THE MOBILITY OF TRYPTOPHAN AND DANSYL FLUOROPHORES IN LABELLED POLY(*N*-ETHYLACRYLAMIDE) AND POLY(*N*-ETHYLMETHACRYLAMIDE)*

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Dedicated to Professor Otto Wichterle on the occasion of his 80th birthday.

The mobility of tryptophan fluorophore in *N*-butyl-N^{α}-acetyltryptophanamide and in side chains of labelled poly(*N*-ethylacrylamide) and poly(*N*-ethylmethacrylamide) was investigated by the fluorescence depolarization method. The mobility of the fluorophore in the low-molecular-weight model is much higher than in side chains of the polymers. Different steric hindrance by the polymer backbone can explain the higher mobility of the fluorophore in poly(*N*-ethylacrylamide) than in poly(*N*-ethylmethacrylamide). The mobility of 5-dimethylamino-1-naphthalenesulfonamide (dansyl) fluorophore in side chains of labelled poly(*N*-ethylmethacrylamide) in the high-viscosity range increases with increasing number of methylene groups in side chain. In the low-viscosity range, the rates of conformational changes in short side chains (n = 2 – 7) are approximately constant and significantly decrease in long side chains (n = 10, 12). The drop in the rates is probably due to intramolecular association of the long hydrophobic chains in water.

Polarized luminescence may provide valuable information on the structural and dynamic properties of biological and synthetic macromolecules. In our previous publications[1,2] we have used dansyl fluorophore to estimate the mobility of variously long side chains. Similarly, experimental data in the present study were analyzed according to the generalized Perrin equation[3,4]

$$Y = \frac{2/3}{1/\tau \int_0^\infty \langle \cos^2 \alpha(t) \rangle \exp(-t/\tau) \mathrm{d}t - 1/3} = \left[\sum_j \frac{f_j}{1 + 3\tau/\sigma_j} \right]^{-1}, \qquad (1)$$

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where $Y = (1/P - 1/3) / (1/P_0 - 1/3)$ for polarized excitation, t is time, P is the limiting fluorescence polarization, τ is the excited-state lifetime of the fluorescent group, f_j is the relative content of processes with the rotational relaxation times $\sigma_j (\Sigma f_j = 1)$ and the time function of fluorophore rotation $\alpha(t)$ is expressed by the following equation:

$$\langle \cos^2 \alpha(t) \rangle = 1/3 + (2/3) \sum_j f_j \exp(-3\, t/\sigma_j) . \qquad (2)$$

Some average characteristics of the rotational relaxation spectrum may be obtained by analyzing two limiting cases of the dependence $Y = Y(T/\eta)$ at $(T/\eta) \to 0$ and $(T/\eta) \to \infty$, where η is the solvent viscosity and T is temperature.

In the case of a fluorescence label covalently bound to a macromolecule the rotational relaxation spectrum may be divided into "fast" processes with the average relaxation time σ_1 and into "slow" processes with the average relaxation time $\sigma_2 [\sigma_2 >> \sigma_1]$. The generalized Perrin equation for polarized excitation may be then written[4,5] in the two-time approximation as

$$Y = \left[\frac{f}{1 + 3\,\tau/\sigma_1} + \frac{1 - f}{1 + 3\,\tau/\sigma_2} \right]^{-1} , \qquad (3)$$

where f is the total contribution of "fast" processes to the decrease in fluorescence polarization P. From the slope of the asymptote of the $Y = Y(T/\eta)$ dependence at $(T/\eta) \to 0$ (where $\sigma_2 >> \tau$) it is possible to obtain the average relaxation time of "fast" processes σ_1 at any solvent viscosity. From the slope of the asymptote of the $Y = Y(T/\eta)$ dependence at $(T/\eta) \to \infty$ (where $\sigma_1 << \tau$) it is possible to obtain the average relaxation time of "slow" processes σ_2 at any solvent viscosity and the total contribution f of "fast" processes to the decrease in fluorescence polarization (the intercept of this asymptote is $Y_0' = 1/(1 - f)$). It is natural to expect that the rotational relaxation time σ_1 characterizes torsional vibrations of single bonds within the framework of rotational-isomeric states. The rotational relaxation time σ_2 characterizes the rate of conformational changes of the chain where the fluorophore is attached.

The mobility of a fluorescence label bound at the end of side chain of a flexible polymer molecule is affected by several factors. "Slow" relaxation processes σ_2 in the two-time approximation include intramolecular motions with the average relaxation time σ_i and the rotation of a macromolecule as a whole with the average relaxation time σ_m. If each of these processes can lead to fluorescence depolarization, the overall relaxation time σ_2 is

$$1/\sigma_2 = 1/\sigma_i + 1/\sigma_m . \qquad (4)$$

The time of rotational diffusion of the macromolecule as a whole can be estimated approximately by the equation

$$\sigma_m = b \frac{M [\eta] \eta}{RT}, \quad (5)$$

where M is molar mass of the polymer, $[\eta]$ is the limiting viscosity number and b is the coefficient which depends on the shape of the macromolecule ($b = 2$ for coil macromolecules and $b \approx 1$ for compact spherical particles). By estimating σ_m it is possible to calculate the average relaxation time σ_i of intramolecular motions in which the fluorophore participates. σ_i may be expressed by

$$1/\sigma_i = 1/\sigma_s + 1/\sigma_b, \quad (6)$$

where σ_s and σ_b are average times of the "slow" relaxation processes in the polymer side chains and in its backbone, respectively.

The present study was designed to explore the mobilities of tryptophan and dansyl fluorophores and to gain experimental data for determination of the axial depolarization factors of tryptophan fluorophore in copolymers *V* and *VI* and dansyl fluorophore in copolymers *VII*. The axial depolarization factors[6] are used to calculate the distance between tryptophan and dansyl fluorophores in copolymers *VII* by means of nonradiative energy transfer method[7]. The distance of tryptophan and dansyl fluorophores as a function of the number of methylene groups in the side chain may confirm assumed intramolecular association of long aliphatic chains in a polar medium (water).

EXPERIMENTAL

Monomers and Models

N-Butyl-N$^{\alpha}$-acetyltryptophanamide (I) was prepared by the reaction of N$^{\alpha}$-acetyl-DL-tryptophan 4-nitrophenyl ester[8] (2.425 g, 6.6 mmol) with butylamine (0.578 g, 7.9 mmol) in acetone (50 ml) at 50 °C for 5 h. The reaction mixture was chromatographed on silica gel using acetone as eluent. After recrystallization from acetone, *I*, melting at 157 °C (DSC), was pure (TLC). For $C_{17}H_{23}N_3O_2$ (301.4) calculated: 67.75% C, 7.69% H, 13.94% N ; found: 67.93% C, 7.51% H, 14.10% N.

N$^{\alpha}$-Methacryloyl-DL-tryptophan phthalimidyl ester (II). To a solution of 0.037 mol of N$^{\alpha}$-methacryloyl-DL-tryptophan[9] in ethyl acetate (100 ml), 0.037 mol *N,N'*-dicyclohexylcarbodiimide in ethyl acetate (30 ml) and 0.04 mol *N*-hydroxyphthalimide in DMF (50 ml) was added dropwise at −10 °C. The reaction mixture was stirred at −10 °C for 3 h, then at room temperature for 10 h and for another 3 h after the addition of 0.1 ml of glacial acetic acid. After removing dicyclohexylurea by filtration and evaporation of the solvent, the viscous residue was dissolved in ethyl acetate (180 ml), and the solution was extracted with a cool (≈ 0 °C) saturated aqueous $NaHCO_3$ (3 × 80 ml) and water (≈ 0 °C) (2 × 100 ml). Acetone solution of the crude product was fractionated by column chromatography on silica gel and the purified *II* was recrystallized from an ethyl acetate–hexane mixture; m.p. 88 – 89 °C. For $C_{23}H_{19}N_3O_5$ (417.4) calculated: 66.18% C, 4.59% H, 10.00% N; found: 66.02% C, 4.59% H, 9.94% N. UV spectra: in MeOH λ_{max} = 282 nm (ε = 7.20 cm^2 mol^{-1}), in DMSO λ_{max} = 284 nm (ε = 7.70 cm^2 mol^{-1}).

N-Ethylacrylamide and N-ethylmethacrylamide were prepared[10] by the reaction of the corresponding chlorides with ethylamine in diethyl ether, in the presence of triethylamine. All solvents used in the preparation of copolymers were dried prior to use.

*Poly(N-ethylacrylamide-co-*II) (III) *and poly(N-ethylmethacrylamide-co-*II) (IV). A mixture of active ester *II*, *N*-ethylacrylamide or *N*-ethylmethacrylamide, AIBN (0.5 wt.% per monomer feed) and acetone (250 wt.% per monomer feed) in a glass ampoule was subjected to three cooling-evacuation-heating-N_2 purging cycles and polymerized at 60 °C for 13 or 4 h, respectively. The total conversions were 29 or 22%, respectively. The prepared copolymers were twice reprecipitated from methanol into diethyl ether. The characteristics of the copolymers *III* and *IV* are given in Table I.

Fluorescence-labelled copolymers V, VI *and* VII. A mixture of copolymer *III* or *IV*, fivefold molar excess (per active ester units in *III* or *IV*) of octylamine or *N*-(ω-aminoalkyl)-5-dimethylaminonaphthalenesulfonamide[11] (Dns–NH–$(CH_2)_n$–NH_2), DMSO (ca 10 ml per 1 g of the reaction components) and imidazole (1 wt.% per reacting components) were heated in a sealed glass ampoule at 60 °C for 30 h. Then, 0.2 g of ethylamine was added and, after 5 h, the reaction mixture was diluted with DMSO. Low-molecular-weight compounds were removed by dialysis against 0.015 M HCl, or against aqueous ethanol or methanol (1 : 1 v/v) acidified with HCl. Final dialysis was always performed against water. Aqueous polymer solutions were lyophilized and *V*, *VI* and *VII* were reprecipitated twice from methanol into diethyl ether.

Methods

Determination of fluorophores. The content of the tryptophan or dansyl fluorophores in copolymers was determined by UV spectrometry in methanol, assuming that the molar absorption coefficient of the monomeric unit in copolymers *III* and *IV* was the same as that of monomer *II* and the molar absorption coefficient of the dansyl unit in copolymers *VII* was the same as that of Dns–NH–$(CH_2)_n$–NH_2 (ε_{338}= 4.40 cm^2 mol^{-1}). Analysis of copolymers *VII* revealed that aminolysis of phthalimidyl ester units in *III* and *IV* with dansylamines proceeds almost quantitatively.

Measurement of fluorescence polarization. The stationary fluorescence polarization was measured with a Hitachi–Perkin–Elmer MPF-2A and a 44B spectrophotometers in an L-arrangement. The temperature of solutions in a quartz cell (1 × 1 × 4 cm) was recorded with a thermocouple placed directly in the cell.

TABLE I
Copolymerization characteristics of poly(*N*-ethylacrylamide-*co-II*) (*III*) and poly(*N*-ethylmethacrylamide-*co-II*) (*IV*)

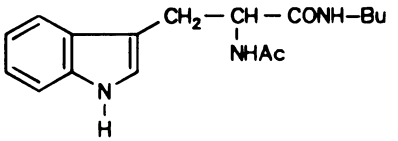
Copolymer	*II* Content, mole %		$[\eta]^a$ cm^3 g^{-1}	M. w.
	in feed	in copolymer		
III	1.5	2.7	27	65 000[b]
IV	1.2	2.0	18	60 000[c]

[a] In water at 25 °C; [b] M_w, by light scattering; [c] from the Mark–Houwink equation[12], $[\eta] = 2.42 \cdot 10^{-3} M_v^{0.81}$.

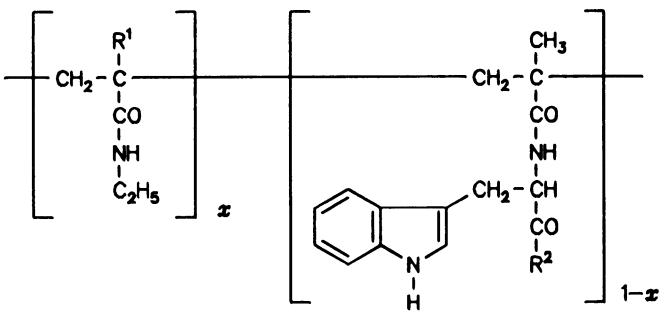
Fluorescence polarization P was calculated as

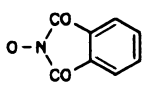
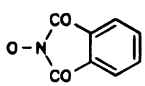
$$P = \frac{I_{VV} - G I_{VH}}{I_{VV} + G I_{VH}}, \tag{7}$$

where I_{VV} is the emission intensity corresponding to vertically polarized excitation and vertically polarized emission and I_{VH} is the emission intensity corresponding to vertically polarized excitation and



I



	R^1	R^2
III	H	
IV	CH_3	
V	H	$NH(CH_2)_7CH_3$
VI	CH_3	$NH(CH_2)_7CH_3$
VII	CH_3	$NH(CH_2)_nNHDns$

horizontally polarized emission. The factor G is the sensitivity ratio of the detection system to vertically and horizontally polarized light[13,14]. The factor G ($G = I_{HV}/I_{HH}$) was determined in methanol using N^{α}-acetyl-DL-tryptophan, carbazole and Dns–NH–$(CH_2)_2$–NH_2 at 30 °C (G_{350} = 1.08, G_{520} = 0.89). Dansyl fluorophore was excited at 338 nm and its emission was recorded at 520 nm. Tryptophan fluorophore was excited at 282 nm and its emission was recorded at 350 nm (slits 10 nm). The measurements were carried out at 20, 30, and 50 °C and the viscosity of the medium was controlled by varying the composition of the mixed solvent glycerol–water[15].

Measurement of excited-state lifetime. The lifetimes of the excited states of dansyl and tryptophan fluorophores were determined by the pulse technique. A free running discharge lamp was the source of excitation radiation. Excitation and emission wavelengths for both fluorophores were the same as in measurement of fluorescence polarization. The interference filters used were Corning 7-54 Dell Optics (337 and 282 nm) for excitation, Dell Optics (350 and 520 nm) for emission and two gelatin-type sharp cut filters. Detection was performed with a single photon counting system Ortec. The decay curves were analyzed by the least-square deconvolution.

RESULTS AND DISCUSSION

Actual Lifetime of Excited States

Actual lifetimes of tryptophan fluorophore in low-molecular-weight model *I* and in copolymers *V* and *VI* are approximately the same in methanol, water and aqueous glycerol. The fluorescence decay was described by double exponential function with nanosecond (τ_1) and subnanosecond (τ_2) components. The latter ($\tau_2 = 0.50 \pm 0.15$ ns) contributes to the tryptophan fluorescence with less than 5% of the total emission intensity and therefore only the values τ_1 are summarized in Table II. The measured values are in good agreement with reported data[16,17].

Actual lifetimes of dansyl fluorophore in copolymers *VII* (Table III) in methanol are approximately the same and the fluorescence decay was described by single exponential function. Actual lifetimes of dansyl fluorophore in water and in aqueous glycerol are also approximately the same but longer than in methanol. The fluorescence decay in this case was described by double exponential function. Only the τ_1 values are sum-

TABLE II
Actual excited-state lifetimes (τ_1, ns) of the tryptophan fluorophores in model *I* and copolymers *V* and *VI* in various media

Model or copolymer	Methanol	Water	Aqueous glycerol	
			15 wt.%	87 wt.%
I	3.3	3.1	3.0	3.3
V	3.6	3.4	3.4	3.6
VI	3.8	3.3	3.6	3.7

marized in Table III because the contributions of the component τ_2 were negligible. The measured values are in good agreement with published data[18 - 20].

Rotational Diffusion of Tryptophan Fluorophore

The mobility of tryptophan fluorophore in model compound *I* was investigated by using the fluorescence depolarization method. Fast Brownian rotation of the tryptophan structure unit in *I* brings about an almost total fluorescence depolarization of tryptophan fluorophore for $T/\eta > 70$ K mPa^{-1} s^{-1}. From the dependence $1/P = 1/P(T/\eta)$, the value $1/P_0 = 5.0$ was determined for tryptophan fluorophore. From the equation $P_0 = 3\cos^2\delta - 1/(\cos^2\delta + 3)$ we can calculate the angle δ between absorption and emission transition moments for a completely retarded fluorescent group as $\delta = 41°$. In the literature[5], $1/P_{n0} = 7.8$ was determined for L-tryptophan using excitation with natural light. Our result is in good agreement with the recalculated value for polarized excitation $1/P_0 = 4.4$; $[P_{n0} = P_0/(2 - P_0)]$. The experimental curve $Y = Y(T/\eta)$ is shown in Fig. 1. The dependence is in a broad range of viscosities ($T/\eta = 3 - 60$ mPa s) linear and only for very high viscosities it is curved. The isotherms for 20, 30 and 50 °C are identical within the

TABLE III
Actual excited-state lifetimes (τ_1, ns) of the dansyl fluorophores in copolymers *VII* in various media

n	Methanol	Water	Aqueous glycerol 75 wt.%
2	12.7	14.1	14.7
6	12.3	14.3	14.0
12	12.1	13.9	13.9

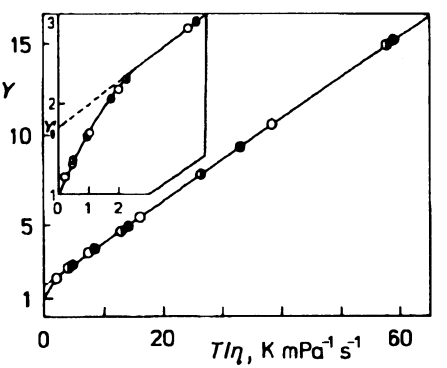


FIG. 1
The dependence $Y = Y(T/\eta)$ for the tryptophan fluorophore in low-molecular-weight model *I* (○ 293.1 K, ◑ 303.1 K, ● 323.1 K)

accuracy limits of the method. This means[4] that we cannot evaluate the height of potential barrier of internal rotation of fluorophore in model *I*. In our previous report[1] we have found the potential barrier of rotation of dansyl fluorophore in homologous Ac–Trp–NH–$(CH_2)_n$–NH–Dns, ΔU_r = 13, 31, 38, and 52 kJ mol^{-1} for n = 2 – 7, 8, 10, and 12, respectively. In comparison with these values the activation energy of viscous flow for 54.1 wt.% aqueous glycerol was ΔU_v = 27 kJ mol^{-1} and the rotational relaxation times σ_2 followed the Arrhenius-type equation

$$\sigma_2 = A\,(\eta/T)\exp[(\Delta U_r + \Delta U_v)/RT]\,. \qquad (8)$$

It seems that the behaviour of model compound *I* is more similar to the behaviour of a sphere in viscous medium than in the case of models which contain both the fluorophores. The mobility of tryptophan fluorophore in model *I* is probably not substantially affected by a relatively small rest of the molecule.

In addition to the study of the tryptophan fluorophore in model *I*, also the mobility of the same fluorophore in side chains of copolymers *V* and *VI* was investigated. The experimental curves $Y = Y(T/\eta)$ are shown in Figs 2 and 3. The dependences are in a broad range of viscosities (T/η = 20 – 250 K mPa^{-1} s^{-1}) linear and are curved only for very high viscosities. Fast Brownian rotation of tryptophan structural units in both copolymers brings about an almost total fluorescence depolarization of emitted radiation for T/η > 300 K mPa^{-1} s^{-1}. The measurements of copolymer *V* were performed at 20, 30 and 50 °C and the isotherms were identical. The measurements of copolymer *VI* were performed at 20 and 30 °C (the isotherms were identical); at 50 °C the copolymer in cuvette precipitated and the results were confusing (polymer with a lower critical solution temperature). From the dependence $Y = Y(T/\eta)$ the average characteristics of the rotational relaxation spectrum (the two-time approximation) were calculated (Table IV).

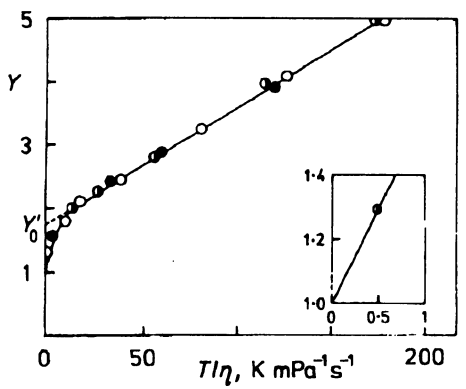


FIG. 2
The dependence $Y = Y(T/\eta)$ for the tryptophan fluorophore in the side chain of copolymer *V* (○ 293.1 K, ◑ 303.1 K, ● 323.1 K)

The average values σ_1 were calculated from the initial slope of the function $Y = Y(T/\eta)$ up to $T/\eta = 2.0$ K mPa^{-1} s^{-1}. The average values σ_2 were obtained from the slope of the linear part of the dependence $Y = Y(T/\eta)$; the Y_0' value was determined by extrapolating this linear dependence to $(T/\eta) \rightarrow 0$.

From the values in Table IV it can be seen that the tryptophan fluorophore in side chains of copolymer *V* exhibits a higher mobility than that in side chains of copolymer *VI*. The mobility of fluorescent label in copolymers *V* and *VI* significantly decreases in comparison with the label in low-molecular-weight model *I* (σ_2= 1.8 ns at T/η = 40 K mPa^{-1} s^{-1} was calculated; τ = 3.2 ns, from Fig. 1). Similarly to the model *I*, we could not evaluate the potential barrier of rotation of tryptophan fluorophore in copolymers *V* and *VI*.

Using stereochemical models Finkelstein[21] studied the internal rotation of side groups of polypeptide chains. For the tryptophan units it was found that the main hindrance of rotation around the $C_\alpha - C_\beta$ and the $C_\beta - C_\gamma$ bonds is steric repulsion of the indole rings and polypeptide backbone. On the basis of this analysis and from published

TABLE IV
Average characteristics of the rotational relaxation spectra of tryptophan fluorophores in copolymers *V* and *VI* (in the two-time approximation)

Copolymer	τ, ns	T/η = 2.0 K mPa^{-1} s^{-1}		T/η = 200 K mPa^{-1} s^{-1}			
		Y	σ_1, ns	Y_0'	Y	f	σ_2, ns
V	3.5	1.70	15	1.75	5.40	0.43	5.0
VI	3.6	1.40	27	1.45	4.30	0.31	5.5

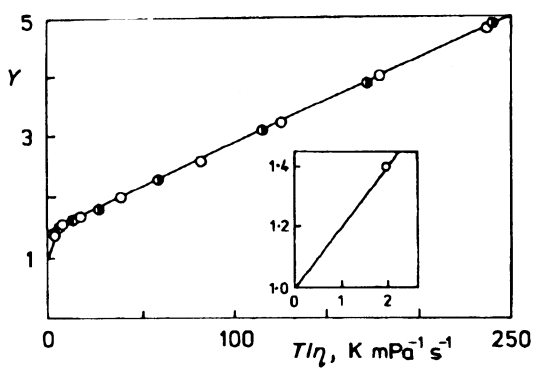


FIG. 3
The dependence $Y = Y(T/\eta)$ for the tryptophan fluorophore in the side chain of copolymer *VI* (○ 293.1 K, ◑ 303.1 K)

experimental data Semisotnov et al.[5] estimated the potential barrier of rotation of tryptophan fluorophore in the copolymer of tryptophan with glutamine (ΔU_r = 8.37 kJ mol^{-1}). They did not try, however, to calculate this value from their own experimental data. The potential barriers of rotation around the $C_\alpha - C_\beta$ and the $C_\beta - C_\gamma$ bonds in tryptophan are also discussed elsewhere[22].

From the examination of space-filling models of copolymers *V* and *VI* it was concluded that no conformational changes of the side chain with tryptophan unit located near the polymer backbone are possible. From this point of view, the main contribution to the mobility of tryptophan is the flexibility of the chain segments to which the fluorophore is attached. A similar behaviour, i.e. the absence of potential barrier of rotation was observed in the case of fluorescence label placed in the backbone of poly(methyl methacrylates) and poly(alkyl acrylates) with a long side chain[23,24]. This observation led to the conclusion that it is possible to describe the motion of the backbone in these polymers by cooperative continual mechanism of motion along the equipotential planes in conformational space of the chain. This means that the motion of relatively long segments of the chain is involved and this motion does not depend on rotational barriers of the backbone but it depends on medium viscosity. This conclusion is valid for the segmental motions within a time range $\sigma \approx 10^{-9} - 10^{-8}$ s. We assume a similar mechanism of relaxation behaviour in copolymers *V* and *VI*. This quasi-continuous motion of the polymer backbone is probably caused by the suppression of the rotational isomerism owing to the interaction of side chain methylene groups and polymer backbone units.

On the other hand, we must admit that, due to the limited accuracy of the used method, we cannot evaluate ΔU_r less than 10 kJ mol^{-1} using the described experimental arrangement.

Rotational Diffusion of Dansyl Fluorophore

The mobility of 5-dimethylamino-1-naphthalenesulfonamide (dansyl) fluorophore in the side chains of copolymer *VII* was investigated. From the dependence of $1/P$ on T/η we were able to confirm the known value of $1/P_0 = 2.8$ for the dansyl fluorophore. The angle between absorption and emission transition moments for completely retarded fluorescent group was calculated: $\delta = 28°$. The average characteristics of the rotational relaxation spectrum (the two-time approximation) at 20 °C can be seen in Table V. The average values σ_1, σ_2 and Y_0' were determined in a similar way as above. The experimental curves $Y = Y(T/\eta)$ at 20 °C are drawn in Fig. 4. The dependences are linear in the range of T/η 80 – 300 (400) K mPa^{-1} s^{-1}; the linear region of the curves increases with decreasing number of methylene groups in the side chain of copolymer *VII*.

The rotational relaxation time of diffusion of macromolecule as a whole (σ_m), calculated from Eq. (5), is approximately 860 ns. In view of the fact that the time σ_m is large compared with the measured σ_2, the latter approximately equals σ_i. Side chains of dif-

ferent length carrying the tryptophan and dansyl fluorophores were bound to the same polymer backbone having the same molar mass. At a given temperature the polymer backbone affects the mobility of the fluorophore by a constant contribution, independent of the number of methylene groups in the side chain, since the side chains lie sufficiently far apart and the measured solution is dilute (the copolymer concentration is 1 – 2 g l^{-1}). Hence, the differences in the σ_2 values given in Table V really reflect the mobility of different side chains.

TABLE V
Average characteristics of the rotational relaxation spectra of the dansyl fluorophores[a] in copolymers *VII* at 293.1 K (in the two-time approximation)

n	T/η = 7.51 KmPa^{-1} s^{-1}		T/η = 300 K mPa^{-1} s^{-1}			
	Y	σ_1, ns	Y_0'	*Y*	*f*	σ_2, ns
2	1.26	162	1.3	2.8	0.23	36
3	1.32	131	1.5	3.1	0.33	39
4	1.38	111	1.5	3.1	0.33	39
5	1.42	100	1.5	3.1	0.33	39
6	1.48	88	1.7	3.6	0.41	38
7	1.74	57	2.3	4.7	0.57	40
8	2.42	30	5.6	8.5	0.82	81
10	4.18	13	6.8	8.5	0.85	168
12	5.56	9	6.9	8.0	0.86	263

[a] τ = 14 ns.

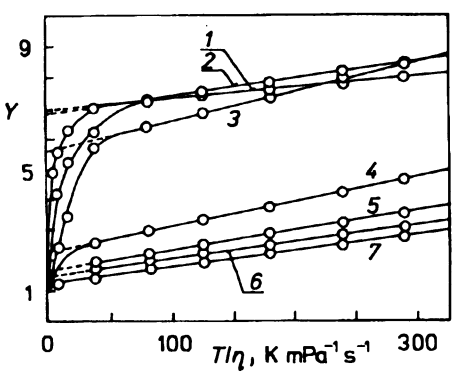


FIG. 4
The dependence $Y = Y(T/\eta)$ for the dansyl fluorophore in the side chain of copolymer *VII* (T = 293.1 K). The number of methylene groups in side chain, *n*: *1* 12; *2* 10; *3* 8; *4* 7; *5* 6; *6* 3, 4, 5; *7* 2

Similarly[2] to poly(*N*-ethylacrylamide) and poly[*N*-(2-hydroxypropyl)methacrylamide] the values of σ_2 in *VII* did not change significantly for $n = 2 - 7$. On the other hand, in *VII* with $n = 8$, 10, and 12 the mobility of the fluorophore was considerably limited. With increasing number of methylene groups in the side chain its hydrophobic character increases and the essentially aqueous medium in the low-viscosity range is a thermodynamically poor solvent for the methylene chain. The drop in the rate of conformational changes is probably due to intramolecular association of long hydrophobic methylene chains in the polar medium[1,2]. In the high-viscosity range it is assumed that the motion of segments of the polymer backbone can be neglected during the lifetime of the fluorophore excited state, while in side chains only torsional vibrations of single bonds within the rotational isomeric states are operative. As a result, the value f of the fraction of "fast" processes of the two-time approximation increases and the value σ_1 decreases with increasing length of the side chain (Table V).

The total mobility of the side chains due to both torsional vibrations and conformational changes of the side chain increases with increasing number of methylene groups in the side chain. At 30 °C and particularly at 50 °C precipitation of polymer takes place in a similar way as in the case of other polymers with lower critical solution temperature such as poly(*N*-isopropylacrylamide). Owing to the polymer precipitation we could not obtain reliable experimental data for the calculation of average characteristics of the rotational relaxation spectrum and, consequently, of the values ΔU_r, $\Delta H^{\neq}$ and $\Delta S^{\neq}$.

CONCLUSIONS

1. The determination of the actual excited-state lifetime of fluorophores made it possible to use the dependence $Y = Y(T/\eta)$ for analysis of experimental data.

2. In the low-viscosity range the molecule of model *I* behaves like a spherical particle with dominant influence of the indole moiety.

3. The mobility of tryptophan fluorophore in copolymers *V* and *VI* significantly decreases in comparison with that in low-molecular-weight model *I*. The decrease is obviously caused by the attachment of tryptophan to polymer backbone.

4. The tryptophan fluorophore in side chain of copolymer *V* exhibits a higher mobility than that in side chain of copolymer *VI* which can be explained by different steric hindrance of the polymer backbones.

5. The height of potential barrier of rotation of tryptophan fluorophore in model *I* and copolymers *V* and *VI* could not be evaluated. This problem will be studied in future by means of time-resolved fluorescence anisotropy measurements.

6. The mobility of dansyl fluorophore in copolymers *VII* in the low-viscosity range is approximately the same for short side chains but significantly decreases for long side chains. The drop in the mobility is probably caused by intramolecular association of long hydrophobic methylene chains in the polar medium. In the high-viscosity range,

the fraction of "fast" processes in the two-time approximation increases with increasing length of the side chain.

REFERENCES

1. Mikeš F., Vyprachtický D., Szabo A. G., Labský J., Wakayama N.: Macromolecules, submitted.
2. Vyprachtický D., Mikeš F., Szabo A. G., Wakayama N., Labský J.: Macromolecules, submitted.
3. Weber G.: Biochem. J. *51*, 145 (1952).
4. Anufrieva E. V., Gotlib Yu. Ya.: Adv. Polym. Sci. *40*, 1 (1981).
5. Semisotnov G. V., Zikherman K. Kh., Kasatkin S. B., Ptitsyn O. B., Anufrieva E. V.: Biopolymers *20*, 2287 (1981).
6. Dale R. E., Eisinger J., Blumberg W. E.: Ann. N. Y. Acad. Sci. *366*, 155 (1981).
7. Vyprachtický D., Mikeš F., Hoang Lam, Sanyová J.: Unpublished results.
8. Zerner B., Bond R. P. M., Bender M. L.: J. Am. Chem. Soc. *86*, 3674 (1964).
9. Fréchet J. M. J., Mikeš F., Vyprachtický D., Hoang Lam, Labský J.: Makromol. Chem. *189*, 671 (1988).
10. Ulbrich K., Kopeček J.: Makromol. Chem. *178*, 2159 (1977).
11. Nilson J. L. G., Stenberg P., Ljunggren Ch.: Acta Pharm. Suec. *8*, 497 (1971).
12. Bohdanecký M.: Private communication.
13. Azumi T., McGlynn S. P.: J. Chem. Phys. *37*, 413 (1962).
14. Chen R. T.: Science *147*, 729 (1965).
15. Landolt H. H., Börnstein R.: *Physikalisch-chemische Tabellen*, 6th ed., Vol. II/Va. Springer, Berlin 1950.
16. Ghiggino K. P., Roberts A. J., Phillips D.: Adv. Polym. Sci. *40*, 93, 136 (1981).
17. Lakowicz J. R.: *Principles of Fluorescence Spectroscopy*, p. 363. Plenum Press, New York – London 1984.
18. Ying-Hsia Li, Lia-Man Chan, Tyer L., Moody R. T., Himel Ch. M., Hercules D. M.: J. Am. Chem. Soc. 97, 3118 (1975).
19. Gill III T. J., Ladoulis Ch. T., Kunz H. W., King M.: Biochemistry *11*, 2644 (1972).
20. Conrad R. H., Brand L.: Biochemistry *7*, 777 (1968).
21. Finkelstein A. V.: Mol. Biol. (Moscow) *10*, 507 (1976).
22. Szabo A. G., Rayner D. M.: J. Am. Chem. Soc. *102*, 544 (1980).
23. Gotlib Yu. Ya., Darinskii A. A.: Vysokomol. Soedin., A *12*, 2263 (1970).
24. Anufrieva E. V., Pautov V. D., Freibzon Ya. S., Shibaev V. P., Plate N. A.: Vysokomol. Soedin., A *17*, 586 (1975).

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