THE EFFECT OF COMPLEXING BETWEEN POLY-A AND POLY-U ON THE RATE OF THE SLOW $^1H \rightarrow {}^3H$ EXCHANGE IN ADENYLIC ACID

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1. Introduction

The slow isotope hydrogen exchange between purine nucleotides and water involves hydrogen atoms at $C_{(8)}$ of the purine ring [1,2]. The rate of this exchange must depend on the electron density distribution in the purine rings. Since the latter is changed with the change in macromolecular conformation, one could expect the modification of the exchange rate with the alteration of the macromolecular structure.

The results of our previous investigation [3] demonstrate that the rate of the slow hydrogen exchange does depend on the conformation of the polynucleotide molecule. The stacking of the purine bases in poly-A at or near pH 7 results in a pronounced retardation of the $^1H \rightarrow {}^3H$ exchange as compared with AMP; the involvement of $N_{(7)}$ in the hydrogen bonding results in much stronger retardation of the $^1H \rightarrow {}^3H$ exchange in poly-A (pH 4) as compared with AMP.

The purpose of this investigation is to obtain more quantitative information about the effect of the base stacking on the retardation of the hydrogen exchange in polynucleotides and to correlate the magnitude of the retardation with some structural parameters of the complexes involving purine polynucleotides.

2. Materials and methods

The incubation was carried out in 0.05 M acetate buffer (pH 7.1) prepared with tritiated water with different concentrations of sodium ions. The specific radioactivity of these solutions was 100 mC/ml. The formation and stability of the complexes between poly-A and poly-U (poly(A+U) or poly(A+2U)) has been determined spectrophotometrically using melting curves at 259 and 280 mμ. The experimental values of T_m in 0.15 M NaCl for poly(A+U) and in 0.3 M NaCl for poly(A+2U) (57° and 63° respectively) were in good agreement with the data published [4–8]. The incubation was carried out at 45–50° during 72 hr.

The concentration of the complexes has been determined spectrophotometrically using the following values of the molar extinction coefficients: $E_{259}^{poly(A+U)} = 14 \times 10^3$ and $E_{259}^{poly(A+2U)} = 18.6 \times 10^3$ which were calculated per two and per three moles of nucleotides respectively on the basis of the hyperchromic effect of the isomolar solutions prepared in 0.15 M NaCl. The following molar extinction coefficients for poly-A and poly-U, determined on the basis of the phosphorus content, have been used: $E_{257}^{poly\text{-}A} = 10 \times 10^3$ and $E_{261}^{poly\text{-}U} = 8.1 \times 10^3$.

The properties of the preparations used as well as the methods of their isolation from the incubation medium have been described in the previous paper [3].

The retardation of the $^1H \rightarrow {}^3H$ exchange in poly-A due to the interaction with poly-U has been expressed as retardation coefficient K_r (see [3]). The specific radioactivity of poly-U was operationally assumed to be equal to zero since under our experimental conditions in seven independent experiments it did not exceed 1×10^4 while the standard deviation was $\pm 10^4$ dpm/μmole.

3. Results and discussion

The results presented in table 1 demonstrate that

Table 1
The values of specific radioactivity(sp.a.) of poly-A and its complexes with poly-U after the incubation (for conditions of incubation see text).

No. of exp.	sp.a. (dpm/μmole) X 10^{-5}			$K_r^{poly(A+U)}$	$K_r^{poly(A+2U)}$
	Poly-A	Poly(A+U)	Poly(A+2U)		
1	2.2	1.3	1.3	1.7	1.7
2	1.6	0.8	1.1	2.0	1.5
3	2.4	1.2	0.8	2.0	3.0
4	1.6	0.7	0.6	2.3	2.7
				Aver. 2.0 ± 0.3	2.2 ± 0.7

$^1H \rightarrow {}^3H$ exchange in poly(A+U) at 45°C is retarded approximately twice as compared with exchange in the single stranded poly-A. Poly(A+U) complex has a double stranded structure similar to that of native DNA [9]. The amino groups and $N_{(1)}$ of adenine residues in this complex are hydrogen bonded to the $C_{(6)}$ carbonyl groups and $N_{(1)}$ of uracil residues respectively and below 45° all bases of the complex are stacked. According to Poland's calculation [10] however in single stranded poly-A only 36% of bases are stacked under these conditions. Therefore, the formation of the complex between poly-A and poly-U results in the formation of much more regular structure and is accompanied by retardation of $^1H \rightarrow {}^3H$ exchange in adenylic acid. The comparison of the specific radioactivity of poly(A+U) with the specific radioactivity of AMP at 50° (1.2 X 10^5 and 4.4 X 10^5 dpm/μmole respectively) indicates that the transition from the random orientation of the purine rings to practically regular structure in poly(A+U) retards the $^1H \rightarrow {}^3H$ exchange by a factor of 3.7. The same conclusion has been drawn in the preceding paper [3] by the extrapolation of the value of K_r for a single stranded poly-A to the temperature below 0°, where the regularity in poly-A approaches 100%. These data suggest that the retardation of $^1H \rightarrow {}^3H$ exchange in both poly-A and poly(A+U) is due to the increase in the regularity of the polymer structure and that the dependence between the K_r and the percentage of stacked bases is approximately linear (see fig. 1).

It should be mentioned however that the structure of the three-stranded complex poly(A+2U) as well as the nature of forces responsible for its stability are yet unknown [11,12]. It was established that the addition of the second poly-U strand to poly(A+U) complex

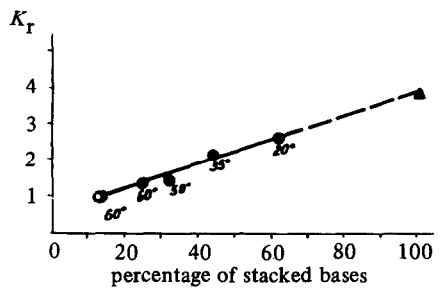

Fig. 1. The dependence of K_r on the amount of stacked bases (in %) in poly-A. ○ – value of McDonald and Phillips [19] for 92°. ● – data of our previous investigation [3]. ▲ – value for the poly(A+U).

resulting in the formation of poly(A+2U) complex does not alter the bulk conformation of the molecule; the analysis of thermodynamic parameters of poly(A+U) and poly(A+2U) suggests a relatively weak interaction between the double helix of poly(A+U) and the supplementary strand of poly-U [13–16]. According to Felsenfeld [17] the second strand of poly-U occupies the deep groove of the double helix, displacing water molecules from it. This may result in the formation of the stable hydrogen bridges between adenine residues of poly-A and uracil residues of the supplementary poly-U strand. On the bases of IR spectral studies Miles [18] concluded that such bridges were indeed formed; however they involved $C_{(2)}$ but not $C_{(6)}$ carbonyl groups of uracils as in Watson-Crick pairing. Further it was postulated that the position of $N_{(7)}$ and the amino group of adenine are responsible for the interaction between the double stranded poly(A+U) structure and the supplementary poly-U strand.

The data of table 1 demonstrate that the $^1H \rightarrow {}^3H$ exchange in poly(A+2U) at 45° just as in poly(A+U) is retarded approximately twice as compared with poly-A. Similar magnitude of retardation in both poly(A+U) and poly(A+2U) as compared with poly-A might indicate the absence of hydrogen bonding between $N_{(7)}$ of adenine and $N_{(1)}$ of uracil of the second poly-U strand, since the participation of $N_{(7)}$ in hydrogen bonding within double stranded poly-A results in a very strong retardation of the hydrogen-tritium exchange [3].

4. Summary

The retardation of the hydrogen-tritium exchange in adenylic residues of poly(A+U) and poly(A+2U) as compared with poly-A and AMP was demonstrated.

The formation of the double stranded complex poly(A+U) is accompanied by about fourfold retardation of $^1H \rightarrow {}^3H$ exchange in adenylic acid residues as compared with AMP. The addition of the second poly-U strand to this complex resulting in the formation of poly(A+2U) does not lead to further retardation of the exchange. This may indicate the absence of stable hydrogen bonding between $N_{(7)}$ of poly-A adenine and $N_{(1)}$ of uracil in the second poly-U strand.

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