FLUORESCENCE STUDY OF S_4 AND S_7 RIBOSOMAL PROTEINS COMPLEXED TO 16 S RNA

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1. Introduction

Many works have been devoted to the ribosome's architecture [1–5] but few informations are available on the nature of interactions between proteins and the rRNAs, due to the scarcity of the investigation methods: the important absorbance of RNA excludes, for instance, most of the spectroscopic techniques such as ultraviolet absorption and circular dichroism.

Intrinsic fluorescence, orginating from aromatic residues, is of particular interest to approach this problem since the protein emission is detectable in the complex with rRNA and may therefore provide indications on the global behaviour of the protein and on the specific role of aromatic residues in the binding mechanism. We started such a fluorescence study by S_4 and S_7, two of the five 30 S proteins which directly bind to the 16 S RNA.

Previous works, using chemical labelling, showed the involvement of lysine and tyrosine residues of these proteins in the complex formation [6], and in the particular case of S_4, whose primary structure is known [7], allowed to propose some rRNA binding sites of the protein [8].

In order to obtain informations on the bound proteins, we compared the fluorescence characteristics of complexed proteins with those of isolated ones – previously investigated in this laboratory [9]. This study allowed to show that, for S_7, some of the 3–4 tryptophans of the protein are also involved in the interaction mechanism, whereas for S_4 the emitting residues (the only tryptophan and some of the eight tyrosines) *do not* participate to the binding process, and the overall conformation is not altered by the complexation.

2. Materials and methods

2.1. *Ribosomal material*

S_4 and S_7 were prepared from *E. coli* MRE 600: after separation of the 30 S subunit in a zonal rotor, proteins were separated chromatographically and characterized as previously described [10]. 16 S RNA has been obtained from the 30 S subunit by SDS-phenol extraction [11].

Protein–rRNA complexes were prepared in TMK buffer (KCl 0.35 M, $MgCl_2$ 0.02 M, Tris 0.01 M, pH 7.4) according to Garrett et al. [12] except that unbound proteins were removed by sedimentation of the complex for 6 hr, at 0°C and 40 000 rev/min, in a Spinco 40.3 rotor. Qualitative control of the protein binding was achived by gel electrophoresis as described by Garrett et al. [12].

Complex solutions were obtained by redissolving the sedimented complex in TMK buffer, in such quantity that the protein concentration was 100–200 γ/ml.

2.2. *Fluorescence measurements*

Fluorescence spectra were obtained with an absolute differential spectrofluorimeter FICA 55, for two excitation wavelengths: 280 ± 7.5 nm and 295 ± 7.5 nm. The important Raman diffused light, which is 30 mn red shifted from the excitation wavelength was subtracted either by differential recording or by graphic difference, using 16 S RNA solution as reference.

3. Results

Fluorescence spectra of S_4 and S_7 proteins com-

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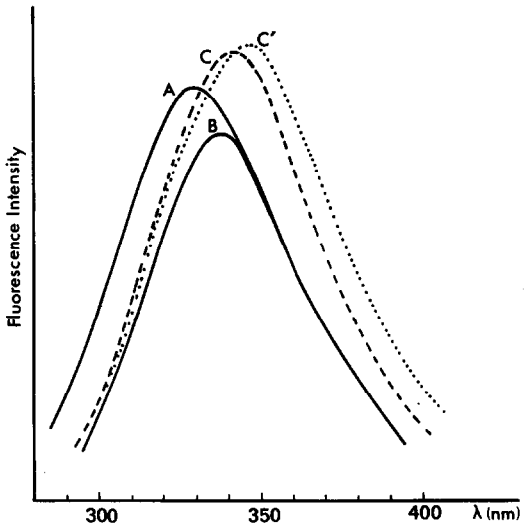

Fig. 1. Fluorescence spectra of ribosomal proteins. S_4, free or RNA bound: curve A (λ_{exc} = 280 nm), curve B (λ_{exc} = 295 nm). S_7, RNA bound: curve C (λ_{exc} = 280 or 295 nm). S_7, isolated: curve C' (λ_{exc} = 280 or 295 nm).

plexed with 16 S RNA are presented on fig.1. They significantly differ from those of the free (uncomplexed) proteins only in the case of S_7, whose spectrum in the free state is also given in fig.1.

Main spectral characteristics of the free and rRNA bound proteins are summarized in table 1. The RNA absorption considerably lowers the emission intensity from complexes, for which the results – peak positions (λ_{max}) and spectrum widths ($\Delta\lambda$) – are less precise, in this case the accuracy being however better for 295 nm than for 280 nm excitation, due to the smaller RNA absorbance.

It is seen that complexation does not modify the fluorescence of S_4 but induces for S_7 a blue-shift and a width reduction of the spectrum.

4. Discussion

These data show that natural fluorescence of rRNA bound proteins can be detected in spite of the important absorption of the exciting light by RNA and they moreover give some informations on the RNA-protein binding process.

4.1. *S_7 protein*

In our previous study [9], the fluorescence of the free S_7 protein has been shown to originate from two kinds of tryptophan residues, emitting at different wavelengths:

(i) exposed to the aqueous medium (class a, λ_{max} = 350–355 nm),

(ii) partially buried (class b, λ_{max} = 340 nm).

The modifications induced by complexation may therefore be ascribed either to a partial burial (due to a protein conformational change or to the contact with 16 S RNA) or to a quenching of the most exposed (class a) tryptophans. The last hypothesis is favoured by the fact that the spectral variations due to com-

Table 1
Fluorescence characteristics of free and 16 S RNA-complexed S_4 and S_7 proteins

Protein	State	λ_{exc} (nm)	Emitting residues in the free state	λ_{max} (nm)	$\Delta\lambda$ (nm)
S_4	free	280	Tyr + Trp	332	63
		295	Trp	339	56
	rRNA–bound	280		332 ± 4	60 ± 3
		295		340 ± 2	56 ± 2
S_7	free	280 or 295	Trp, class a (λ_m = 355 nm) + Trp, class b (λ_m = 340 nm)	345	60
	free + CsCl 1 M	idem	Trp, class b	340	57
	rRNA–bound	280 or 295		340 ± 2	57 ± 1

plexation are very close to those we previously observed when the free protein was submitted to external quenchers (see table 1 and ref. [9]).

Such a quenching, on the other hand, is consistent with a localization of these residues in the binding sites of the protein, since Helene and co-workers [13–15] showed that aromatic residues are quenched by specific interactions with nucleic acids, such as stacking (for tryptophan and tyrosine) and hydrogen bonding (for tyrosine only). We are therefore led to propose that to exposed (class a) tryptophan residues of S_7 are involved in the binding mechanism, through the insertion of their indole ring between nucleic bases.

4.2. *S_4 protein*

Fluorescence of the free S_4 protein originates from tyrosines and tryptophan when excited at 280 nm, and from tryptophan only when excited at 295 nm [9]. Since emission spectra of these two residues are quite different, any important quenching by 16 S RNA would notably modify the fluorescence characteristics (spectrum shape and position). The identity of complexed and free protein spectra, therefore indicates that binding does not induce fluorescence quenching, from which it can be inferred, according to Helene's work, that emitting residues are not specifically involved in the binding process.

This result is interesting since it means that interaction sites of this protein do not include the only tryptophan (position 167) nor the sequence containing most of tyrosine residues (positions 50–100). It is to be noted that this conclusion is consistent with a recent paper [8], proposing as binding regions the two aminoacid sequences 109–129 and 137–157 and also with a study of Garrett concluding that the tryptophan residue is not involved in the binding mechanism [16].

The identical emission of the free and complexed protein also indicates that the general conformation of S_4 is close in the two states. The streched shape previously proposed for the free protein [9] must therefore be preserved in the complex. On the other hand, protein conformational changes (α helix melting accompanied by partial destabilization) have been evidenced at 42°C in the 30 S subunit reconstitution conditions [9,10,17]. The present result shows that such changes are only transient since the initial structure is found again after binding, they however may be essential to allow specific recognitions between RNA and some residues of the protein, unaccessible in the native structure.

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