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CONFORMATION OF ACID FORMS OF POLY C: TEMPERATURE AND IONIC STRENGTH DEPENDENCE OF PROTONATION OF CYTIDINE AND CYTIDINE-5′-PHOSPHATE

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1. The pK values for protonation of the ring N_3 nitrogen in cytidine-5′-phosphate have been measured over a temperature range of 10 - 80° and at ionic strengths of 0.1, 0.2 and 1.0. 2. The influence of a divalent cation, 10 mM-Mg^{2+}, has also been investigated. 3. The thermodynamic constants for the protonation reaction have been calculated. 4. The significance of the results in relation to the helix-coil transitions of the acid, twin-helical forms of various poly C analogues is discussed. 5. The absorption spectra of both the fully neutral and the fully protonated forms of cytidine have been shown to exhibit a small, but definite, intrinsic temperature dependence similar to that exhibited by other pyrimidines and purines. Although the origin of this temperature effect on an electronic absorption spectrum remains to be clarified, it is shown to be significant enough to be taken into account in some studies on helix-coil transitions in polynucleotides by UV absorption techniques.

The conformations of the so-called "acid" forms of poly rC, poly dC, and the corresponding poly 5MerC and poly 5MedC[1], have been the object of numerous investigations. In the case of poly rC the acid form has been shown by X-ray diffraction to consist of a twin-stranded helix in which two cytosine residues form a "base-pair" *via* hydrogen bonding between the C_2 carbonyls and the amino hydrogens, and the formation of an ionic bond by the sharing of a proton between the N_3 nitrogens, as illustrated in Scheme 1 (Langridge & Rich, 1963). Similar structures prevail in solution for poly rC (Akinrimisi, Sander & Ts'o, 1963), poly 5MerC (Szer & Shugar, 1966), poly dC (Inman, 1964) and poly 5MedC (Żmudzka, Bollum & Shugar, 1969). However, whereas the "acid" forms of the polyribonucleotides are formed only in acid medium, those of the polydeoxyribonucleotides begin to form in slightly alkaline medium (hence the use, above, of the term "so-called acid forms").

[1] The following abbreviations are used in this text: poly rC, polyribocytidylic acid; poly dC, polydeoxycytidylic acid; poly 5MerC, poly 5-methylribocytidylic acid; poly 5MedC, poly 5-methyldeoxycytidylic acid; poly U, polyuridylic acid; poly A, polyadenylic acid; pK_a, apparent pK value; pK, thermodynamic value.