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Competition Between Energy Transfer and Deactivation During Quenching of Tryptophan Fluorescence of Albumin by Dye Molecules

N. L. Vekshin,[1,3] V. I. Sukharev,[1] A. van Hoek,[2] and A. J. W. G. Visser[2]

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Using quenching of Trp fluorescence by different dyes, bound to bovine serum albumin, it is shown that the spectral overlap between emission and absorption spectra does not necessarily lead to energy transfer as the sole quenching mechanism. A correlation of the limiting quenching value with the Förster overlap integral was not observed. The energy transfer efficiency was less than the quenching efficiency because of competitive radiationless deactivation processes induced by dye binding.

KEY WORDS: Bovine serum albumin; energy transfer; fluorescence quenching.

INTRODUCTION

Quenching of fluorescence from tryptophan (Trp) in proteins by dyes and other chromophoric compounds is usually explained as the result of resonance energy transfer (RET). However, the overlap between the Trp emission spectrum and the absorption spectrum of a dye is one of the conditions required for RET (and hence for the extent of quenching), but this is not sufficient to explain all aspects of quenching [1]. Competitive processes (dynamic deactivation, exciplex formation, and others) are in many cases dominant, as was shown for potential Förster donor–acceptor pairs: Tyr–Trp [2], Trp–pyrene [3,4], and Trp–NADH [5].

In order to illustrate these effects, bovine serum (BSA) was selected because of its extraordinary ligand-binding properties. BSA has two tryptophan residues: Trp-135 and Trp-214 [6]. BSA and the highly homologous human serum albumin (HSA), which contains only Trp-214, have served in the past as model proteins to study time-resolved fluorescence properties of Trp in proteins [7].

EXPERIMENTAL

BSA (5th fraction; Serva) was dissolved in a buffer (20 m*M* Tris–HCl, pH 8.0) solution of ~0.1 g/L at 20°C, a concentration at which inner filter effects and reabsorption were negligible. Perkin Coleman Model M40 and M80 spectrophotometers were used for recording the absorption spectra. A Perkin Elmer Model MPF 44B fluorometer was used for recording the fluorescence spectra. The excitation wavelength was 286 nm; bandwidths were 4 nm. Fluorescence lifetimes were determined using an SLM 4800 phase and modulation spectrofluorometer.

RESULTS AND DISCUSSION

Trp-135 is buried inside the globule, while Trp-214 is located near the surface and hence more accessible for various quenchers. Almost all dyes, used at concentrations of ~1–10 μM, quench the Trp fluorescence of

[1] Institute of Cell Biophysics, Moscow Region, Pushchino, 142292, Russia.
[2] Department of Biomolecular Sciences, Agricultural University, Wageningen, The Netherlands.
[3] To whom correspondence should be addressed.

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BSA (see Table I). Typical dissociation constants (K_d) are therefore of the order of 10^{-6} *M*, which is not unusual (see Ref. 6). No increase in Trp fluorescence was observed in the experiments. Any spectral shift of the remaining Trp emission was absent. Therefore it was concluded that the binding of dye molecules does not cause a global change in the protein conformation.

Negatively charged and uncharged dye molecules result in more quenching of Trp fluorescence in albumin than positively charged dyes. This observation is due to the presence of one (or more) positively charged group(s) in the binding pocket, at which both Trp residues are located in the near-vicinity. Since the three-dimensional structure of HSA is known [6], we can relate this observation to structural aspects. The three-dimensional structure is composed of three homologous domains (I, II, and III). Each domain is subdivided in to two helical subdomains (A and B) which are connected by disulfide bridges. The site where negatively charged aromatic residues are bound is localized in a hydrophobic pocket in subdomain IIA. Subdomain IIA indeed contains a positively charged group, namely, Lys-199. For detailed structural features of other binding sites, see Ref. 6.

The limiting value of quenching, reached at a molar excess of a dye with respect to protein (see Fig. 1), is characteristic for each dye. The limiting value is constant and reflects the accessibility of Trp for a given dye. A high quenching activity was detected (in decreasing order): eosin, thiasine red, ANS, titanium yellow, trypan red, diS-C_3-(5), pyrene, and thymol blue. Among these, diS-C_3-(5) has no light absorption in the region of the Trp emission: the spectral overlap integral is about zero. Parallel to a decrease in the Trp fluorescence in BSA during titration with dye molecules, a reduction of fluorescence lifetimes was observed. For instance, saturating binding of pyrene or diS-C_3-(5) reduces the average fluorescence decay time of Trp from 5.5 to 3.8 ns. For the Trp–pyrene pair, the Förster radius (R_0) is equal to 28.6 Å [2]. However, the efficiency of RET, detected by the pyrene excitation spectrum, was lower than the efficiency of quenching. Similar results were obtained for all dyes used. Consequently, quenching of Trp fluorescence in BSA most likely proceeds via radiationless deactivation and exciplex formation.

Binding of eosin, ANS, titanium yellow, acridine orange, coryphosphine, and pyrene to BSA resulted in a sharp increase in their fluorescence (see Table I). This emission was not sensitized (to a considerable extent) but was caused by direct excitation of the dye molecules at 286 nm. Trypan red, pyronine B, auramine 00, and diS-C_3-(5), upon binding to BSA, show a weak fluorescence upon 286-nm excitation. The latter fluorescence intensity was much lower than the decrease in Trp fluorescence, indicating that the quenching is caused by both radiationless deactivation and RET. In the case of binding of thiasine red, thymol blue, and chryzoidin to BSA, the

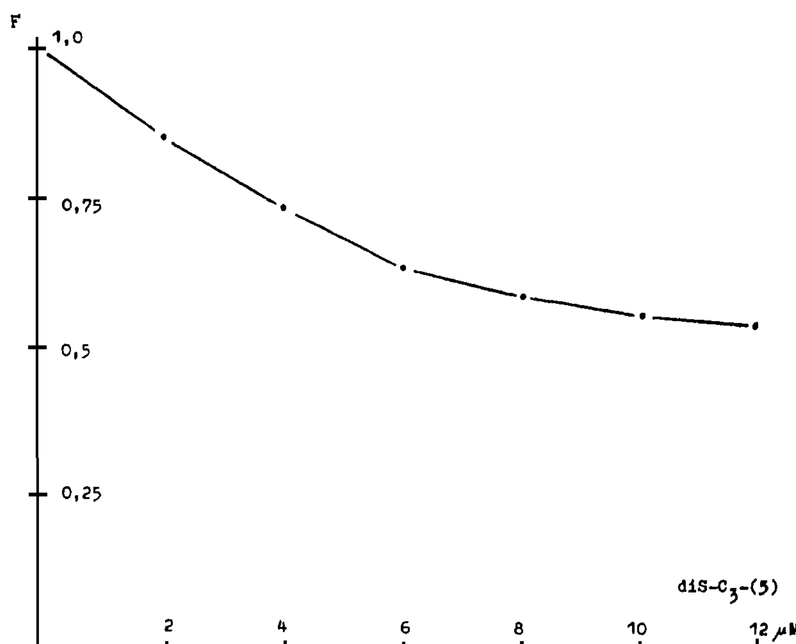

Fig. 1. Normalized integrated fluorescence intensity of BSA–tryptophan as a function of the concentration of added diS-C_3-(5).

Table I. Data from Quenching Experiments of BSA Tryptophan Fluorescence by Different Dye Molecules[a]

Dye	Charge	RSOI (%)	$1 - (F/F_0)$ (%)	F_{dye} (rel. units)
Eosin	2−	30	80	2
Thiasine red	2−	47	76	0
ANS	1−	20	70	1
Titanium yellow	2−	100	64	1
Trypan red	5−	19	48	0.2
diS-C_3-(5)	1+	~0	46	0.5
Pyrene	0	25	44	1
Thymol blue	1−	28	32	0
Acridine orange	1+	~0	24	1
Pyronine B	1+	19	15	0.1
Chryzoidin	1+	75	14	0
Coryphosphine	1+	38	6	3
Auramine 00	1+	9	0	0.05

[a] The charge of the dye molecules is given, together with the relative spectral overlap integral (RSOI) of Trp emission and dye absorption, the amount of quenching $1 - (F/F_0)$, and the relative dye fluorescence upon excitation at 286 nm. The overlap integral $\int F(\nu)\, E(\nu)\, d\nu$ is contained in the critical transfer distance R_0. The overlap integral for the pair Trp–titanium yellow is largest and, for that reason, has been set to 100%.

fluorescence of these dyes at 286-nm excitation was not observed, although quenching of Trp fluorescence took place. The latter observation indicates that the quenching is caused preferentially by radiationless deactivation via both chromophoric and nonchromophoric groups in these dye molecules.

CONCLUSIONS

A correlation between the limiting quenching values and the Förster spectral overlap integral was not found. Quenching of Trp fluorescence in BSA can take place even without a significant spectral overlap. Hence, it is impossible to identify a quenching efficiency with that of RET. Efficiencies of RET are noticeably lower than those of quenching because radiationless deactivation of Trp is induced by the dye molecule itself and possibly by some surrounding amino acids in the protein. Since HSA has only a single Trp (Trp-214), complementary experiments with HSA can lead to more details of this mechanism, which in turn can be connected with structural features.

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