FLUORESCENCE STUDIES ON RIBONUCLEASE T_1

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Summary: The fluorescence spectra of native and denatured ribonuclease T_1 (RNase T_1) have been recorded under various conditions. Solvent perturbation of the fluorescence of RNase T_1 has been studied. Its effect is opposite to that observed with model compounds. The results show, that the single tryptophan residue of RNase T_1 is partially buried in the enzyme and that most of the tyrosine residues are not outside of the protein molecule. It is concluded that these tyrosine residues are involved in side chain-side chain interactions with acidic amino acid residues.

Fluorescence spectroscopy provides a suitable method to study the environment of tyrosine and tryptophan residues in proteins, since solvent and environmental factors have a profound effect on the fluorescence intensities of these amino acid residues (1,2). Thus, the influences of denaturing agents and of solvent perturbation on the tyrosyl and tryptophanyl fluorescence of ribonuclease T_1 (RNase T_1) have been investigated.

Based on titration data, it was recently reported that the pK-values of an unusually large number of acidic amino acid and tyrosine residues in RNase T_1 are perturbed (3). From the fluorescence data, which we wish to report, the conclusion can be drawn that this perturbation is the result of a rather uncommon characteristic of the tertiary structure of RNase T_1. In contrast to many other proteins, most of the tyrosine residues of RNase T_1 are not outside of the protein molecule and are involved in side chain-side chain interactions withacidic amino acid residues. Also, the data reported show, that some of the 9 tyrosine residues of RNase T_1

are located in the neighbourhood of the single tryptophan residue (Trp 59), whereas others are away from Trp 59.

Experimental

Lyophilized and salt free RNase T_1 (Sankyo Co., Ltd., Tokyo, Japan, Lot No. 8R20) was used without further purification. The fluorescence spectra were recorded with an Aminco-Bowman spectrofluorometer at room temperature. In all studies, the enzyme (0.1 mg/ml) was dissolved in 0.1 M KCl. A Radiometer pH-meter model 25 equipped with a combination electrode (Fa. Ingold, Frankfurt, Germany, type No. 405-M-312-a) was used for the pH-measurements. The fluorescence spectra of denatured protein were recorded one hour after addition of the denaturing agents.

Results and Discussion

The fluorescences of tryptophan and tyrosine have a quantum yield of 21 and 20 per cent, respectively, and their emission maxima are separated by about 50 nm (4). In proteins, the peak of tyrosyl fluorescence is usually masked by a stronger emission of the tryptophanyl residue (5). Thus, the fluorescence spectrum of RNase T_1 (Fig.1) is similar to those reported for other proteins (6). However, while the molar Tyr/Trp ratio of this enzyme is 9:1 (7), the ratio of their fluorescence intensities is about 1:50 as derived from the data at 304 nm and 330 nm in Fig.1. This means, that the quantum yield of the tyrosine residues in RNase T_1 is unusually low.

When the fluorescence spectrum of RNase T_1 is recorded in an 8 M urea solution (Fig.1), a separate emission maximum is observed due to the increase of fluorescence intensity at 304 nm. On the other hand, the tryptophanyl fluorescence intensity decreases and its emission maximum is shifted to 360 nm. This indicates, that the hydrophobic microenvironment of Trp 59 has changed to a hydrophilic one (6). Evidently, in an 8 M urea solution, the single tryptophan residue

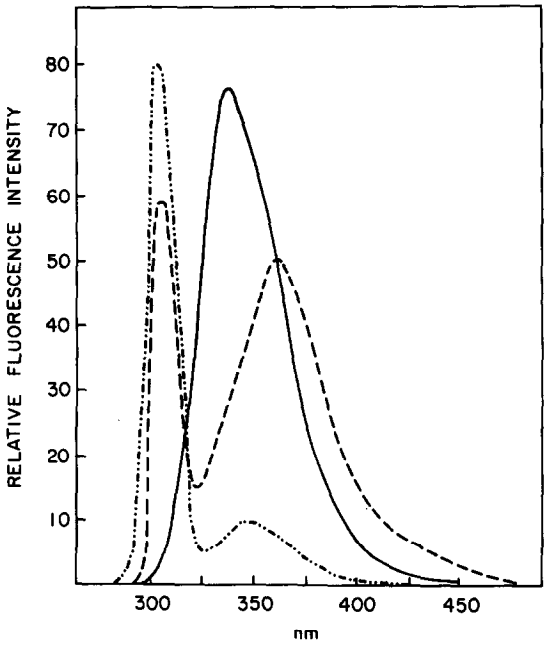

Fig. 1. Fluorescence spectra of native RNase T_1 at pH 6.4 in 0.1 M KCl solution (——), of RNase T_1 denatured by 8 M urea (– – –) and denatured by 5% sodium lauryl sulfate (-···-). Solutions contained 0.1 mg/ml enzyme. The wave length of excitation was 280 nm.

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of RNase T_1 becomes more exposed to the solvent water due to unfolding of the protein. ORD-measurements have indicated, that the tertiary structure of native RNase T_1 is altered in an 8 M urea solution (8). The fluorescence data suggest the same conclusion.

More striking is the effect of detergent on the tyrosyl and tryptophanyl fluorescence of RNase T_1 (Fig.1). In the presence of sodium lauryl sulfate, the fluorescence spectrum of RNase T_1 has two clearly separated peaks, one for the tyrosyl fluorescence at 304 nm and one for the tyrptophanyl fluorescence at 340 nm. The fluorescence intensity ratio of 8:1 of these peaks is close to the theoretical ratio of 9:1 based on the amino acid composition (7). If detergent was added to a 10^{-5} M solution of tryptophan or tyrosine, no effect on the fluorescence intensity was observed. Evidently, the strong quenching of the

fluorescence of the tyrosine residues of RNase T_1 is strictly correlated to the folding of this enzyme. Hence, in contrast to many other proteins most of the tyrosine residues of RNase T_1 are not exposed to the outside of this protein molecule in its native tertiary structure.

Usually, the fluorescence intensities of tyrosine and tryptophan are enhanced by solvents of low polarity (1,9). However, the fluorescence intensities of the tyrosyl and tryptophanyl residues of RNase T_1 decrease with decreasing solvent polarity (Fig.2). Furthermore, this decrease is not linear and the appearance of this non linearity is pH-dependent (10). These discontinuities seem to be caused by conformational changes of the enzyme, which are strongly cooperative and hence produce a non-linear change of the tyrosyl and tryptophanyl fluorescence.

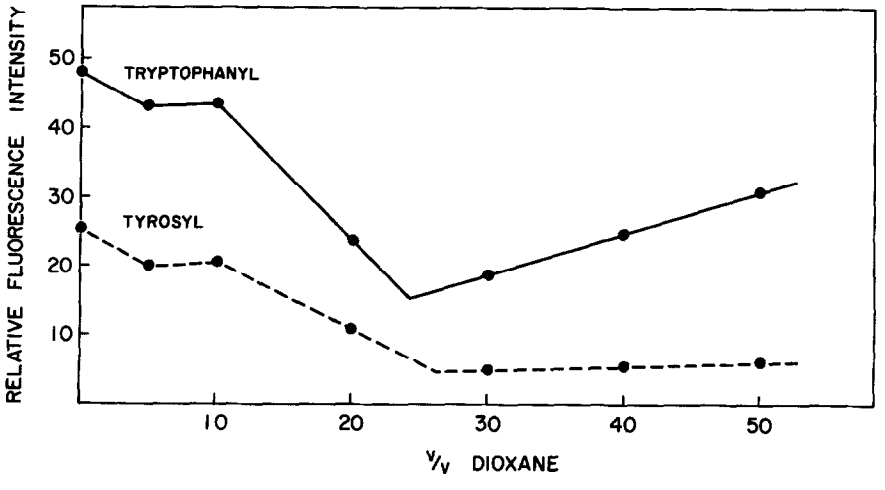

Fig. 2. The effect of dioxane on the tryptophanyl (——) and tyrosyl fluorescence (– – –) of RNase T_1 at pH 6.5. Solutions contained 0.1 mg/ml enzyme in 0.1 M KCl. The wave length of excitation was 280 nm. The wave lengths of emission were 295 nm for the tyrosyl fluorescence and 360 nm for the tryptophanyl fluorescence. The fluorescence intensity of tyrosine was measured with a 25 times higher amplification relative to that of tryptophan.

With respect to the behaviour of model compounds, e.g. N-acetyl tryptophanamide (1), the tryptophanyl fluorescence normalizes at perturbant concentrations above 25 per cent. This is indicated by the slope of the increase in fluorescence intensity (Fig.2), which is now similar to the one of N-acetyl tryptophanamide, and by a red shift of the emission maximum to 360 nm (Fig.3). Compared to the fluorescence of N-acetyl tyrosinamide (9), the tyrosyl fluorescence of RNase T_1 behaves different up to 50 per cent perturbant concentration (Fig.2).

Fig.1 and Fig.3 show that the changes of tryptophanyl fluorescence caused by 8 M urea and by solvent perturbation are comparable. This is not the case for the tyrosyl fluorescence. Since the tyrosyl and tryptophanyl fluorescence do not behave in unison, some of the tyrosine residues of RNase T_1 are not affected by the neighbourhood of trypto-

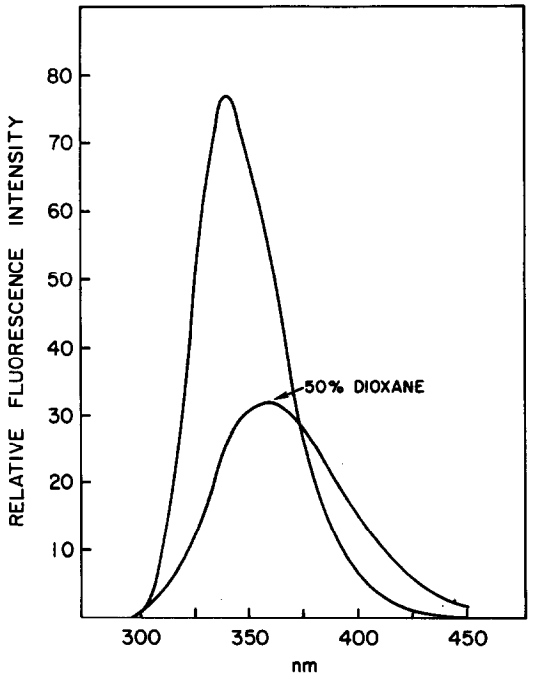

Fig. 3. Fluorescence spectra of RNase T_1 in 0.1 M KCl and in 50 %v/v dioxane at pH 6.4. Solutions contained 0.1 mg/ml enzyme. The wavelength of excitation was 280 nm.

phan. This conclusion is supported by recent measurements of the fluorescence excitation spectrum of RNase T_1. According to these data approximately one third of the energy absorbed by the tyrosines is transferred to tryptophan and two thirds is deactivated without radiation (11). Hence, it is possible to distinguish between two different classes of tyrosine residues in RNase T_1: 1) tyrosine residues, which are in the neighbourhood of Trp 59; 2) tyrosine residues, which are still inside the protein molecule, but away from Trp 59.

The low quantum yield of fluorescence of the first class of tyrosine residues is due to an energy migration to Trp 59 (12). The low quantum yield of the second class of tyrosine residues of RNase T_1 is a result of the nature of their environments. Two different environments have to be considered for the explanation of the observed quenching: 1) side chain-side chain interactions of the tyrosyls with carboxylate anions of acidic amino acid residues (2); 2) interactions with the peptide backbone in the hydrophobic part of the protein (13).

Evidence for the first mechanism is supported by the following observations. Most of the tyrosine and the acidic amino acid residues do not titrate normally (3). Denaturation of the enzyme by urea, a molecule which competes for hydrogen bonds, markedly increases the fluorescence intensity of the tyrosine residues. On the other hand, a decrease in solvent polarity results in an additional quenching of the tyrosyl fluorescence. Such a decrease in solvent polarity weakens hydrophobic interactions and strengthens interactions via hydrogen bonding. Thus it is concluded, that most of the tyrosine residues of RNase T_1 are involved in side chain-side chain interactions with carboxylate anions of acidic amino acid residues.

The reported increased reactivity of 5 out of 9 tyrosine residues upon reaction with nitrous acid (14) also fits very well in this interpretation of the state of the tyrosine residues of RNase T_1.

Since modification of the tyrosine residues inactivates RNase T_1 (14), this rather uncommon state of the tyrosine residues seems to be important for maintaining the tertiary structure of this enzyme.

Acknowledgements

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