EVIDENCE FOR A THREE STRANDED COMPLEX BETWEEN POLY I AND POLY C

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The conditions for the formation of a triple stranded semi-protonated complex between polyinosinic (poly (I)) and polycytidylic acid (poly (C)) are described. From potentiometric titrations and mixing curves, the stoichiometry and a phase-transition diagram is established between the partly protonated triple stranded and the unprotonated and completely protonated double helical complexes.

1. Introduction

Giannoni and Rich [1] found that poly (I) and poly (C) * formed a double stranded complex at pH 3 different from that formed at neutral pH [2]. Potentiometric titration showed that one proton was bound for each cytidine residue and gradient centrifugation gave a single peak with the stoichiometry 1 : 1. The titration curve, however, passed through an intermediary step with an apparent pK = 4.9 (in 0.05 M Na^+) at 0.5 protons per cytidine residue. Although no experimental proof was given, Giannoni and Rich [1] assigned an incompletely protonated double stranded structure to this form.

In a recent review [3] this proposal has been criticized on the ground that it seemed rather unlikely that a complex with alternatingly protonated bases would be stable, especially since the melting profiles showed sharp transitions. It also appeared improbable that a rearrangement between the neutral and the acid form could take place without some abrupt changes. According to the proposed scheme for the acid form [1] it seemed impossible to make this arrangement without complete dissociation of the polymers poly (I) and poly (C) and thus formation of the highly stable acid form poly (C). poly (C^+) [4]. Also rotation of the bases seemed rather difficult and thus unlikely. It was therefore suggested [3] that the intermediary form might be a triple stranded structure between one strand of poly (I) and two strands of poly (C), one of the latter carrying a proton (fig. 1). The exact location of the proton would not necessarily be fixed to the N-1 of the cytidine shown, but could be on N-1 of the other cytidine as well, although this seems less likely.

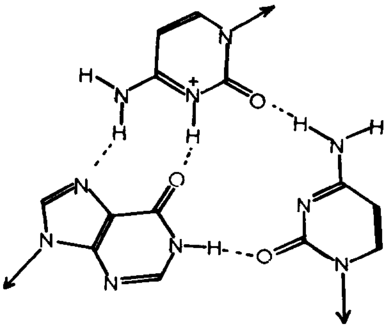

Fig. 1. Possible arrangement between one chain of poly (I), one chain of poly (C) and one protonated chain of poly (C^+) [3].

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* Boursier du S.C.P.R.I. (Ministère de la Santé) (1967-1968).

** The abbreviations are those approved by the IUB as used in ref. [3].

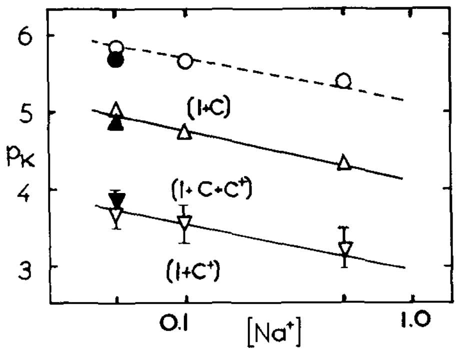

Fig. 2. Variation of potentiometrically measured transition pK
(---o---) poly (C) → poly (C) . poly (C^+)
(—△—) poly (I) . poly (C) → poly (I) . poly (C) . poly (C^+)
(—▽—) poly (I) . poly (C). poly (C^+) → poly (I) . poly (C^+)
filled symbols: results of Giannoni and Rich [1].

2. Material and methods

Poly (I) and poly (C) were purchased from Miles Laboratories, Elkart, USA, or were gifts of Drs. Grunberg-Manago and Michelson.

Potentiometric titrations were performed on a Radiometer recording titrimeter TTT1. Mixing curves [5] were made by mixing appropriate amounts of the two polymers at pH 7, titrating these directly to the desired pH and recording ultraviolet absorption spectra on a Cary 14 recording spectrophotometer. It was found unnecessary to dialyse the solutions against the desired medium [1], the results being the same with both methods.

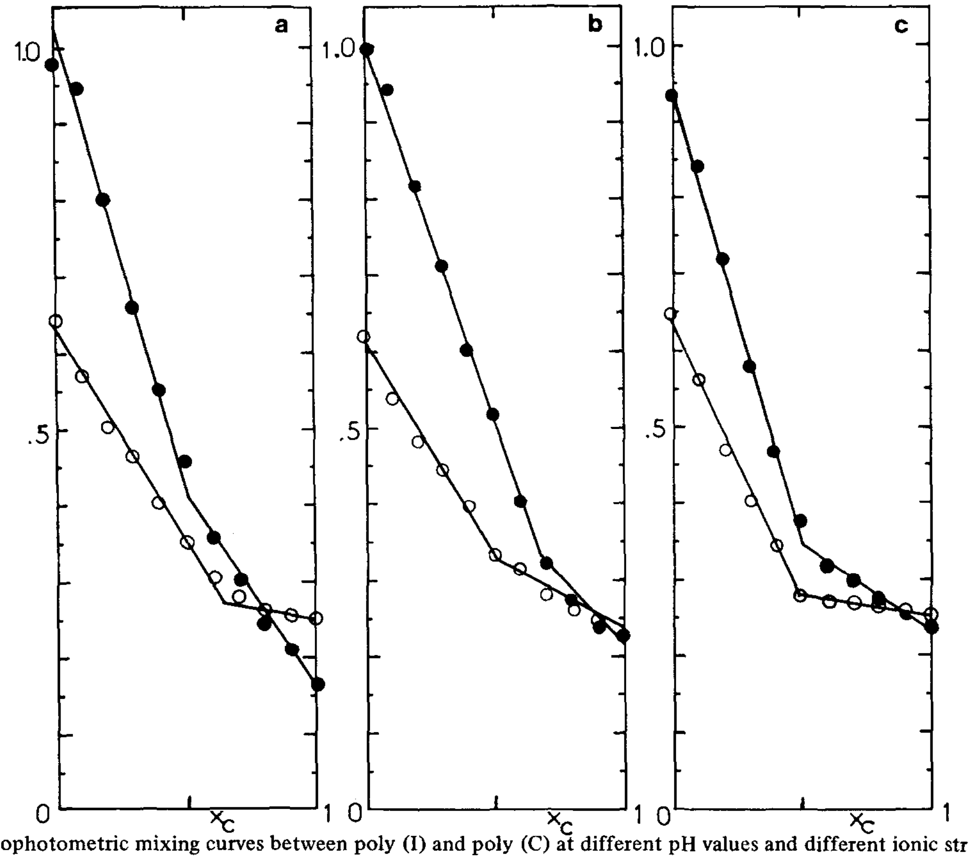

Fig. 3. Spectrophotometric mixing curves between poly (I) and poly (C) at different pH values and different ionic strength.
a/●: pH 3.0, 0.03 M Na^+; ○: pH 3.9, 0.6 M Na^+
b/●: pH 4.7, 0.03 M Na^+; ○: pH 5.2, 0.6 M Na^+
c/●: pH 7.25, 0.03 M Na^+; ○: pH 7.25, 0.6 M Na^+.

3. Results and discussion

The potentiometric titration of the complex poly (I) . poly (C) showed strong dependence of the transition pK on ionic strength (fig. 2), parallel to that for the formation of poly (C) . poly (C^+) which is shown for comparison. From this figure, it can be deduced that formation of the triple stranded complex should be observed at higher pH values, the lower the ionic strength. Fig. 3 confirms this supposition: in 0.03 M Na^+ the triple stranded complex is formed at pH 4.7, while the unprotonated poly (I) . poly (C) is formed in neutral medium and the completely protonated double stranded poly (I) . poly (C^+) at pH 3.0, the latter in agreement with previous results [1]. In 0.6 M Na^+ the triple stranded complex is formed only below pH 4, while at pH 5.2 the unprotonated double helical form is still stable. The mixing curves in fig. 3 show sharp intersections which indicate that thermodynamical equilibrium has been reached [6]. Corresponding results have been obtained at other wavelengths. While the absorbances of poly (C) at any given pH are similar at high and low ionic strength [4], those of poly (I) are much lower in high salt medium (fig. 3). This is in agreement with previous results [7–9] showing that poly (I) forms a multistranded structure at high ionic strength.

Although the paradox of the intermediary semiprotonated complex between poly (I) and poly (C) seems to be solved, it would be premature, however, to speculate about the way this disproportionation takes place.

Acknowledgement

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