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DEMONSTRATION OF BASE-PAIRING BETWEEN 2-SUBSTITUTED ADENINE AND URACIL DERIVATIVES IN AQUEOUS AND NON-AQUEOUS SOLUTION

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Interaction of 2-substituted adenine derivatives were studied spectrophotometrically. They are able to base-pair with uracil either of a polymerized form in aqueous solution or of a monomeric form in non-aqueous solution. The Hoogsteen type base-pairing is possibly formed between the 2-substituted adenine and uracil. A model is raised in which a conformational change of tRNA anticodon region occurs through the formation of base-pairing between the modified adenosine and uridine.

A number of modified bases are found in tRNA molecule. In particular, hypermodified purine nucleotides are frequently found to be located on the 3'-side of the anticodon sequence. We have investigated the interaction of such adenine derivatives with uracil which is either polymerized or monomeric. The study of complex formation between polynucleotides and their complementary monomers in aqueous solution is useful in providing the information on molecular interaction in helical structure.[1] Also, the study of interaction between complementary monomers in non-aqueous solution is relevant to elucidate the properties of planer hydrogen bonding.[2]

Polyribo-5-bromouridylic acid (poly(rBU)) was used as a polymeric form of uracil because of the thermal stability of the complexes, and 1-cyclohexyluracil (ch^1U) was used as monomeric uracil for its solubility in $CDCl_3$.

The circular dichroism (CD) and absorption spectra of an equimolar mixture of 2-methyladenine (m^2A) and poly(rBU) in 0.4 M Na^+ (pH 7.0) at 0°C and 25°C are

shown in Fig. 1a. Fairly large positive bands ($[\theta]_{292}$, 1.5×10^4 and $[\theta]_{229}$, 1.1×10^4) and a negative band ($[\theta]_{256}$, -1.3×10^4) are observed by lowering the temperature to 0°C. Concomitantly, the absorbance at 265nm which represents an absorption maximum of the m^2A shows about 30% hyperchromic effect, indicating that the m^2A is incorporated into helical complex. As Fig. 1b shows, the heating curve is conclusively monophasic responsible for a single step of the melting process.

Similar results are obtained for an equimolar mixture of 2-methylthioadenine (ms^2A) and poly(rBU) under the same condition with the m^2A-poly(rBU) mixture. As Fig. 2a shows, the positive CD band at 291nm ($[\theta]$, 1.5×10^4) seems to be a composite of several chromophores, and a small positive band at 260nm is presumably the same one as those observed in the adenosine-poly(rBU) complex[3]. The absorption maxima at 279nm and 236nm exhibit 15-20% hypochromicity due to the complex formation. Fig. 2b shows the heating curve has a monophasic profile also indicating the process is a cooperative one.

The possibility of self-association of the poly(rBU) is eliminated for both experiments by the facts that the CD peaks are entirely different from those of the helical poly(rBU) as well as the characteristic features of the CD spectra.

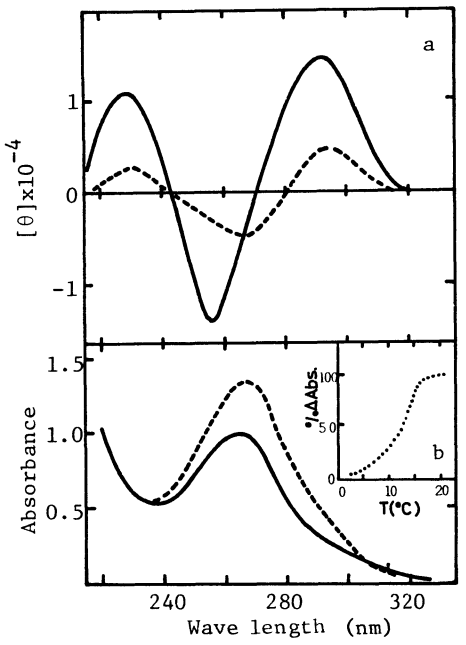

Fig. 1: a, CD and absorption spectra of a 1:1 mixture of m^2A-poly(rBU) in 0.4 M NaCl -0.01 M Na-cacodylate, pH 7.0; —— 0°C, ---- 25°C. b, Heating curve at 268nm.

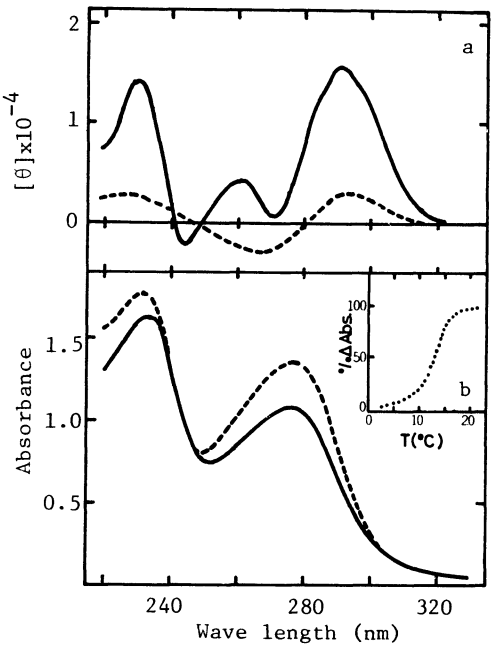

Fig. 2: a, CD and absorption spectra of a 1:1 mixture of ms^2A-poly(rBU) in 0.4 M NaCl -0.01 M Na-cacodylate, pH 7.0; —— 0°C, ---- 25°C. b, Heating curve at 280nm.

The stoichiometry of the complexes are not determined yet. However, it is pointed out that the melting temperatures (Tm) of the m^2A-poly(rBU) and ms^2A-poly(rBU) complexes are 13°C each. This is significantly low compared with the triple-helical adenine-poly(rBU) complex of which Tm was 20°C under the same condition. This suggests the stoichiometry of 2-substituted adenine-poly(rBU) complexes above mentioned is presumably 1:1 instead of 2:1, with reservations that the unknown factors may affect the stability of such helical structures.

It should be referred that these adenine derivatives are devoid of ribose moiety at position N9 and may provide an extra site for hydrogen bonding. Therefore, 9-methyl analogues of m^2A and ms^2A were synthesized and studied in a similar fassion. The CD and absorption spectra illustrated clearly that the helical complexes were formed between 9-methyl-2-substituted adenine and poly(rBU)[4]. This is compatible with the fact that the base-pairing using N3 and N9-H of a purine ring is impossible stereochemically in the case of the 2-substituted adenines.

In connection with the above investigations, the interaction of 2-methyl-9-n-propyladenine (m^2p^9A) and 2-methylthio-9-n-propyladenine (ms^2p^9A) with ch^1U were studied by the infrared(IR) spectral measurements to elucidate the properties of hydrogen bonding in non-aqueous solution. Fig. 3 shows the IR spectra of the mixtures of such adenine and uracil monomers in $CDCl_3$ as well as the spectrum of an unmodified adenine-uracil mixture. The bands at 3525 cm^{-1} and 3420 cm^{-1}, which are associated with the antisymmetric and symmetric stretching vibrations of the amino group of adenine respectively, are shifted to 3485 cm^{-1} and below 3330 cm^{-1} through forming hydrogen bonding with the ch^1U.

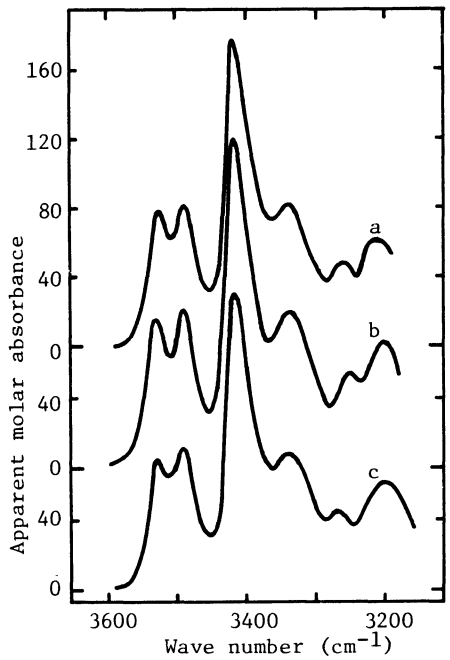

Fig. 3: IR spectra of 1:1 mixtures of ch^1U and a, m^2p^9A, b, ms^2p^9A, c, p^9A in $CDCl_3$. Total concentration, 0.04 M; path length, 1.0 mm (quartz cell).

It is seen in Fig. 3 that the mode of interactions is strikingly similar and the association constants would be in a same order for the three kinds of the

complexes. This is further verified by the experiments of concentration change, and the association constants are measured to be in a range of 180-220 liter/mole. Thus, the scheme of base-pairing for these adenine derivatives may be essentially similar to that of the adenine's[2].

Considering the above results together, it seems likely that the Hoogsteen type base-pairings are formed for the 2-substituted adenine-uracil complexes in which N7 of the adenine ring is utilized for one of the hydrogen bonding[5]. The Watson-Crick type base-pairing is excluded because the bulky substituents at position 2 hinder it sterically.

N^6-Methyladenine and N^6-isopentenyladenine as well as their 9-alkyl analogues are capable of forming the 1:1 base-pairing with either polymeric or monomeric uracil[6]. This fact also corroborates the above interpretation because the results of X-ray diffraction analysis of N^6-substituted adenines revealed that the free N6-H is projected to the imidazole ring which makes it feasible to form the Hoogsteen type base-pairing.

In all the nucleotide sequences of tRNA determined so far except for some eukaryotic initiator tRNAs, the uracil which is located next to the 5'-side of the anticodon (U33 in yeast $tRNA^{phe}$) is known as an invariant nucleoside in the clover leaf structure of tRNA[7]. On the other hand, the modified adenosines which occupy the next position of 3'-side of the anticodon would possibly base-pair with uridine residue nearby it by forming the Hoogsteen type base-pairing, if the environmental condition allows. In a three dimensional structure of tRNA molecule solved by X-

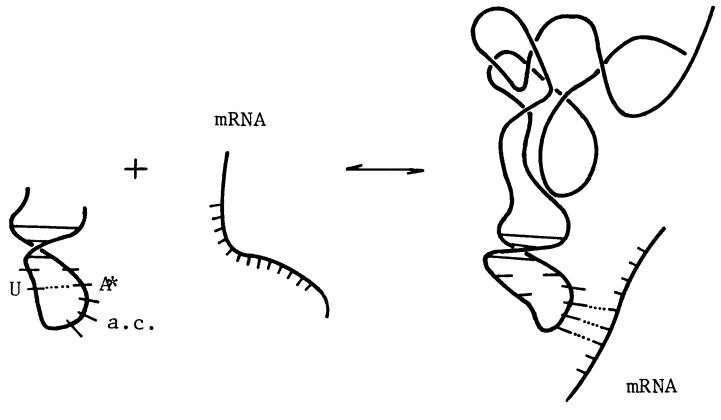

Fig 4: A two state model of the anticodon conformation of tRNA molecule.

ray diffraction method, features of the anticodon conformation is characterized fundamentally as a stacked array of five nucleotides including anticodon triplet. There is no hydrogen bonding between the bases[7]. However, if this stacked state is dissolved for any reason, the hydrogen bonding between the invariant uridine and the modified adenosines must be definitely possible. It should be pointed out that the same situation is seen in the T-Ψ-C loop where 1-methyladenosine base-pairs with thymidine beyond three nucleotides[7].

On this basis, a two state model which describes a conformational change of the tRNA anticodon region accompanied by a release of mRNA is presented in Fig. 4. This model appears quite relevant for tRNA molecule to carry out its biological functions, since it is widely accepted to suppose a cycle of the attachment and release of mRNA on the anticodon. The functional roles of the substituents are possibly, 1) to prevent mRNA from mispairing with the modified adenosines and to send it in a correct site, and 2) to modulate the degree of stacking within the anticodon-array through perturbing the electronic state of the modified adenosine nucleotides.

Recently, some reports appeared describing considerable conformational changes of the tRNA molecule or its crucial portion in the course of its functioning cycle [8,9]. Our model is fully consistent with these suggestions, although the conformational change of the anticodon is not reported yet. Furthermore, there is a report which claims that even a very dilute organic solvent such that used in the crystallization of tRNA may cause significant alterations of the tRNA structure[10]. This sort of experimental results should be taken into account when the tRNA structure in crystal is discussed in relation to the structure in solution where it is biologically active.

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