TYROSINE FLUORESCENCE OF S_8 AND S_{15} *ESCHERICHIA COLI* RIBOSOMAL PROTEINS

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1. Introduction

Nomura and others [1–3] showed that 5 of the 21 different proteins in the 30 S subunit of the *Escherichia coli* ribosome (S_4, S_7, S_8, S_{15} and S_{20}) can bind directly to the 16 S RNA in the absence of other proteins. A fluorescence study of the two tryptophan-containing proteins S_4 and S_7 [4] provided information on their conformation and on the localization of the emitting residues. Further work on the other RNA-independent binding proteins is reported here. Since proteins S_8 and S_{15} (S_{20} was not studied because of the difficulty of obtaining it in the native state) do not contain tryptophyl residues emission must be due to tyrosyl residues only (class A proteins). In order to obtain information on these proteins, we compared various emission characteristics (emission spectra, fluorescence quantum yields and decay times, and effects of external quenchers and of temperature) with those of the denatured proteins. The data show that emitting Tyr residues are rather exposed and characterized by high quantum yield values.

2. Materials and methods

2.1. *Ribosomal proteins*

Ribosomal proteins S_8 and S_{15} were prepared from *E. coli* MRE 600 as previously described [5]. The two proteins were studied in buffer (0.35 M KCl; 0.02 M $MgCl_2$; 0.005 M Tris, pH 7.4), which is an optimal reconstitution solvent. Each sample of lyophilized protein (100–250 μg) is first dissolved in 10–20 μl of 6 M guanidium chloride and then diluted with 1 ml buffer. Denatured protein solutions were prepared by dissolving the protein in 6 M guanidium chloride at pH 7.4.

Ultra-pure guanidium chloride and Tris were purchased from Schwartz-Mann.

2.2. *Tyrosine content*

The proteins S_8 and S_{15} have been recently sequenced: S_8 contains 3 tyrosyl residues at positions 64, 85, and 109 [6]; S_{15} contains two tyrosines at positions 67 and 76 [7].

2.3. *Fluorescence measurements*

Absorption spectra were recorded with a Cary 15 Spectrophotometer and corrected from the scattered light [8]. We obtained fluorescence spectra with an absolute spectrofluorimeter (Fica 55) and determined quantum yields (ϕ) as previously described [4], taking free tyrosine as a reference ($\phi = 0.14$) [9]. Quantum yields were corrected to account for the screening effect of scattered light [10]. Lifetimes (τ) were measured by the single photoelectron technique [11]. Protein solutions were excited at 275 nm and the fluorescence was detected at 305 nm. The temperature coefficients at 30°C, as defined by Laustriat and Gerard were determined as previously described [12].

All the measurements were done on the native and denatured protein solutions at concentrations of 0.2–0.4 mg/ml.

3. Results

3.1. *Absorption and fluorescence spectra*

The absorption spectra of proteins S_8 and S_{15} are very close to the spectrum of free tyrosine in water (λ_{max} = 275 nm, shoulder at 280 nm). The proteins'

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fluorescence spectra present a maximum near 305 nm, characteristic of tyrosyl residues. The spectral parameters were unchanged after denaturation of the proteins, as expected for tyrosine emission.

3.2. *Fluorescence quantum yields and decay times*

Values of quantum yields and decay times are given in table 1. Three comments can be made about these results:

(i) For both of these native ribosomal proteins, emission yields are unusually high as compared with those obtained for most of the tyrosine-containing proteins [13] (for example, ribonuclase A, $\phi = 0.012$; insulin, $\phi = 0.022$).

(ii) The ribosomal protein quantum yields decrease after protein denaturation, contrary to the behaviour commonly observed for this kind of protein [13] (denatured ribonuclease A, $\phi = 0.036$; denatured insulin, $\phi = 0.035$).

(iii) In denatured S_8 and S_{15} proteins, the fluorescence lifetimes are not changed in the same way as the emission yields are (no variation in lifetime for S_8 and little decrease for S_{15}).

3.3. *Influence of external quenchers*

Molecules or ions can be added externally to test the degree of accessibility of the protein fluorophores. We used two oppositely charged ionic quenchers, I^- and Cs^+, to take into account a possible electrostatic effect from the environments of the emitting residues. Addition of 0.15 M KI or 1 M CsCl (concentrations that induce about the same quenching effect on the fluorescence of free tyrosine) lowers the protein fluorescence intensities by about the same amount for native and denatured proteins, as indicated by I_0/I values (left-most column, table 2). As equimolar concentrations of a nonquenching salt (NaCl) do not modify the fluorescence emission of the proteins, the decrease indicates that the emitting tyrosyl residues in the native proteins are quite accessible. However, the values of the Stern-Volmer constant K and the kinetic constant K_q (table 2) show that the effect of Cs^+ is twice as high for denatured as for native proteins; the difference probably denotes some electrical repulsion effect in the native state.

3.4. *Influence of temperature*

For the two ribosomal proteins, there is no discontinuity in the linear decrease of fluorescence with increasing temperature (10–45°C), indicating a conformational stability around the tyrosyl residues in this temperature range. However, information on the emission mechanism can be obtained from the temperature coefficients of the studied proteins and of free tyrosine in an aqueous environment. The protein S_8 exhibits the same coefficients ($C = -1.4\%\ °C^{-1}$) in the native and denatured states, whereas that of the protein S_{15} increases upon denaturation (from -1.2 to $-1.6\%\ °C^{-1}$).

4. Discussion

4.1. *Exposure of the emitting residues*

Since the emission maximum of tyrosyl residues does not depend on the environment, their localiza-

Table 1
Fluorescence parameters of S_8 and S_{15} ribosomal proteins

		ϕ (± 0.005)	τ (ns) (± 0.2)	$(\phi/\tau)(s^{-1}) \times 10^{-9}$ (± 0.004)
S_8	Native	0.047	2.7	0.017
	Denatured	0.037	2.7	0.013
S_{15}	Native	0.051	2.6	0.019
	Denatured	0.036	2.3	0.016
Free L-tyrosine		0.140 (ref. [9])	3.5	0.040

Table 2
Effects of external quenchers on the fluorescence of S_8 and S_{15} proteins

	I_0/I [a]	K (M^{-1})	k_q (M^{-1} s^{-1}) $\times 10^{-9}$	τ_0 (ns)	p	A
I^- Quenching						
Free L-tyrosine	3.3	15.5	4.4	3.5	0.37	4π
S_8 Native	1.4	2.3	0.9	2.7	0.37	1.0π
S_8 Denatured	1.5	3.2	1.2	2.7	0.37	1.4π
S_{15} Native	1.4	2.7	1.0	2.6	0.37	1.2π
S_{15} Denatured	1.4	2.7	1.2	2.3	0.37	1.4π
Cs^+ Quenching						
Free L-tyrosine	4.0	3.0	0.9	3.5	0.12	4π
S_8 Native	1.3	0.2	0.1	2.7	0.12	0.7π
S_8 Denatured	1.5	0.5	0.2	2.7	0.12	1.3π
S_{15} Native	1.3	0.3	0.1	2.6	0.12	0.7π
S_{15} Denatured	1.4	0.4	0.2	2.3	0.12	1.3π

[a] I_0/I, ratio of the fluorescence intensities in the absence (I_0) and in the presence (I) of quenchers (KI, 0.15 M; CsCl, 1 M). Other parameters are defined in the text

tion can only be deduced from the effects of external quenchers. Exposure of these residues to the solvent is correlated to the solid angle A under which a quencher can freely approach an excited residue. Values of A can be deduced from the expression of the kinetic quenching constant k_q, given by [14]:

$$k_q = A\frac{N}{1000}\,[D_F + D_Q]\,R\,[1 + R[(D_F + D_Q)\tau_0]^{-\frac{1}{2}}]\,p$$

where N is the Avogadro number, R the interaction distance (~ 5Å), D_F the diffusion constant of the fluorophore (= 0.7×10^{-5} cm^2 s^{-1} for free tyrosine and ~ 0 for tyrosyl residues), D_Q the diffusion constant of the quencher (= 2×10^{-5} cm^2 s^{-1} and 0.9×10^{-5} cm^2 s^{-1} for I^- and Cs^+ respectively) (15), τ_0 the lifetime of the fluorescent molecules in absence of quencher, and p the probability of deactivation during an encounter.

For free tyrosine, where $A = 4\pi$, the probability is 0.37 and 0.12 for I^- and Cs^+, respectively. Assuming similar values of p in the case of tyrosyl residues, values of A for proteins can be deduced from those of K_q. The results (table 2) show that:

(i) In denatured S_8 and S_{15} proteins, tyrosyl residues are about equally accessible to both quenchers ($A = 1.3$–1.4π);

(ii) for both proteins, I^- can approach tyrosyl residues nearly as easily in the native as in the denatured state, indicating an important exposure of these residues in the native state;

(iii) in contrast, Cs^+ approaches tyrosyl residues much less easier in native proteins than it does in denatured ones; the difference probably reflects the presence of positively charged groups in the vicinity of the emitting tyrosyl residues in the native state. This result is strongly corroborated by the fact that the

tyrosyl residues are located in the COOH-terminal parts of S_8 and S_{15} proteins, which contain most of basic aminoacids and therefore many positive charges [6,7]. Such an influence of the sign of the ion charge was not observed for S_4 and S_7 proteins [4].

4.2. *Number and localization of emitting residues*

As is common for tyrosine-containing proteins, dynamic and static quenchings occur in S_8 and S_{15} proteins.

Dynamic quenching is indicated by the low values of the lifetimes as compared with the lifetime in free tyrosine, and also by the relatively high absolute value of the temperature coefficients (1.2–1.4% $°C^{-1}$), which has been shown to be ~ 0.85% $°C^{-1}$ for free tyrosine in water and to increase in the presence of quenchers [12].

The efficiency of *static quenching* can be estimated from the ϕ/τ ratios, since the fraction of fluorescent residues submitted to this process is given by [11]:

$$\omega = 1 - \frac{(\phi/\tau)p}{(\phi/\tau)m}$$

where subscripts p and m refer respectively to the residues and to the corresponding model system, free tyrosine in water in the present case.

Data in table 1 lead to $\omega = 0.58$ (0.66) for S_8 and $\omega = 0.51$ (0.61) for S_{15}, values between brackets referring to denatured proteins. These results would indicate that, on the average, one (out of two) tyrosyl residues emit in native S_{15} protein, and 1.3 (out of three) in native S_8, the emitting residues being characterized by an emission quantum yield value of 0.10. That the emission of S_8 could be assigned to 1.3 residues denotes that quantitative treatment of tyrosyl emission can remain ambiguous, principally because the processes of quenching of tyrosine in protein are not yet fully understood. In the case of S_8, one major emission could be due largely to a single residue, and only a little to the other residues.

Coming back to the quantum yields, value of 0.10 for tyrosyl residues can be considered as anomalously high in comparison with those generally observed in native proteins and in tyrosine peptides [13]. Such a high quantum yield can be related to the fact that, contrary to what is commonly observed, the protein yield decreases upon denaturation. This indicates that, in the native state, emitting tyrosyl residues of S_8 and S_{15} are preserved from a quenching process that normally occurs in most of native proteins. Similar features have been found for muscle proteins containing 90% of α helix (tropomyosin and paramyosin) [16], in which tyrosyl residues are favorably situated in an α helical structure, where the static quenching by peptide carbonyl groups – resulting from the formation of a hydrogen bond with the phenol group of tyrosine – cannot occur. It could therefore appear reasonable to consider that the emitting residues of S_8 and S_{15} are in a helical part of the proteins. In addition, this proposal is consistent with the fact that decay time does not vary after denaturation for S_8 and exhibits only a small decrease for S_{15}: the quenching process induced by denaturation is mainly static, suggesting the loss of a particular conformation. These conclusions, however, are not in agreement with theoretical predictions of secondary structure carried out for both proteins (see ref. [6] for S_8 and ref. [7] for S_{15}). It is nevertheless to be noted that for S_8 protein two tyrosines (residues 64 and 85) are precisely located near predicted α helical structures, which could in part explain our results.

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