FLUORESCENCE ANISOTROPY AND MOBILITY OF DANSYL FLUOROPHORE IN LABELLED HOMOLOGOUS ALKANES

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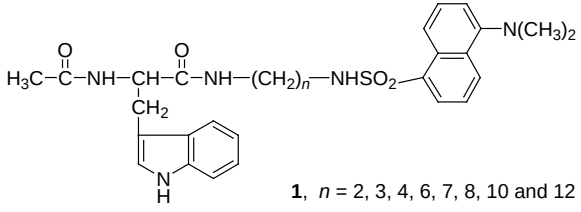
Using the steady-state and time-resolved fluorescence anisotropy, the mobility of 5-(dimethylamino)naphthalene-1-sulfonyl (dansyl) fluorophore in homologous 1-[2-acetamido-3-(1*H*-indol-3-yl)propanamido]-*n*-[5-(dimethylamino)naphthalene-1-sulfonamido]alkanes **1** was studied in binary solvents glycerol–water. Steady-state fluorescence data were evaluated by the generalized Perrin equation and the micro-Brownian motion of dansyl fluorophore was described by means of average characteristics (rotational relaxation times) of the rotational relaxation spectrum. The rotational relaxation time of "fast" motions caused by torsional vibrations of single bonds within the rotational-isomeric states decreases with increasing number of methylene groups in homologous compounds. The rotational relaxation time of "slow" motions due to conformational changes of the chain between the tryptophane and dansyl fluorophore remains at first approximately constant with increasing number of methylene groups but increases considerably for long aliphatic chains. The observed decrease in the rate of conformational changes of a long aliphatic chain is probably due to intramolecular interaction of parts of the methylene chain in a medium with high water content. The values of activation enthalpy $\Delta H^{\neq}$ and activation entropy $\Delta S^{\neq}$ calculated from experimental data corroborate such interpretation. Time-resolved anisotropy of dansyl fluorophore at a particular binary solvent composition confirmed the shape of rotational relaxation spectrum and the measured rotational correlation times have been discussed. The time-dependent decays of anisotropy supported our previous interpretation in terms of intramolecular association of the long aliphatic chain in polar medium.

Key words: Fluorescence anisotropy; Dansyl fluorophore; Time-resolved fluorescence; Rotational correlation time; Micro-Brownian motion; Fluorescence spectroscopy.

Fluorescence anisotropy (depolarization) may provide valuable information on the structure and dynamic properties not only of synthetic and biological macromolecules[1–3], but also of low-molecular-weight compounds[4]. This study has been motivated by our previous investigation[5,6] of the rate of enzymatically (chymotrypsin) catalyzed hydrolysis of peptide bond placed at

the ends of side chains of varying lengths in copolymers of the methacrylamide type. The decreasing rate of hydrolysis of substrates with long aliphatic chains was interpreted by an intramolecular association of the side chain. According to the generally accepted mechanism[7] of enzymatically catalyzed hydrolysis, the rate constant of formation of the necessary enzyme–substrate complex is affected, among other factors, by a change in the conformation[8] of the substrate with respect to the active site of the enzyme. The intramolecular association of the side chain in copolymers of the methacrylamide type causes a change in its hydrodynamic volume. Therefore, different mobilities of the dansyl fluorophore were found out, depending on the length of the side chain[9]. To estimate the effect of attachment of the side chain to a polymer backbone, the mobility was measured of dansyl fluorophore in low-molecular-weight homologous compounds **1**, modelling the side chains of copolymers containing an enzymatically hydrolyzable peptide bond. Both steady-state and time-resolved fluorescence techniques were applied to estimate the micro-Brownian motion of dansyl fluorophore in compounds **1**.



1, *n* = 2, 3, 4, 6, 7, 8, 10 and 12

Rotational diffusion of fluorophores is a dominant cause of fluorescence depolarization, which is for a spherical molecule described by the Perrin equation[3]. Analysis of the micro-Brownian motion and the theory of relaxation phenomena in polymers show that the Brownian rotation of a fluorescent label covalently bound to the chain sometimes obeys a more complicated time dependence than in the case of free spherical particle. The considerations led to the so-called generalized Perrin equation[1,10–13]

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

where Y is a reduced fluorescence polarization ($Y = (1/P - 1/3)/(1/P_0 - 1/3)$), P the fluorescence polarization, P_0 the fluorescence polarization at $T/\eta \to 0$ (T is absolute temperature and η is viscosity coefficient of the medium),

τ the average lifetime of the excited state, f_j the relative content of processes with the rotational relaxation times ρ_j ($\Sigma f_j = 1$) and the time function of fluorophore rotation is expressed by $\langle \cos^2 \alpha(t) \rangle = 1/3 + (2/3)\, \Sigma f_j \exp(-3t/\rho_j)$.

Determination of the shape of rotational relaxation spectrum (all f_j and ρ_j) from the experimental curve $Y = Y(T/\eta)$ is in general a complex mathematical problem. As shown by Weber[2,11], some average characteristics of the rotational relaxation spectrum may be obtained by analyzing the two limiting cases of the dependence $Y = Y(T/\eta)$ at $(T/\eta) \to 0$ and $(T/\eta) \to \infty$. From the slope of the asymptote at $(T/\eta) \to 0$ (where $\tau < \rho_j$), it is possible to obtain the reciprocal average rotational relaxation time ρ_{-1}

$$\frac{\mathrm{d}Y}{\mathrm{d}(T/\eta)_{T/\eta \to 0}} = (3\eta\tau/T)\sum_j f_j/\rho_j = (3\eta\tau/T)\langle 1/\rho \rangle \equiv (3\eta\tau/T)(1/\rho_{-1}) \,. \quad (2)$$

From the slope of the asymptote at $(T/\eta) \to \infty$ (where $\tau > \rho_j$), it is possible to obtain the number average of the rotational relaxation times of the spectrum ρ_n, the intercept of this asymptote being Y_0' (or $1/P_0'$ for the plot $1/P = 1/P(T/\eta)$). Thus, we have

$$\frac{\mathrm{d}Y}{\mathrm{d}(T/\eta)_{T/\eta \to \infty}} = (3\eta\tau/T)\left(\sum_j f_j \rho_j\right)^{-1} = (3\eta\tau/T)\, 1/\langle \rho \rangle \equiv 3\eta\tau/T\rho_n \quad (3)$$

$$Y_0' = \frac{1/P_0' - 1/3}{1/P_0 - 1/3} = \frac{\sum f_j \rho_j^2}{\left(\sum_j f_j \rho_j\right)^2} = \frac{\langle \rho^2 \rangle}{\langle \rho \rangle^2} \equiv \rho_w/\rho_n \,, \quad (4)$$

where ρ_w is the weight average of the rotational relaxation times of the spectrum

$$\rho_w = \left[\sum_j f_j \rho_j^2\right] / \left[\sum_j f_j \rho_j\right] = \langle \rho^2 \rangle / \langle \rho \rangle \quad (5)$$

and Y_0' is the measure of the distribution width of the time spectrum valid for polarized excitation. The rotational relaxation spectrum may be divided into "fast processes" with the average relaxation time ρ' and into "slow processes" with the average relaxation time ρ'' ($\rho'' >> \rho'$). Equation (*1*) may be then written in a two-time approximation[1,13,14] as

$$Y = \left[\frac{f}{1 + 3\tau / \rho'} + \frac{1 - f}{1 + 3\tau / \rho''} \right]^{-1} , \qquad (6)$$

where f is the total contribution of the "fast processes" to the decrease in the fluorescence polarization. In the region of low viscosities ($\rho' << \tau$), Eq. (*6*) becomes

$$Y(1 - f) = 1 + 3\tau / \rho'' . \qquad (7)$$

In the region of high viscosities ($\rho'' >> \tau$), the "slow processes" are not operative during the fluorescence lifetime τ. Equation (*6*) then becomes

$$\frac{Y - 1}{1 - (1 - f)Y} = 3\tau / \rho' . \qquad (8)$$

An investigation of the dependences $Y = Y(T/\eta)$ and $1/P = 1/P(T/\eta)$ in the region of low viscosities allows the average relaxation time of the "slow processes", ρ'', and the overall contribution of the "fast processes", f, to be determined. An investigation carried out at high viscosities makes it possible to determine the average relaxation time of the "fast processes", ρ'.

The time-resolved decays of fluorescence anisotropy, $r(t)$, provide considerable additional information about the diffusive motions of the fluorophore. These data can reveal whether a fluorophore is free to rotate over all angles, or if the environment surrounding the fluorophore restricts its angular displacement. In addition, these measurements can reveal the anisotropy decays as a result of a single rotation, or whether several rotational processes are present.

EXPERIMENTAL

Materials

N-Acetyl-DL-tryptophane (Ac-Trp) was prepared by reacting DL-tryptophane with acetic anhydride according to Berg *et al.*[15,16]. M.p. 206–207 °C. For $C_{13}H_{14}N_2O_3$ (246.3) calculated: 63.41% C, 5.69% H, 11.38% N; found: 63.59% C, 5.61% H, 11.60% N. UV (methanol): λ_{max} 282 nm, ε_{282} 5 590 l mol^{-1} cm^{-1}.

N-Acetyl-DL-tryptophane 4-nitrophenyl ester (Ac-Trp-ONp) was prepared by esterification of Ac-Trp with 4-nitrophenol using dicyclohexylcarbodiimide (modified procedure[17]). M.p. 136 °C. For $C_{19}H_{17}N_3O_5$ (367.4) calculated: 62.12% C, 4.67% H, 11.44% N; found: 62.21% C, 4.84% H, 11.51% N. UV (methanol): λ_{max} 282 nm, ε_{282} 14 700 l mol^{-1} cm^{-1}.

Aliphatic diamines, $H_2N(CH_2)_nNH_2$, n = 2, 3, 4, 6, 7, 8, 10 and 12, were purchased from Aldrich, Fluka AG and Koch-Light Laboratories.

5-(Dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride, DNS-Cl) was a commercial product (Fluka AG).

N-(ω-Aminoalkyl)-5-(dimethylamino)naphthalene-1-sulfonamides (dansyl amines, DNS-NH$(CH_2)_nNH_2$), were prepared by a reaction of DNS-Cl with aliphatic diamines $H_2N(CH_2)_nNH_2$ according to published procedures[18,19]. UV (methanol) were the same for all: λ_{max} 282 nm, ε_{282} 1 560 l mol^{-1} cm^{-1}; λ_{max} 338 nm, ε_{338} 4 400 l mol^{-1} cm^{-1}.

1-[2-Acetamido-3-(1H-indol-3-yl)propanamido]-n-[5-(dimethylamino)naphthalene-1-sulfonamido]alkanes, Ac-Trp-NH$(CH_2)_nNH$-DNS, **1**, were prepared by aminolysis of Ac-Trp-ONp with dansyl amines DNS-NH$(CH_2)_nNH_2$. An equimolar mixture of Ac-Trp-ONp and dansyl amine in acetone or ethyl acetate (n = 2, 7) was heated to 50 °C (12 h). The solvent was evaporated and the residue was dissolved in chloroform. The solution was washed with a saturated aqueous solution of sodium hydrogencarbonate and water. The product was chromatographed on silica gel and crystallized from ethyl acetate. Melting points were 174, 198, 71, 72.5, 69, 68, 63 and 60 °C for n = 2, 3, 4, 6, 7, 8, 10 and 12, respectively. The structure of the compounds was confirmed by ^{1}H NMR spectra (Varian XL-100) and IR spectra (Perkin–Elmer 325). Calculated and found values of elemental analysis for C, H, N and S were in very good agreement for all the prepared **1**. All compounds were homogeneous according to TLC.

Glycerol, 99.5% spectrophotometric grade (Aldrich), was used without further purification.

Methods and Procedures

The steady-state fluorescence polarization was measured with a Hitachi Perkin–Elmer MPF-2A spectrophotometer in L-format. The temperature of solutions in a quartz cell (1 × 1 × 4 cm) was recorded with a thermocouple placed directly in the cell. The fluorescence polarization P was calculated as

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}, \qquad (9)$$

where I is the steady-state emission intensity and the subscripts V, H refer to vertical and horizontal set up of the polarizers, respectively. The sensitivity factor G ($G = I_{HV}/I_{HH}$) was determined[3,20,21] in methanol with DNS-NH$(CH_2)_2NH_2$ at 30 °C (G_{520} = 0.89). Dansyl fluorophore was excited at 338 nm and its emission was recorded at 520 nm. The measurements were carried out under isothermal conditions at three temperatures (20, 30 and 50 °C) and the viscosity of the medium was controlled by varying the composition of the binary solvent, glycerol–water[22]. The concentration of homologous compounds **1** was 10^{-5}–10^{-4} mol l^{-1}.

The fluorescence lifetime of dansyl fluorophore was measured with a time-resolved fluorimeter FL 900 CDT (Edinburgh Analytical Instruments, Edinburgh, U.K.) using described procedure[23]. The decay curves were analyzed by deconvolution of the lamp pulse with the impulse response of the sample and were single-exponential. The fluorescence lifetime (τ) of dansyl fluorophore in compounds **1** was found to be nearly independent of the composition of the binary solvent, glycerol–water (Table I). The average value τ = 14 ns was used in the calculations.

The time-resolved fluorescence anisotropy, $r(t)$, was calculated point by point using the equation

$$r(t) = \frac{GI_{VV}(t) - I_{VH}(t)}{GI_{VV}(t) + 2I_{VH}(t)}, \qquad (10)$$

where $I(t)$ is the time-resolved emission intensity and $G = \Sigma I_{HH}(t)/\Sigma I_{HV}(t)$. The subscripts V, H have the same meaning as in Eq. (*9*). The emission anisotropy was analyzed using the FL900CDT software with experimental function

$$r(t) = r_\infty + \sum B_i \exp\left(-t / \Phi_i\right) \qquad (11)$$

without deconvolution, where B_i is the preexponential factor representing the fractional contribution of the component with rotational correlation time Φ_i to the anisotropy decay $r(t)$ (rel $B_i = (B_i\Phi_i/\Sigma B_i\Phi_i) \times 100\%$), r_∞ is the limiting anisotropy and t is time. Least-squares analysis of time-resolved decay was used for estimation of $r(t)$. The goodness of fit (χ^2) was calculated from

$$\chi^2 = \sum_t \frac{\left[r(t) - r_c(t)\right]^2}{r(t)}, \qquad (12)$$

where $r_c(t)$ is the calculated decay of anisotropy. Excitation of the samples was carried out at 338 nm with a pulse lamp nF 900 controlled by a thyratron tube having a repetition frequency 40 kHz. The lamp was filled with hydrogen (>99.995%) at 50 kPa. The intensity level of the emission at 520 nm was adjusted so that 800 fluorescence photons or less were observed per second. The time-resolved anisotropy measurements were performed in T-format at 30 °C.

UV-VIS spectra were taken on a Hewlett–Packard 8451A spectrophotometer.

TABLE I
Fluorescence lifetimes (τ) of dansyl fluorophore in compounds **1** measured in aqueous glycerol at 30 °C (ex = 338 nm, em = 520 nm)

Aqueous glycerol wt.%	τ, ns		
	n = 4	*n* = 6	*n* = 10
99.5	14.2	14.0	13.9
90.0	14.1	13.7	13.8
80.0	13.8	14.5	14.3
70.0	13.6	14.1	14.4
55.0	14.1	13.8	13.9

RESULTS AND DISCUSSION

Steady-State Fluorescence Depolarization Study

The fluorescence polarization (Eq. (*9*)) of dansyl fluorophore in compounds **1** was measured as a function of T/η. For $T/\eta > 60$ K mPa^{-1} s^{-1}, the fast Brownian rotation of the dansyl structure unit brings about an almost total fluorescence depolarization. From the dependence $1/P = 1/P(T/\eta)$, the value $1/P_0 = 2.80$ was determined for dansyl fluorophore in agreement with the published[11,24] results. Experimental curves $Y = Y(T/\eta)$ (Figs 1 and 2 represent typical data) are linear in the low-viscosity range ($T/\eta \approx 10$–50 K mPa^{-1} s^{-1})

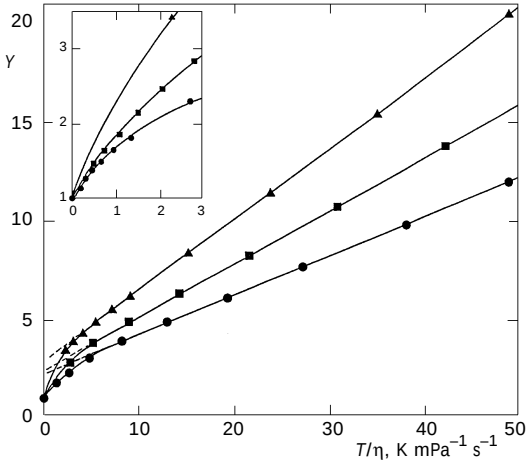


FIG. 1
Steady-state fluorescence depolarization, $Y = Y(T/\eta)$, of dansyl fluorophore in compound $n = 6$ at three temperatures (● 293.15 K, ■ 303.15 K, ▲ 323.15 K) and $1/P_0 = 2.8$

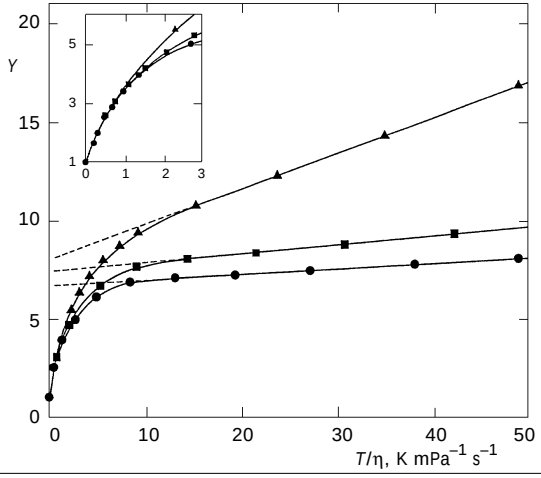


FIG. 2
Steady-state fluorescence depolarization, $Y = Y(T/\eta)$, of dansyl fluorophore in compound $n = 12$ at three temperatures (● 293.15 K, ■ 303.15 K, ▲ 323.15 K) and $1/P_0 = 2.8$

and non-linear in the high-viscosity range ($T/\eta < 10$ K mPa^{-1} s^{-1}). Thus, the studied systems can be accurately described in the whole viscosity range by using the spectrum of rotational relaxation times, although a single value of rotational relaxation time would be sufficient within the observed broad range of linear dependence. The mobility of dansyl fluorophore was therefore characterized by the values ρ', ρ'', ρ_{-1}, ρ_n, ρ_w, Y_0' and f obtained by analysing the experimental curves $Y = Y(T/\eta)$. The average values of ρ_n and ρ_w (= ρ'' here only as a result of linearity) 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 $T/\eta \to 0$ (Eqs (*3*), (*4*) and (*7*)). The values ρ' and ρ_{-1} were calculated from the initial slope of the function $Y = Y(T/\eta)$ (Eqs (*2*) and (*8*)). The measured and calculated average characteristics of the rotational relaxation spectrum for $T/\eta = 0.4$ and 40 K mPa^{-1} s^{-1} are summarized in Table II.

In the high-viscosity range, neither the motion of whole molecules nor a change in the rotational-isomeric states is possible during the fluorophore lifetime. Only torsional vibrations of single bonds within the framework of certain rotational-isomeric states of the molecule occur. With increasing number of methylene groups in the molecule, the mobility of dansyl fluorophore increases (ρ_{-1} decreases), and so does the contribution of the "fast processes" to the fluorescence depolarization. A rise in temperature brings about a decrease in the ρ_{-1} values in the compounds with $n \leq 8$. In the compounds where $n = 10$ or 12, the rise in temperature probably causes a decrease in the ρ_{-1} values but this cannot be experimentally observed in the arrangement employed.

In the low-viscosity range, the motions leading to changes in rotational-isomeric states of the molecule are released and the whole molecule can also move in the medium. The rotational relaxation times ρ_w and ρ_n characterize the rate of conformational changes and the mobility of the molecule as a whole. The latter may be approximately described by a time of rotational diffusion according to the Debye relation. By estimating the molecular volume from the measured distances between the tryptophane and dansyl fluorophores using the nonradiative energy transfer[25], it is possible to calculate the time of rotational diffusion at $T/\eta = 40$ K mPa^{-1} s^{-1}. The calculations show that for $n = 2$ and $n = 12$, the time of rotational diffusion increases approximately two-fold. However, the ρ_w and ρ_n values in Table II indicate that for the same compounds, the ρ_w increases almost thirty times and ρ_n nearly seven times. Hence, in compounds with long methylene chains, the increase in the size of the molecule itself does not bring about the observed increase in the values of ρ_w and ρ_n.

TABLE II
Average characteristics of the rotational relaxation spectrum of dansyl fluorophore in homologous compounds **1**, calculated for average lifetime τ = 14 ns

n	T/η = 0.4 K mPa^{-1} s^{-1}			T/η = 40 K mPa^{-1} s^{-1}				
	Y	ρ'/τ	ρ_{-1}/τ	Y_0'	f	ρ_n/τ	ρ_w/τ	Y
				T = 293.15 K				
2	1.19	4.7	15.8	1.7	0.41	0.5	0.8	7.8
3	1.23	4.6	13.0	1.9	0.47	0.4	0.8	8.7
4	1.28	4.2	10.7	2.1	0.52	0.4	0.9	9.5
6	1.32	4.0	9.4	2.3	0.57	0.4	0.9	10.0
7	1.34	3.9	8.8	2.4	0.58	0.4	1.0	10.0
8	1.39	3.9	7.7	2.8	0.64	0.8	2.3	6.5
10	1.80	2.7	3.8	6.5	0.84	1.9	12.2	8.1
12	2.30	1.5	2.3	6.7	0.85	3.3	22.3	7.6
				T = 303.15 K				
2	1.26	3.9	11.5	1.9	0.47	0.3	0.6	11.1
3	1.31	3.6	9.7	2.1	0.52	0.3	0.7	10.9
4	1.32	3.7	9.4	2.2	0.55	0.3	0.7	12.3
6	1.37	3.7	8.1	2.5	0.60	0.3	0.7	13.0
7	1.39	3.6	7.7	2.6	0.62	0.3	0.8	12.1
8	1.42	3.6	7.1	2.9	0.66	0.4	1.1	10.5
10	1.80	2.8	3.8	6.9	0.85	1.2	8.0	9.5
12	2.30	1.6	2.3	7.4	0.86	1.9	13.9	9.0
				T = 323.15 K				
2	1.32	4.0	9.4	2.3	0.57	0.2	0.6	14.2
3	1.35	3.8	8.6	2.4	0.58	0.2	0.6	15.1
4	1.37	3.7	8.1	2.5	0.60	0.2	0.6	15.5
6	1.42	3.7	7.1	2.9	0.66	0.2	0.6	17.0
7	1.44	3.7	6.8	3.1	0.68	0.2	0.6	18.0
8	1.47	3.6	6.4	3.4	0.71	0.2	0.7	18.9
10	1.80	2.9	3.8	7.5	0.87	0.4	3.1	14.8
12	2.30	1.6	2.3	8.0	0.88	0.4	3.4	15.0

In this case, an effect is operative which is related to the preference of certain rotational-isomeric states, and thus to the conformation of the molecule. The total energy of the conformer may be regarded as the sum of the rotational potential of the bond and of the energy of interaction between nonbonded atoms or groups[26]. The solvent can obviously affect both terms to a considerable extent. In the low-viscosity range, the employed binary solvent, glycerol–water, contains mainly water. In a highly polar medium, the methylene chain is not sufficiently solvated because an essentially aqueous medium is not a thermodynamically good solvent for the hydrophobic aliphatic chains. If only interactions of the first and second order are considered, the size of the molecule greatly depends on the magnitude of the interaction, similarly to synthetic polymers[26]. Since the gauche conformers are energetically favored over the trans conformers in highly polar media, the population of the former in the system will increase under such conditions. In molecules with long methylene chains, n = 8–12, this results in a decrease in the average dimensions of the molecule and in strong intramolecular interactions between methylene groups. Conformations which make intensive intramolecular interactions possible are not very likely to occur in compounds with a small number of methylene groups since the chain is too short to allow the methylene groups to interact. It should be borne in mind that the total mobility of the dansyl fluorophore in the low-viscosity range is given both by torsional vibrations and by changes in rotational-isomeric states.

In the linear range, the potential barrier of rotation of the dansyl fluorophore can be calculated from the temperature dependence of the relaxation time. ρ_w may be expressed as[13,27,28]

$$\rho_w = \rho_0 \exp\left(\Delta U/RT\right), \qquad (13)$$

where $\Delta U = \Delta U_R + \Delta U_V$, ΔU_R is the potential barrier of rotation and ΔU_V is the activation energy of viscous flow. To eliminate the activation energy of viscous flow, the ρ_w/τ values in Table II were recalculated to the same viscosity (η = 7.33 mPa s) and the ΔU_R values were obtained as slopes of the dependences ln (ρ_w/τ) *vs* $1/T$ (Table III). The potential barrier of rotation ΔU_R is much smaller for compounds n = 2–7 than for compounds n = 8–12. The increase in ΔU_R for compounds with long methylene chains is probably due to interactions of methylene groups and is obviously closely related with the mechanism which governs the conformation change of the meth-

ylene chain. ΔU_R can be regarded as the activation energy of transition from the starting conformation into the activated state. To obtain data on conformational changes, the relaxation time ρ_w was also expressed in terms of the theory of absolute reaction rates. Here

$$\rho_w = (h/kT)\exp\left(-\Delta S^{\neq}/R\right)\exp\left(\Delta H^{\neq}/RT\right), \qquad (14)$$

where h is the Planck constant, k is the Boltzmann constant, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are the activation enthalpy and activation entropy, respectively. Values from Table II were recalculated to the same viscosity (η = 7.33 mPa s) and $\Delta H^{\neq}$ values were obtained as slopes of the dependences ln ($\rho_w T/\tau$) *vs* $1/T$. The $\Delta S^{\neq}$ values were calculated for T = 293.15 K and τ = 14 ns (Table III). The $\Delta H^{\neq}$ value, similarly to ΔU_R, describes the energy levels of the starting and transition (activated) conformational states and, due to interactions inside the long methylene chain, increases for $n \geq 8$. The value of $\Delta H^{\neq}$ involves both the energy difference of conformations between the transition and starting states and the magnitude of interactions of methylene groups to be overcome in the conformational transitions. The quantity $\Delta S^{\neq}$ provides information on the difference in the number of conformations between the transition and the starting states. For n = 2–7, $\Delta S^{\neq} < 0$, and thus the number of possible conformations is smaller in the transition than in the starting state. The decrease in entropy suggests that the transition state is better organized. With increasing number of methylene groups in the chain, $n > 8$, the number of conformations of the starting state is restricted due to strong interactions of the methylene chain and the change in the activation entropy becomes positive, $\Delta S^{\neq} > 0$. This fact is mainly caused by a

TABLE III
Calculated values of ΔU_R, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for homologous compounds **1**

n	ΔU_R, kJ mol^{-1}	$\Delta H^{\neq}$, kJ mol^{-1}	$\Delta S^{\neq}$, J K^{-1} mol^{-1}
2–7	13	10	–48
8	31	28	+4
10	38	36	+20
12	52	52	+67

smaller number of possible conformations in the starting state because for any variation in the rotational-isomeric states, a similar situation in the transition state can be expected for models with both a short and a long methylene chain.

Time-Resolved Fluorescence Anisotropy Study

To support and compare the results obtained from the steady-state approach, the time-resolved technique was applied to the systems representing short, $n = 4$, and long, $n = 10$, aliphatic chains. Time-dependent decays of fluorescence anisotropy $r(t)$ of dansyl fluorophore were measured for five characteristic compositions of binary solvent glycerol–water at 30 °C. Experimental data were analyzed (Eqs (*10*)–(*12*)) to obtain the rotational correlation time (Φ) (Table IV), the limiting anisotropy (r_∞) and the fit parameter (χ^2) (Figs 3 and 4 show typical experimental data). Useful and interesting data are obtained when $\tau \approx \Phi$ and rotational diffusion contributes to the time-dependent decay of anisotropy. If $\tau << \Phi$, then the fluorescence intensity has decayed prior to significant loss of anisotropy and, conversely, if the rotational correlation time is very short ($\tau >> \Phi$), then $r(t) = 0$. If we compare the Φ values from Table IV with the average fluorescence lifetime of dansyl fluorophore ($\tau = 14$ ns), we can assume to have reliable data. The rotational correlation time (Φ) of dansyl fluorophore in the compound $n = 4$ decreases with increasing value of T/η, however, the Φ is not inversely proportional to T/η, but the times Φ are somewhat longer in di-

TABLE IV
Rotational correlation times (Φ) of dansyl fluorophore in compounds **1** determined by time-resolved technique in aqueous glycerol at 30 °C (ex = 338 nm, em = 520 nm)

Aqueous glycerol wt.%	T/η K mPa^{-1} s^{-1}	Φ, ns	
		$n = 4$	$n = 10$
99.5	0.50	39.8	32.5
90.0	2.78	9.7	9.3
80.0	8.94	3.8	4.6
70.0	21.5	1.9	9.8
55.0	56.5	1.8	11.7

lute binary solvents. In other words, the rotational diffusion of dansyl fluorophore in the model $n = 4$ is not a simple function of T/η. We have described a similar observation by the steady-state fluorescence depolarization as a spectrum of rotational relaxation times. For the compound $n = 10$, the phenomenon is even more enhanced.

In a high-viscosity solvent (99.5 wt.% glycerol), the Φ is longer in $n = 4$ than that in $n = 10$ because the increasing number of methylene groups in the molecule increases the mobility in certain rotational-isomeric states. As the binary solvent glycerol–water becomes less viscous (T/η increasing, higher content of water), the time Φ of $n = 10$ becomes longer than that of $n = 4$. The viscosity decrease leads to changes in rotational-isomeric states and the more aqueous medium is not a thermodynamically good solvent for the hydrophobic aliphatic chain. Possible intramolecular interactions between methylene groups of a longer compound, $n = 10$, reduces then the rotational diffusion of dansyl fluorophore.

When the rotational motions of a fluorophore are hindered, one finds that the anisotropy does not decay to zero. Such behaviour was observed in 99.5 wt.% glycerol for both $n = 4$ and $n = 10$ as well as in 90 wt.% glycerol for $n = 4$ and the limiting anisotropies (r_∞) were found to be 0.06, 0.02 and 0.03, respectively (Fig. 3). By hindrance, we mean that the angular range of rotational motion is limited and limiting anisotropy is observed at times which are long compared with the fluorescence lifetime. The found limiting anisotropies in highly viscous solvents support our interpretation of

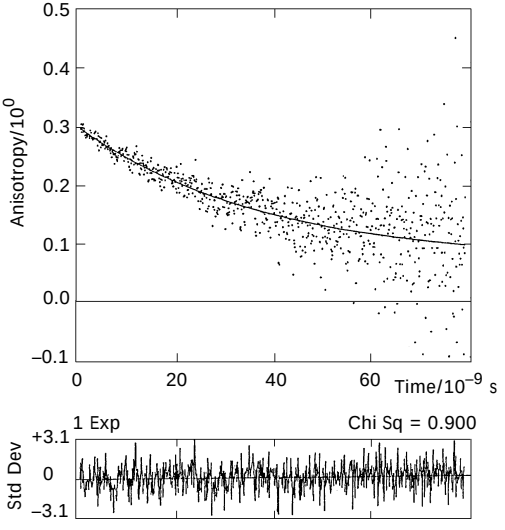


FIG. 3
Time-resolved emission anisotropy, $r(t)$, of dansyl fluorophore in compound $n = 4$ measured in 99.5 wt.% glycerol at 303.15 K (Φ = 39.8 ns, G = 2.56, r_∞ = 0.06, χ^2 = 0.900, B_1 = 0.231)

mobility in certain rotational-isomeric states. Clearly, in less viscous binary solvents, we have found the limiting anisotropy equal to zero (Fig. 4). All the measured decays $r(t)$ were found to be single-exponential with fit parameter $\chi^2 < 1.2$. This was expected in very viscous medium (99.5 wt.% glycerol), where slow processes are impossible and also in less viscous media (< 80 wt.% glycerol), where the fast processes are not operative. We assumed that in the transition area (around 90 wt.% glycerol), we could probably distinguish a double-exponential decay of $r(t)$. The measurements were repeated on different days with several prepared solutions but the results remained the same – single-exponential. We cannot explain this difficulty at present.

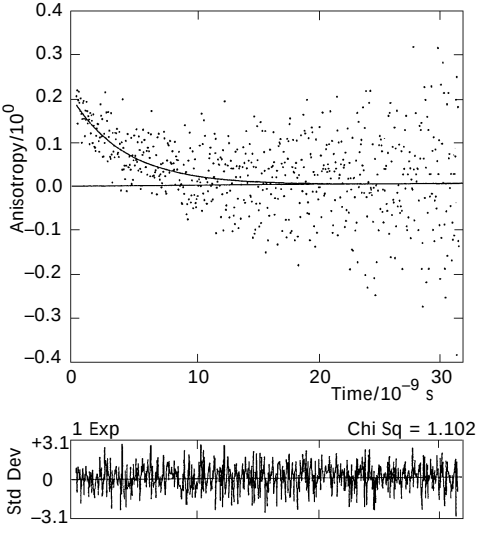


FIG. 4
Time-resolved emission anisotropy, $r(t)$, of dansyl fluorophore in compound n = 10 measured in 80 wt.% glycerol at 303.15 K (Φ = 4.6 ns, G = 2.13, r_∞ = 0.00, χ^2 = 1.102, B_1 = 0.187)

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SYMBOLS

B_i	preexponential factor
f	total contribution of fast processes to fluorescence depolarization
f_j	relative content of processes with rotational relaxation time ρ_j
G	sensitivity factor
h	Planck constant, J s

I	fluorescence intensity, a.u.
k	Boltzmann constant, J K^{-1}
P	fluorescence polarization
P_0	fluorescence polarization in frozen state ($T/\eta \to 0$)
r	fluorescence anisotropy
r_∞	limiting fluorescence anisotropy
R	universal gas constant, J K^{-1} mol^{-1}
T	temperature, K
t	time, s
Y	reduced fluorescence polarization
Y_0'	measure of the distribution width of the time spectrum
$\Delta H^{\neq}$	activation enthalpy, kJ mol^{-1}
$\Delta S^{\neq}$	activation entropy, J K^{-1} mol^{-1}
ΔU_R	potential barrier of rotation, kJ mol^{-1}
ΔU_V	activation energy of viscous flow, kJ mol^{-1}
$\alpha(t)$	time function of fluorophore rotation, °
ε	molar absorption coefficient, l mol^{-1} cm^{-1}
η	viscosity coefficient of the medium, mPa s^{-1}
λ	wavelength, nm
Φ	rotational correlation time, ns
ρ	rotational relaxation time, ns
ρ_{-1}	reciprocal average rotational relaxation time, ns
ρ_n	number-average rotational relaxation time, ns
ρ_w	weight-average rotational relaxation time, ns
ρ', ρ''	values of ρ in a two-time approximation, ns

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