THE EFFECT OF SOLVENT VISCOSITY ON THE FLUORESCENCE OF TRYPTOPHAN DERIVATIVES

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Summary. The relative fluorescence energy yields and fluorescence lifetimes of a series of tryptophan derivatives in propylene glycol and methanol, two solvents of very similar dielectric constant but greatly differing viscosity, have been determined. The results provide direct evidence for an effect of solvent viscosity upon fluorescence emission for these compounds.

The nature of the factors which influence the fluorescence of tryptophan and its derivatives has been a subject of lively research interest (1-8). In particular, Weinryb and Steiner (8) have suggested that the quenching of fluorescence of tryptophan compounds may be temperature- and viscosity-dependent, since differences in fluorescence yield among these derivatives disappear at 91°K. The experiments here reported involve the determination of the relative fluorescence energy yields and fluorescence lifetimes for a series of tryptophan derivatives in two solvents of essentially identical dielectric constant but widely differing viscosity, and provide direct evidence for an effect of solvent viscosity upon the fluorescence of these compounds.

EXPERIMENTAL

All of the tryptophan derivatives were Grade I products of Cyclo Chemical Corp. except for Z-L-trp (Grade II) and indole, L-trp, and DL-trp-OMe (products of Sigma Chemical Co.). They were used as purchased. Spectroquality Grade methanol (MeOH) and Chrmatoquality Grade propylene glycol (PrGly) from Matheson, Coleman & Bell were used as received. These two solvents have very nearly identical dielectric constants (D = 32.5-33) but PrGly is almost sixty times more viscous than MeOH (9).

Fluorescence yields were determined at room temperature with an Aminco-Bowman spectrofluorometer equipped with spectral compensation to correct emission spectra for wavelength variations in detector response. Experiments were conducted in the presence of air, since serious oxygen quenching effects are not likely for these solutions (7,13). Energy yields were normalized to the same solution absorbance at the exciting wavelength (290 nm): actual absorbances varied between 0.10 and 0.20. All yields were corrected for the "inner filter effect" (10). Relative yields were computed both from the ratio of areas under the fluorescence emission curves and from the ratio of fluorescence intensity maxima. These ratios were identical to within experimental uncertainty (cf. Ref. 9).

Fluorescence lifetime measurements were performed in the presence of air at room temperature with a TRW nanosecond spectral source system. A 290-nm interference primary filter and an appropriate secondary filter were employed.

RESULTS AND DISCUSSION

The fluorescence energy yields and lifetimes in PrGly relative to MeOH for several tryptophan derivatives are presented in Table I. The data show clearly that an increase in solvent viscosity (i.e., substitution of PrGly for MeOH) results in an enhancement of fluorescence emission for the compounds considered, as judged by either relative yield or lifetime data (discrepancies between the ratio of relative yields and that of lifetimes for L-trp and gly-L-trp are probably due to the larger uncertainties associated with the determination of lifetimes of less than 2.0 nsec). Such a positive correlation between solvent viscosity and fluorescence emission has usually been interpreted in terms of a diminution of the dissipation of electronic excitation energy *via* intramolecular vibrations and rotations (internal conversion), since molecular flexibility would be decreased in viscous media. Emission from large, bulky molecules with steric constraints should be particularly sensitive to the solvent viscosity. However, Ac-L-trp and Z-L-trp show

TABLE I

EFFECT OF SOLVENT VISCOSITY ON FLUORESCENCE ENERGY YIELD (F) AND LIFETIME (τ) FOR A SERIES OF TRYPTOPHAN COMPOUNDS

Compound	$\frac{F(PrGly)}{F(MeOH)}$ [a]	τ(MeOH) [b], nsec	τ(PrGly) [b], nsec	$\frac{\tau(PrGly)}{\tau(MeOH)}$
Indole	1.2	3.1	3.9	1.3
L-Trp	3.3	1.7	4.0	2.4
DL-Trp-OMe	2.2	1.3	2.8	2.2
Ac-L-Trp	1.9	3.0	5.1	1.7
Ac-L-Trp-NH_2	1.9	2.4	4.2	1.7
Z-L-Trp	1.7	3.0	4.6	1.5
Z-L-Trp-NH_2	1.7	3.2	4.5	1.4
Z-L-Trp-Gly	1.8	3.2	5.0	1.6
Gly-L-Trp	4.1	1.4	4.0	2.9

[a] Estimated precision is ±7%.

[b] Estimated precision for τ is ±0.3 nsec for τ≥2.0 nsec; ±0.4 nsec for τ <2.0 nsec.

similar viscosity enhancement ratios although the amino group substituent on the latter molecule is much larger and bulkier than that on the former. This observation argues against the viscosity dependence of internal conversion as a complete explanation of the results of Table I.

The variation of viscosity enhancement ratio from compound to compound hints at the possibility of a correlation between the molecular nature of the compound and the extent of viscosity enhancement of fluorescence. Such a correlation is suggested cautiously due to the limited number of compounds surveyed; nevertheless, it seems that tryptophan derivatives possessing free amino groups generally show higher viscosity enhancement ratios than do derivatives with acetyl- or benzyloxycarbonyl-substituted amino groups or no amino group. Furthermore, since all available data indicate that the α-amino

group of an amino acid should be protonated in alcoholic media of dielectric constant approx. 33 (11,12), an amino group bearing a dissociable proton appears necessary for a marked viscosity dependence of fluorescence. These considerations point to the existence of a proton-involved, viscosity-dependent quenching process. Further elaboration upon possible viscosity-dependent mechanisms involved would be speculative at this time. However, other investigations on the variation of fluorescence yield with molecular nature for tryptophan compounds have also indicated that a proton-involved quenching process may be operative (1,2,7,8), and hence lend added weight to the conclusions reached here.

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