Biophysical Chemistry 6 (1977) 379–383

TYROSINE FLUORESCENCE OF TWO TRYPTOPHAN-FREE PROTEINS: HISTONES H1 AND H5

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Received 1 December 1976
Revised manuscript received 24 February 1977

The fluorescence intensity of the single tyrosine residue in histone H1 increases from $R_{TYR} = 0.3$ to $R_{TYR} = 1.3$ as the protein undergoes a conformational change from the random coil state to a folded form. Enhanced fluorescence in the folded state has not been observed before in a protein. Histone H5 shows no change in fluorescence intensity on folding. This is interpreted as a result of compensation between enhanced and reduced fluorescence in the three tyrosine residues.

1. Introduction

The study of fluorescence emission intensity from tyrosine is an important method for investigating conformational changes in proteins. In a recent review of tyrosyl fluorescence [1] Cowgill has classified 8 types of tyrosine fluorescence in terms of quenching mechanisms. He concluded that buried tyrosine residues are quenched relative to exposed tyrosine. We report here an unambiguous case in which a buried tyrosine shows enhanced fluorescence.

Histones are suitable molecules for the study of tyrosyl fluorescence since they contain no tryptophan (class A proteins) and furthermore the lysine rich histones H1 and H5 do not contain cystine, a possible quenching residue. H1 from calf thymus is particularly interesting since it contains only one tyrosine in its 215 residues [2]. H5 from chicken erythrocytes contains three tyrosines in 187 residues [3]. Both proteins are random coil when in dilute solution at pH 3.5 and are induced to fold by increase of pH or ionic strength [4,5]. The fluorescence intensity of both proteins has therefore been studied as a function of pH and ionic strength and the conformation of the proteins has been monitored by observing the circular dichroism (CD) spectrum of the same solutions as used for the fluorescence measurements.

2. Materials and methods

Fluorescence intensity is reported as a quantum yield ratio ($R_{TYR} = Q_{histone}/Q_{L\text{-tyrosine}}$) with respect to a 10^{-5} M solution of L-tyrosine in 2×10^{-2} M tris buffer, pH 7 [6]. The fluorescence spectrum was excited at 235 nm, the wavelength of a subsiduary maximum in the tyrosine excitation spectrum. Although this excitation wavelength leads to weaker fluorescence as compared to excitation at the more conventional wavelength of 279 nm, the fluorescence is quite free from overlap of both the Rayleigh and Raman water scattering bands. A check on the use of this excitation wavelength was made with bovine pancreatic ribonuclease A at 10^{-5} M, pH 7. An R_{TYR} value of 0.097 was measured with excitation at 279 nm and a value of $R_{TYR} = 0.099$ was observed with 235 nm excitation. All measurements were carried out at a protein concentration 10^{-5} M. Correction ($\leqslant 10\%$) was always made

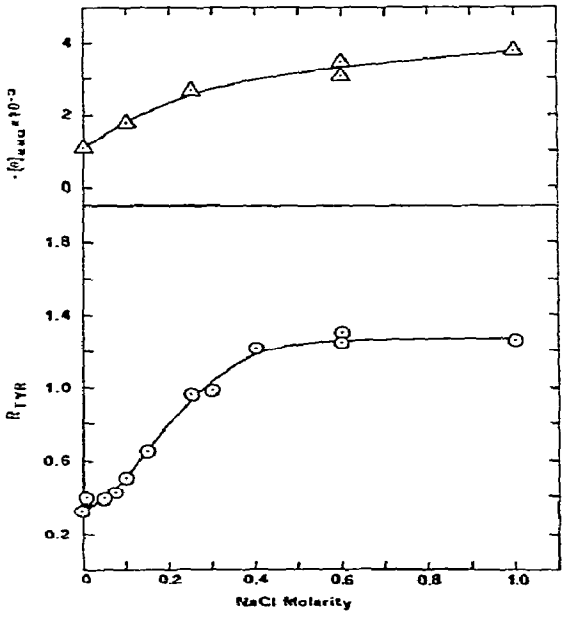

Fig. 1. Ellipticity at 220 nm (upper) and quantum yield ratio (lower) for H1 solutions with changes in NaCl concentration at pH 3.5.

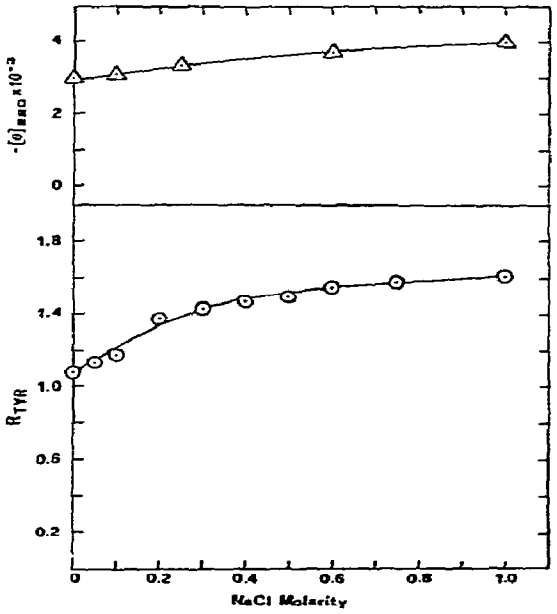

Fig. 2. Ellipticity at 220 nm (upper) and quantum yeild ratio (lower) for H1 solutions with changes in NaCl concentration at pH 7.0.

for the absorption of the excitation light at 235 nm but no correction was required for the emitted light. Permanganate redistilled water was used throughout. No change in the shape or λ_{max} of the fluorescence band was observed with changing protein conformation and solution conditions: fluorescence intensity was therefore measured as the peak height at $\lambda_{max} = 305$ nm. Cylindrical microcells of 5.4 mm diameter were used in a Hitachi–Perkin Elmer MPF-3L spectrofluorometer. CD measurements were made on a Jouan II Dicrographe.

Histone H1 was extracted from calf thymus by the method of Johns [7] and purified by chromatography on Amberlite CG-50 resin. Purity was checked by acrylamide gel electrophoresis according to Panyim and Chalkley [8] using a high loading to check for the presence of other histones. A purity of >95% was estimated by this method. Under the conditions used the Amberlite did not subfractionate H1, but all subfractions have very closely related composition and in particular all have the single tyrosine and identical conformational properties [9]. Histone H5 was obtained from chicken erythrocytes by sulphuric acid extraction [10] and purified on Biogel P10 in 20 mM HCl. Acrylamide gel electrophoresis established a purity of >95%.

3. Results and discussion

A number of preliminary experiments were carried out to check the possible influence on tyrosine fluorescence of several concentrations of NaCl in 10 mM phosphate buffers, using three tyrosine-containing peptides: L-Lysyl-L-tyrosine amide, L-Lysyl-L-tyrosyl-L-lysine and L-Lysyl-L-tyrosine. Only a small change in fluorescence intensity was observed ($\Delta R_{TYR} \leq 0.1$) between pH 3 and 7 and ionic strengths up to 1.0 for the first two peptides. In the case of L-Lysyl-L-tyrosine the fluorescence was greater at pH 7 than at pH 3 by ≈ 0.15. This is presumably due to quenching by the adjacent carboxyl group in this peptide [11]. It was

concluded that no special problems attach to the use of 10 mM phosphate buffers and therefore that all changes in fluorescence intensity of the protein solutions with changing solution conditions are due to protein conformational changes.

Figs. 1 and 2 show quantum yield ratio data obtained on H1 at pH's 3.5 and 7, respectively. The folding of H1 into globular structure by ionic strength or pH increase involving a secondary structure was already shown [4,9,12,13]. It has been established that not all of H1 is included in the tertiary globular fold, but only the section of the chain between residues 39 ± 4 and 116 ± 4, which includes the tyrosine residue at position 72. No β structure was found by infrared and circular dichroism studies in the folded structure of histone H1. The secondary structure of the N-terminal half of H1 can be interpreted in terms of α-helix conformation of the residues 42–55 and 58–75. However the appearance in the NMR spectra of a number of ring-current-shifted resonances upfield of methyl peak, indicates that further tertiary folding takes place to produce a more compact structure. As a result of this, tyrosine 72 is confined to a buried environment. Indirect evidence for a buried environment comes from the observation that nitration or iodination of the residue prevents folding of H1, whilst reduction of the nitro-tyrosine derivative to amino-tyrosine allows the protein to fold but causes changes in the ring-current shifted PMR peaks characteristic of the hydrophobic centre of the fold (G.E. Chapman, unpublished observations). Therefore the change in the ellipticity at 220 nm (fig. 1) reflects the formation of the secondary structure. About 0.5 M NaCl is required for complete folding at pH 3.5, whilst (fig. 2) in low ionic strength at pH 7 the protein is highly folded but still requires added ionic strength to achieve the fully folded state. The changes in fluorescence intensity of H1 clearly parallel the changes in secondary structure (CD) and the associated changes in tertiary structure [4,9,12,13] and must therefore be due to transfer of the single tyrosine residue from a random coil state to a folded state. In the random coil state $R_{TYR} = 0.30$, a value typical for an exposed tyrosine in a denaturated protein [1]. In the folded state, R_{TYR} has an unprecedented value (≈1.3) which is higher than that assumed as representative of non-quenched tyrosyl residues in an aqueous environment [1].

We conclude therefore that in folded H1, the buried

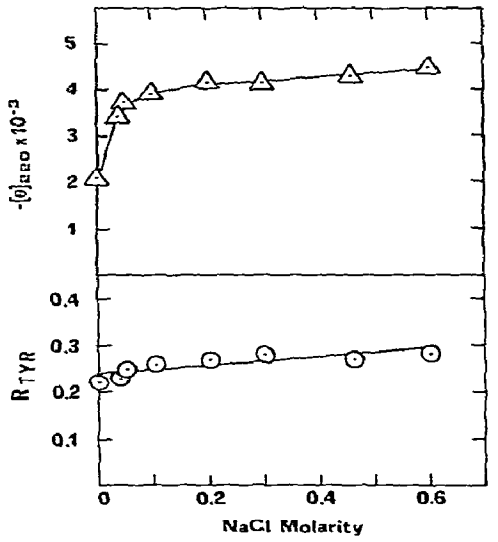

Fig. 3. Ellipticity at 220 nm (upper) and quantum yield ratio (lower) for H5 solutions with changes in NaCl concentration at pH 3.5

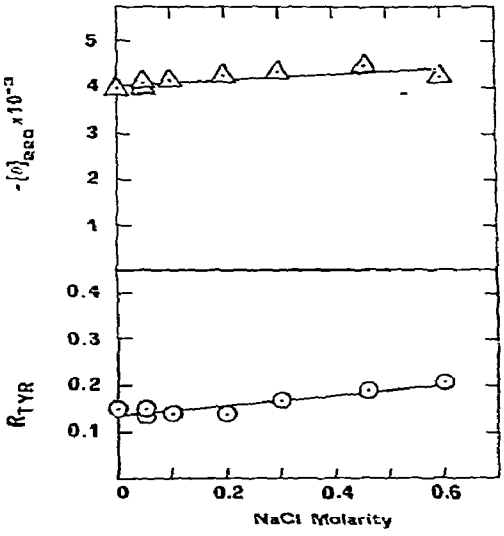

Fig. 4. Ellipticity at 220 nm (upper) and quantum yield ratio (lower) for H5 solutions with changes in NaCl concentration at pH 7.0.

tyrosine is not close to any quenching group so that it displays a much enhanced fluorescence. A new category of buried tyrosine must be added to those already established, i.e. buried in a hydrophobic environment and not hydrogen bonded or close to a quenching group, $R_{TYR} > 1$.

Data for histone H5 are shown in figs. 3 and 4. The conformation of H5 is more sensitive to pH and ionic strength than H1. In the fully folded state the ellipticity at 220 nm is about −4200°. Recent conformational studies have shown that, like H1, not all of the 185 residues of H5 are included in the globular fold [5]. It has been demonstrated that the globular region of H5 involves residues 1 to at least 99 [14]. The extrinsic Cotton effects and the PMR spectrum of H5 show tyrosine perturbations, directly implicating tyrosine in the folded segment [5]. The three tyrosines at positions 28, 53 and 58 are therefore included in the fold. Fig. 3 shows that in the random coil state at pH 3.5 (no NaCl) R_{TYR} is about 0.25, a value reasonable for the denaturated state. The same figure also shows that folding with the increasing of ionic strength has essentially no effect on the fluorescence intensity. At pH 3.5 the lack of change in fluorescence intensity on folding might be the result of a compensating effect in which some tyrosine residues show an enhancement (exposed and non-quenched or buried in a non-polar environment) and others a reduced fluorescence intensity because of the interaction with a carboxyl group. The data of fig. 4 at pH 7 support this tentative interpretation since there is a further reduction of the fluorescence even if the amount of secondary structure is the same of that found at pH 3.5. We are inclined to suggest a higher quenching effect of the dissociated carboxyl group. It is impossible with our data to indicate how many tyrosine groups are involved in the interaction with carboxyl groups. It is also possible, of course, that all 3 residues show no change in fluorescence intensity on folding, but this seems unlikely particularly on the basis of the comparison of the data of figs. 3 and 4. On the other hand, the small increase in fluorescence intensity as the ionic strength rises to 0.6 is less than 0.1 ans is probably a solvent effect and not the consequence of a structural change in H5.

4. Conclusions

A very large enhancement of fluorescence intensity has been demonstrated for the single tyrosine of histone H1 when the protein undergoes a conformational change from a random coil state to a folded form. It is concluded that in the folded form of H1 the tyrosine residue is buried in the core of the protein in a highly hydrophobic environment, not close to any polar quenching groups such as peptide or carboxyl. Such enhancement of tyrosine fluorescence has not been observed before in a protein but is to be expected if the residue is in a truly hydrophobic environment akin to that of an apolar organic solvent [11]. For histone H5 on the other hand, no change is observed in the fluorescence intensity from the 3 tyrosine residues when the protein folds. Since proteins usually show a marked reduction of tyrosine fluorescence on folding, it is concluded that a compensating effect occurs i.e. that whilst certain tyrosine residues in H5 show a reduction in fluorescence intensity at least one must exhibit an enhanced intensity, probably as in H1.

Acknowledgement

This work was supported by Consiglio Nazionale delle Ricerche, Roma, Italy, No. 74.00468.03 and by the Science Research Council of Great Britain. The authors thank Professor R.W. Cowgill for his comments on the manuscript.

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