FLUORESCENCE OF THE ACIDIC FORM OF POLY C

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1. Introduction

Studies of the excited states of the purines, pyrimidines and their nucleosides and nucleotides, at room temperature in aqueous solution, has been retarded due to their low quantum yield of fluorescence ϕ_F in these conditions [1, 2]. Yet, fluorescence measurements have been possible for some modified residues naturally occurring or introduced in transfer RNA. It has been shown in those cases that ϕ_F is very sensitive to conformational changes of nucleic acid [3, 4].

We report here an even more striking example of this behaviour: Poly C fluoresces only in conditions where it is in a semi-protonated double stranded helical form.

2. Experimental

Poly C was a product of Miles Laboratory, Elkhart, Ind., USA. Fluorescence measurements were done in a Jobin–Yvon spectrofluorimeter, or with a FICA 55 absolute spectrofluorimeter as previously described [3]. Measurement of the time-life has been done with the single-photon counting apparatus for the detection of emission events in the nanosecond region, built by Le Bray and J.B. Le Pecq.

3. Results

3.1. *Fluorescence and conformation of Poly C*

No fluorescence can be detected at neutral pH when Poly C is in the single-stranded form. Yet, emission appears between pH 6 and 5 upon acidification (fig. 1a). The variation of fluorescence emission with pH is coincident with the absorption changes that have been related to the formation of the acidic form of Poly C [5]: at low pH this polynucleotide is known to form a double-stranded, semi-protonated helical structure Poly C–Poly C^+ (see references in [5]). Heating a Poly C–Poly C^+ solution results first in a slow regular decrease in the intensity of emission followed by an abrupt transition (fig. 1b). In the pH range 3.5 to 5, the temperature of transition measured by fluorescence is identical (within an experimental error of 0.5°) to that determined by spectrophotometry [5]. It is therefore clear that the fluorescence is detected only in the range of conditions where Poly C is in the double-stranded acidic form. Moreover, in such conditions neither cytidine nor the dinucleotides $C_{3'p5'}C$ and $C_{5'pp5'}C$ are fluorescent.

We have observed that the fluorescence of Poly C–Poly C^+ is insensitive to ionic strength. On the other hand, it is sensitive to temperature (see fig. 1b). Also, a smooth decrease in quantum yield, curve R of fig. 1a, is observed as pH is lowered. This effect is probably due to an increase in the degree of protonation of Poly C–Poly C^+. It has been shown in the similar case of Poly dC that additional protons are accepted by the double-stranded structure as pH is lowered [6]. The pH dependance of Poly C fluorescence at room temp is quite distinct from that observed previously at 77° K in a water–propylene glycol glass [7]. However, it is not clear if the double-stranded structure is stable in those conditions.

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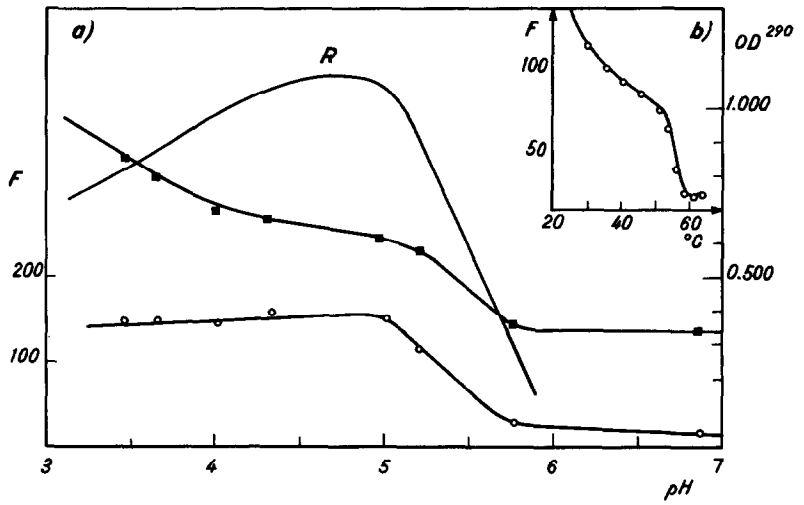


Fig. 1. Fluorescence and conformation of Poly C. a) acidic titration: 1 N HCl is progressively added to a 0.1 N NaCl neutral solution of Poly C (0.390 OD 290). 290 nm absorption (■—■—■) and fluorescence (λexc 290 nm–λem 420 nm) (○—○—○) were then measured as a function of pH. Curve R represents the ratio fluorescence/absorption at 290 nm and varies like Φ_F. b) Effect of temperature on a 1 N NaCl–0.1 N sodium acetate pH 5 solution of poly C.

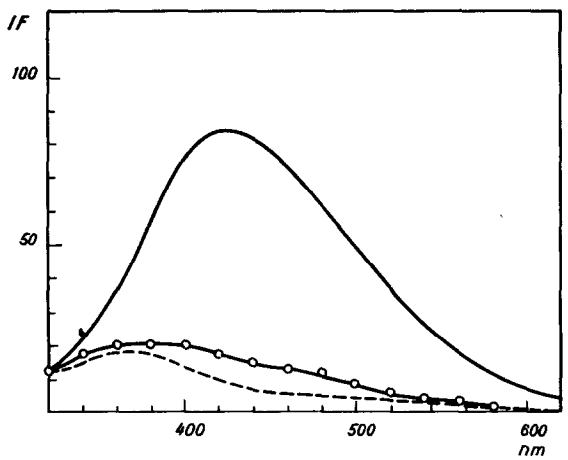


Fig. 2. Emission spectra of Poly C–Poly C^+. Emission of 1 N sodium acetate pH 4.1 solution of Poly C (0.7 OD 260) was measured at 23° with a Jobin–Yvon spectrofluorimeter (λexc 290–Δ λexc = 20 nm–Δ λem = 20 nm). The curves (- - - - - -) and (○—○—○) represent, respectively, the contributions of the pH 4.1 buffer and of Poly C and its buffer at pH 7.

3.2. *Fluorescence characteristics*

The emission band (fig. 2) of Poly C–Poly C^+ is broad (λ^{em}_{max} 420 nm). The energy of the O–O transition calculated from the intersection of the absorption and emission spectra is $3 \pm 0.1\ \mu m^{-1}$. Improvement in the sensitivity of the measurements is necessary to obtain accurate excitation spectra. Our present data indicate that the corrected excitation spectrum is shifted toward the red by some 3 nm with respect to the absorption spectrum of Poly C.

The quantum yield of fluorescence as determined by the ratio method is 4×10^{-3} E $mole^{-1}$. In accord with the current understanding of fluorescence, the time-life is short (less than 2 nsec) and emission is polarized ($p \simeq 24\%$).

4. Discussion

Our observation that Poly C–Poly C^+ fluoresces is particularly interesting in view of the photochemical behaviour of this polynucleotide at room temp. It is known that irradiation at 254 nm of Poly C results in the formation of a cytidine adduct, namely the riboside of 5 (4′ pyrimidin 2′ one) cytosine [8]. We have shown (unpublished work) that this adduct

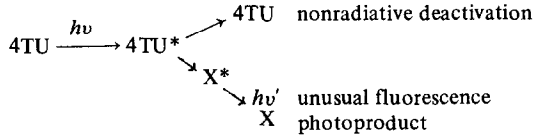
is identical with the 4 thiouridine–cytosine photodimer that we have isolated from *E. coli* tRNA irradiated at 335 nm. This photoproduct is easily identified by its known absorbance properties [4] and ability to be reduced to a fluorescent chromophore by sodium borohydride [3]. We have investigated the formation of this adduct in Poly C and concluded that it forms only under conditions in which Poly C is in the double-stranded semi-protonated acidic structure.

We have also examined the fluorescence and photo-chemical behaviour of Poly I–Poly C complexes at room temp. In this case also a fluorescence [9] similar to that observed in Poly C–Poly C^+ and the formation of the specific adduct is observed only in conditions under which the triple stranded structure Poly I–Poly C–Poly C^+ is stable.

These results are reminiscent of those reported for cytidine at 77° K in frozen aqueous solution at pH values close to 4. Formation of aggregates between one neutral and one protonated cytidine results in the appearance of a new fluorescence [7] distinct from that observed at more acidic or basic pH. At about pH 4, in ice, the emission of cytidine is broad λmax 380 nm) and is very similar to that we report here for Poly C at room temp. Moreover, formation of the specific cytidine adduct at 77° K follows exactly the fluorescence titration curve [8].

It is clear that a particular juxtaposition between one neutral cytidine and one protonated cytidine is needed for the emission of longwave-length fluorescence and for the formation of the specific adduct in both Poly C–Poly C^+ and Poly I–Poly C–Poly C^+ at room temp and cytidine at 77° K. A probable hydrogen bonding scheme between cytosine residues has been proposed for the acidic form of Poly C (see references in [5]). Much less is known for the triple-stranded Poly I–Poly C–Poly C^+ structure [10]. It is rather improbable however, that the arrangement found in Poly C–Poly C^+ remains unchanged in the triple-stranded complex. In the double and triple stranded complexes the interaction between cytidines, necessary for the fluorescence emission, and formation of the specific adduct are, therefore, probably between bases in successive stacked levels. The findings can best be interpreted in terms of charge-transfer complex between one cytidine and its cation as originally proposed by Th. Montenay-Garestier and C. Hélène [7].

In the case of 4 thiouridine (4TU) we have been able to relate the unusual "fluorescence" observed at room temp with the photo-chemistry of this residue. The following scheme where X* is an intermediate excited specie distinct from 4 TU excited singlet or triplet states–4TU*–has been proposed [4].



An analogous scheme is not excluded in the present case. However, the relative position of the emission band as well as its other properties do not rule out the classical scheme where fluorescence and photoproduct formation are competing paths for deactivation of the excited state.

Acknowledgements

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