in buffer solutions. The model *Ia* was chosen not only due to the possibility of measuring its $\text{p}K_a$, but also because in an alkaline medium formed compound, stilbazolium-betaine (*Ia*-SB, Scheme 1) shows a solvatochromic band which is shifted with a change of interaction power (polarity) of the medium. It was shown¹⁰ that, if this solvatochromic molecule is embedded in the polymer chain, it may inform us about the polarity of microenvironment of the polymer chain. With increasing polarity of the solvent the maximum of the solvatochromic absorption band of a reporter of this type is blue shifted. From the relation $E_T = N_A h \nu = 1.197/\lambda_{\text{max}} \cdot 10^5 \text{ kJ mol}^{-1}$ (where E_T is the energy of the solvatochromic maximum, N_A is the Avogadro constant, h is Planck's constant, ν is frequency

and λ_{\max} (nm) is the wavelength of the solvatochromic maximum) it is possible to calculate the polarity of the microenvironment of polymers and water. A value for water ($E_T = 261.9 \text{ kJ mol}^{-1}$) can be compared with the E_T values of the solvatochromic reporter bound on the polymer chain.



SCHEME 1

TABLE I

Characterization of copolymers of N-(2-hydroxypropyl)methacrylamide (HPMA) with 4-nitrophenyl esters of ω -(N-methacryloylamino)acids (*IIIa*–*IIIi*) and pK_a constants of these copolymers labelled with the stilbazolium quaternary salt; c denotes content of 4-nitrophenyl ester, M_w molar mass

Copolymer	c mole %	pK_a^a 20°C	$M_w \cdot 10^{-3}$ g mol^{-1}
<i>II</i> -HPMA ^b	4.02	9.2	30
<i>IIIa</i> -HPMA	3.45	9.0	42
<i>IIIb</i> -HPMA	3.19	—	37
<i>IIIc</i> -HPMA	2.60	8.5	40
<i>IIId</i> -HPMA	2.95	—	34
<i>IIIe</i> -HPMA	3.24	8.6	37
<i>IIIf</i> -HPMA	3.10	8.4	41
<i>IIIg</i> -HPMA	3.00	—	42
<i>IIIh</i> -HPMA	3.10	8.7	41
<i>IIIi</i> -HPMA	2.90	8.7	37

^a ± 0.1 ; 8.3 for low molecular weight model *Ia*; ^b copolymer of 4-nitrophenyl methacrylate with HPMA.

pK_a of the (4-Hydroxystyryl)pyridinium Bromide Structural Unit Bound at the End of the Side Chain of Polymer IV

The 4-(4-hydroxystyryl)pyridinium bromide structural unit in labelled copolymers is ionized according to the same scheme as *Ia*. An attempt to determine pK_a of labelled copolymers was made in a number of buffer solutions ("TRIS", ethyl amine, ethylenediamine, KH_2PO_4). In these solutions components of the buffer solution probably interacted with the solvatochromic molecule, and the results were not reproducible. The measure of these interactions depends on pH of the medium, and therefore the pK_a values could not be evaluated. For this reason, pK_a was determined in water, where satisfactory results were obtained.

The results of determination of pK_a for the low-molecular weight compound *Ia* and for labelled copolymers are summarized in Table I. It can be seen that pK_a of the model compound *Ia* is approximately the same as pK_a of the labelled copolymer *III_f*-HPMA. In all the other cases the value obtained for labelled polymers is higher. An essential difference between the pK_a values was observed for the labelled copolymer *II*-HPMA and for the low-molecular weight model compound *Ia*. In this case the pK_a value of the reporter is higher by almost a unity (reporter being an acid approximately ten times weaker) than the model compound *Ia*.

On the basis of reported data^{11,12} concerning water-aliphatic alcohols mixtures, in which increasing water content in the mixture (i.e. with increasing polarity) the strength of the acids also increases, it may be assumed that also the microenvironment of the reporter bound at various distances from the polymer backbone is different. If pK_a (8.3) of the model compound *Ia* in water is taken as a basis and if the observation reported above is mechanistically applied to our case, the microenvironment of the reporter of the labelled copolymers *II*-HPMA, *III_a*-HPMA, *III_c*-HPMA, *III_e*-HPMA, *III_h*-HPMA, and *III_i*-HPMA should be less polar compared with the polarity of water. This assumption is supported by energy values of the

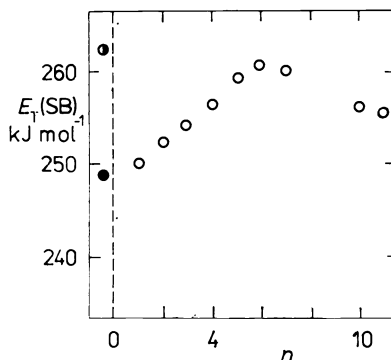


FIG. 1

Dependence of the polarity, $E_T(SB)$, of the microenvironment of polymer on the distance of solvatochromic structural unit from the polymer backbone in water at 25°C (n is a number of the spacer methylene units): ○ polymer IV, ● labelled copolymer II-HPMA; for comparison ● water

maximum of the solvatochromic band of the reporter of labelled copolymers (Fig. 1) compared with the polarity of water ($E_T = 261.9$ kJ/mol) expressed by the energy of the solvatochromic maximum of compound Ia-SB.

Polarity of the microenvironment of the solvatochromic structural unit and the ionization constant of the reporter bound at the side chain of the polymer (in an aqueous solution) increases with increasing length of the side chain, reaching the maximum for a chain possessing six methylene groups (Fig. 1). Polarity of the microenvironment of the polymer attains approximately the same value as that of the polarity of water. With increasing number of methylene groups in the side chain the hydrophobic character of the spacer increases, and since water is not a good solvent for such type of the spacer, an increase in length of the side chain leads to intramolecular interactions of the long aliphatic chain¹³. Due to this, the immediate environment of the solvatochromic reporter becomes less polar, which is reflected in a decrease in the energy of the absorption band of the solvatochromic molecules and in a lower ionization constant of the reporter.

This study provides a basic view of the effect of environment of the polymer chain and of the spacer length on pK_a of groups bound at the end of the side chains. To obtain unambiguous conclusions, however, it is necessary to prepare labelled copolymers also of other water-soluble polymers.

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