INTERACTION OF POLYNUCLEOTIDES: A HYPOTHESIS ON REASONS FOR THE CHANGES IN THE ULTRAVIOLET SPECTRUM UPON COMPLEX FORMATION

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The method of following the interaction between polynucleotides by estimation of their UV absorption spectra, first introduced by Warner [1] and Davis and Rich [2], is now in common use. Thanks to the development of enzymic methods for the synthesis of polynucleotides, different kinds of polymers have been obtained and their complexing abilities have been investigated.

On complex formation there is decreased absorption at some wavelengths and changes in the shape of the spectrum. The reason for this hypochromism of synthetic polynucleotides, as well as that of DNA and RNA, has been frequently discussed. It is believed that the main forces contributing to hypochromism are the coulombic interactions in the chromophores of the base pairs [3–6]. Moreover, Rich and Kasha [7] have distinguished a hyperchromic region due to $n-\pi$ transitions of helical polynucleotides.

The contribution of solvents to ultraviolet spectra of polynucleotides was clearly shown by Chaney and Gellert [8] who compared the difference spectra of some polynucleotides in coil and helix form with the difference spectra of the appropriate pairs of nucleosides dissolved in water and acetonitrile.

In this paper a possible explanation for the changes in band shape after complex formation is presented.

The dependence of the ultraviolet spectra on the ionic form of the bases is well known. We may assume that during complex formation there are changes in ionic forms of the bases; that the structure of the bases is of a special kind and one base in the presence of another with an exactly complementary structure can give up its proton even in a neutral medium. Such an assumption will make it more understandable why pK values of the bases are so dependent on structure, differing for the nucleosides and the polymers.

According to this hypothesis the changes in UV absorption after complex formation should be correlated with the changes in spectrum connected with transition from neutral forms to ionic ones. Similar kinds of bonds, between the anionic form of thymine and the cationic form of adenine, were proposed by Tsuboi [9] for adenine–thymine pairing in DNA on the basis of infrared spectra investigations.

It seems reasonable to investigate this hypothesis by imitating complex formation using the ultraviolet spectra of the appropriate forms of nucleosides and comparing them with the spectra of polynucleotide complexes.

This has been done as follows: the control spectra, which correspond to the additive spectra of two complementary polymers if there is no interaction between the bases, were estimated from the ultraviolet absorbance spectra of a complementary pair of nucleosides in equimolar amounts in neutral forms; the complex spectra which correspond to the spectra of polymer–polymer complex, were estimated from ultraviolet absorbance spectra of the cationic form of one nucleoside and an anionic form of the other.

The results of such a procedure are given in the figures. The spectra of the nucleoside pairs and those of appropriate pairs of polynucleotides are given. The choice of polynucleotides was dictated by their availability or by the availability of literature data.

As the first example fig. 1a presents the spectra of poly (rA) + poly (rU) for control and complex curves.

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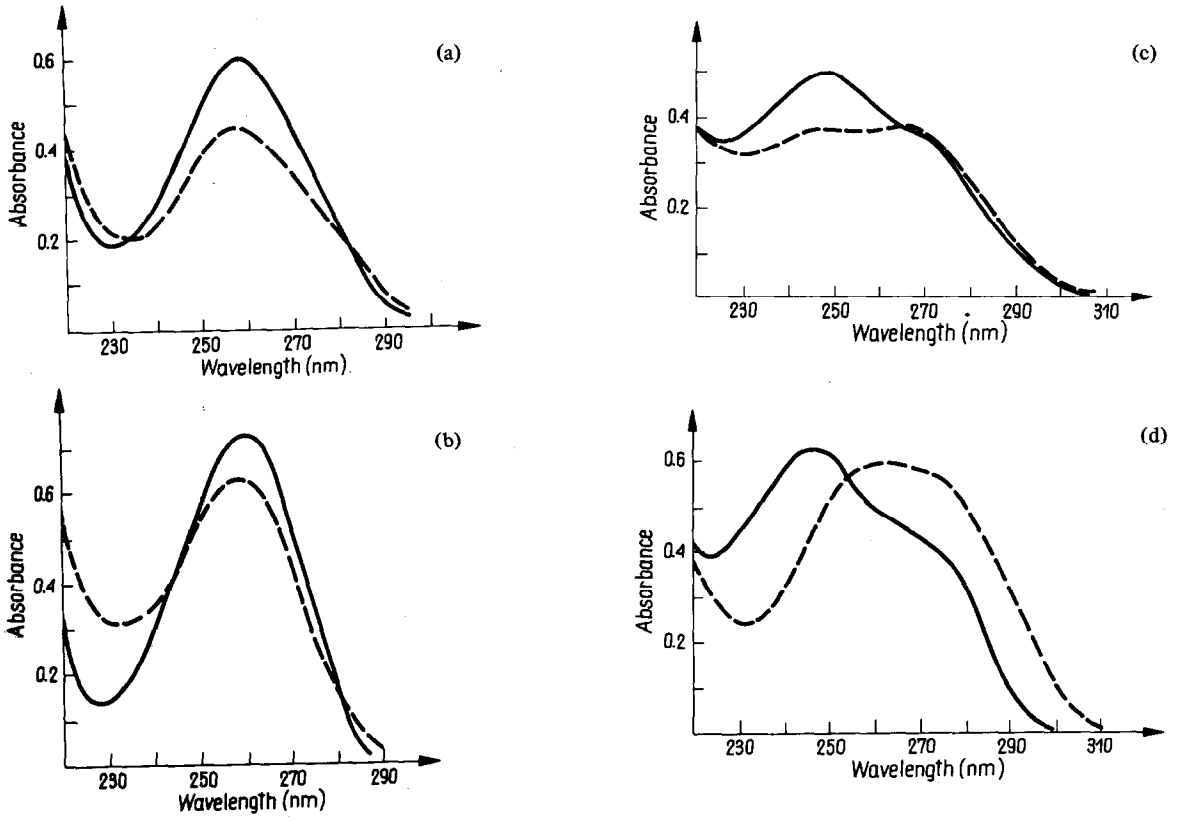

Fig. 1 (a). Ultraviolet spectra of poly U (0.059 mM) + poly A (0.059 mM) in 0.1 M NaCl + 0.01 M phosphate buffer pH 7.0. (——) Theoretical curve: an additive spectrum of the two individual components; (-----) complex curve obtained after mixing of 1 volume of poly U with 1 volume of poly A. (b) (——) Ultraviolet spectrum of neutral form of uridine + neutral form of adenosine, (-----) ultraviolet spectrum of anionic form of uridine + cationic form of adenosine. The ratio is 1:1, and the concentration is the same as in 1 (a).

Fig. 2 (a) Ultraviolet spectra of poly I (0.068 mM) + poly C (0.068 mM) in 0.1 M NaCl, 0.01 M phosphate buffer pH 7.8. (——) Theoretical curve, (-----) complex curve obtained after mixing of 1 volume of poly I with 1 volume of poly C. (b) (——) Ultraviolet spectrum of inosine + cytosine both in neutral forms, (-----) ultraviolet spectrum of anionic form of inosine + cationic form of cytosine. The ratio is 1:1, the concentration is the same as in (a).

Next to it on fig. 1b, there is the algebraic sum of the spectra of adenine + uridine in neutral forms – which corresponds to the control curve, and the spectrum of an anionic form of uridine + the cationic form of adenine – which corresponds to the complexing curve.

In this case, as in the remaining ones, the quantity of nucleosides taken into account is equivalent to the quantity of nucleosides in the complementary polymers.

The same situation is presented in fig. 2 for poly (rI) + poly (rC) (2a) and cytidine + inosine (2b). It is worth noting that protonation of cytidine involves drastic changes in the UV spectrum. For neutral species at pH 7.0 λ_{max} = 271 nm, ϵ_{max} = 9.700; λ_{min} = 250 nm; ϵ_{min} = 6.500. At pH 2.0 λ_{max} = 280 nm, ϵ_{max} = 13.400; λ_{min} = 241 nm, ϵ_{min} = 1.700.

The next example is taken from Ikeda et al. [10] and represents the complex between poly (rU) and poly (r2-amino N^6-MeA) (poly 2-amino, N^6-methyladenylic acid) (fig. 3a). The curves for the appropriate pair of nucleosides are also given (3b). Since the spectra of 2-amino, N^6-methyladenosine are not as well known as the spectra of nucleosides presented heretofore, fig. 4 gives the spectra of both the neutral and acid forms of this compound.

The last example concerns the complex formed between poly (rU) and poly (r2-AP) (poly 2-aminopurine ribotydylic acid). In this case upon interaction

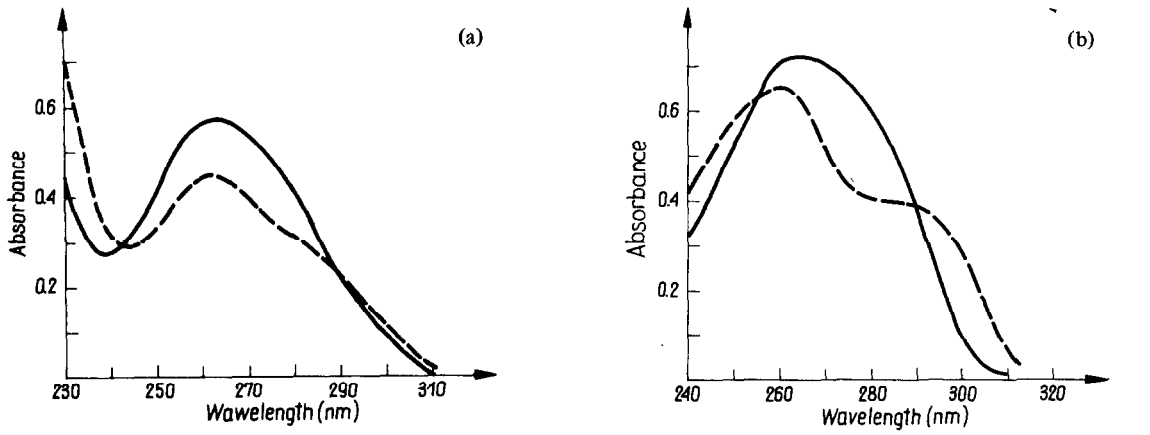

Fig. 3 (a). Ultraviolet spectra of poly $2NH_26MeA$ (0.069 mM) + poly U (0.069 mM) in 0.1 M NaCl, 0.05 M sodium cacodylate pH 7.45. (——) Theoretical curve, (-----) complex curve obtained after mixing these two polymers in the ratio 1:1 (based on the data Ikeda et al. [10]). (b) Ultraviolet spectrum of neutral forms of 2-amino, N^6-methyladenosine + uridine, (-----) ultraviolet spectrum of cationic form of uridine. The ratio is 1:1, the concentration the same as in 3 (a).

of the polymers we can obtain only three stranded complexes where poly U: poly 2-AP is stoichiometrically as 1:2. The method of synthesis of poly 2-AP, as well as its properties will be described elsewhere.

Although tri-stranded helices make the analysis of the complex more difficult, the prominent shift in the spectrum of poly 2-AP far beyond the spectrum of poly U allows for observation of complex formation associated changes in the spectrum of purine components alone.

Protonation of poly-2-AP causes a decrease in absorption spectra with a simultaneous shift of λ_{max} from 305 nm to 312 nm. The same type of change is involved in the protonation of 2-amino-purine riboside where there is only a small difference in the maximum position between the monomer and the polymer (see fig. 5).

Fig. 6a presents the spectrum of the poly (r2-AP) + poly (rU) (2: 1) complex, together with a control curve.

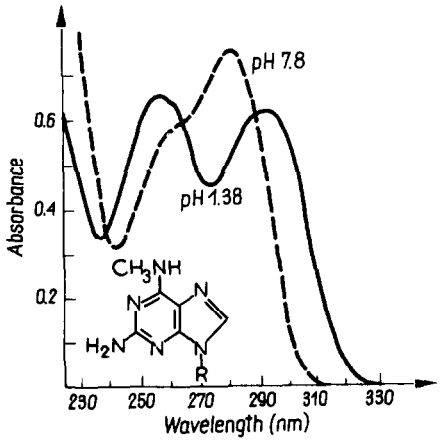

Fig. 4. Ultraviolet spectra of 2-amino, N^6-methyladenosine (——) at pH 1.38 (cationic form) and (-----) at pH 7.8 (neutral form) (from Ikeda et al. [10]).

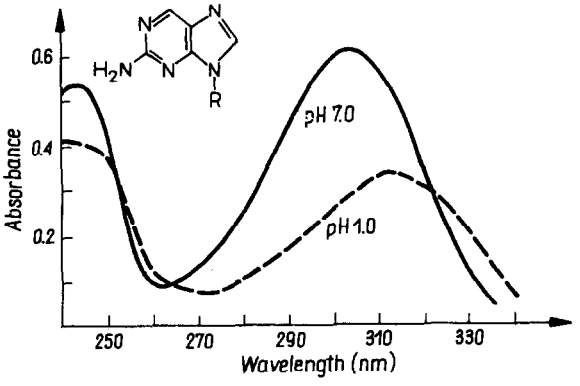

Fig. 5. Ultraviolet spectra of 2-aminopurine riboside (——) at pH 7.4 (neutral form) and at pH 1.0 (acid form).

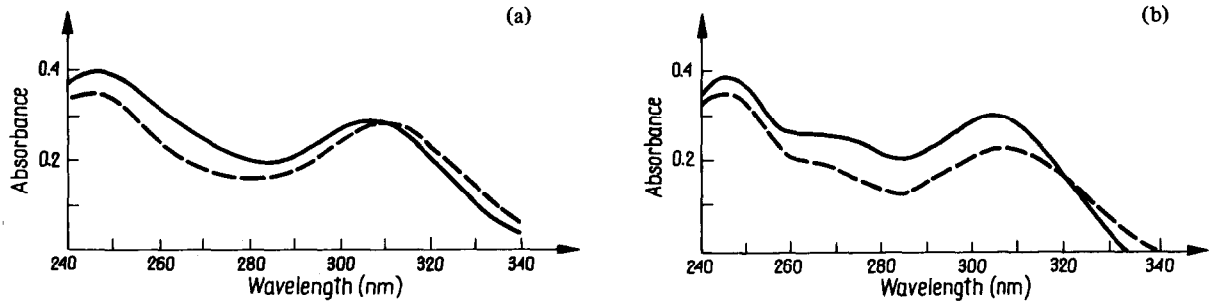

Fig. 6 (a). Ultraviolet spectra of poly 2AP (0.0874 mM) + poly U (0.0436 mM) in 0.5 M NaCl, 0.01 M phosphate buffer, pH 7.25. (——) Theoretical curve; (-----) complex curve, obtained after mixing of 1 volume of poly 2AP with 1 volume of poly U. (b) Ultraviolet spectrum of 2-aminopurine riboside + uridine, both nucleosides in neutral form; (-----) ultraviolet spectrum of acid form of aminopurine riboside + neutral form of 2-aminopurine riboside + anionic form of uridine. In the ratio 0.5:0.5:1. The concentration is the same as in 6 (a).

We can see quite distinctly the shift of maximum in part of the poly r2-AP spectrum characteristic for protonation. This phenomenon is connected exclusively with complex formation. After dissociation of the complex by alkali or heating, this part of the spectrum returns to the previous state.

The similar kind of changes in UV absorption spectra, the appearance of cationic form of 2-aminopurine residue connected with helix formation, has been observed by Ward et al. [11] in alternating polymers: poly r(2-AP-T) and poly r(2-AP-BU).

Fig. 6b presents as usually the spectrum composed of an appropriate form of the nucleoside. Due to three-stranded helices the control curve is composed of the neutral form of uridine and 2-aminopurine riboside in the ratio 1:2, while the complexing curve is composed of an anionic form of uridine, a cationic form of 2-aminopurine riboside, and a neutral form of 2-aminopurine riboside in the ratio (1:1:1).

The similarity between the ultraviolet spectrum after complex formation and the spectrum of nucleosides imitating complex formation is especially striking in this last example. Still some doubts remain whether this consonance is an accidental or a real one. It seems quite reasonable that during the close connection that must exist in di- or three-stranded helices the shift of protons can take place, and influence ultraviolet spectrum. However, the remaining type of bonds, between the keto and amino groups of the complementary bases, must be of hydrogen type.

Simuth et al. [12] working with a complex of poly (rA) · (r4-thioU) have also observed the appearance of an anionic form of 4-thiouracil spectrum. The authors have pointed out that the dissociation of this complex does not lead to differences in absorbance at 260 nm. This will be understable if we assume that the ionic form of the 2-thiouracil residue takes part in complex formation. Comparison of the anionic and neutral spectra of 4-thiouridine (Lipsett [13]) have revealed that at 260 nm the absorbance of both forms is almost equal.

Since Watson and Crick [14] proposed the hypothesis of a "pairing mistake" as an explanation of spontaneous mutations, much attention has been given to the estimation of tautomeric forms of bases, or the frequency with which these two forms occur. In the present situation the problem of tautomerism seems to be of secondary importance. It is more important whether changes in tautomeric forms can really change the complexing properties of the bases. Whether, for instance, the change in cytosine residue from an amino to imino form can automatically change the properties of cytosine from a proton acceptor, which it presumably is when in complex with a hypoxanthine or guanine residue, to a proton donor when paired with adenine. This last possibility sounds rather improbable.

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