STUDIES ON THE EFFECT OF CONFORMATION ON THE RATE OF THE SLOW ISOTOPE HYDROGEN EXCHANGE IN POLYADENYLIC ACID

Maslova R.N., Lesnik E.A. and Varshavsky Ya.M.
Institute of Molecular Biology, Adademy of Sciences USSR, Moscow.

Received December 2, 1968

Several authors have reported that the hydrogen exchange between nucleic acids and water is completed within a few minutes (Sutherland and Tsuboi, 1957; Bradbury et al.,1961; Abaturov and Varshavsky,1965). However Printz and von Hippel (1965) have been able to demonstrate in the native DNA besides this rapid exchange, involving H-atoms of NH and OH groups and "bridge" hydrogens, the slow hydrogen exchange the nature of which remained obscure. Osterman et al. (1967) have presented evidence that the slow exchange could be observed not only in native DNA but also in the denatured DNA, as well as in free nucleoside diphosphates. These authors suggested that H-atoms of CH groups of DNA purine and pyrimidine rings were responsible for this process. This suggestion recently was confirmed by Fritsche (1967) who demonstrated H-D exchange at $C_{(8)}$ of DNA purines.

It is known that the rate of slow hydrogen exchange for CH groups is considerably affected by the electron density at corresponding carbon atoms. For nucleic acids the distribution of electron density depends on the conformation of macromolecules in solution (hydrogen bonding in double-strand helices, stacking of purine and pyrimidine bases etc.). Therefore it was of interest to determine whether the rate of slow exchange

involving CH groups of synthetic polynucleotides would be dependent on the conformation of the molecule.

This paper presents the results of experiments on the rate of $^{1}H \longrightarrow {}^{3}H$ exchange in adenosine-5'-monophosphate (AMP) and in polyadenylic acid (poly-A) under various conditions. This experimental model has been chosen since poly-A in solution can exist in two different interconvertible conformations "neutral" and "acid" depending of the temperature and pH (Steiner and Beers, 1959). In neutral solution the molecules of poly-A exist as single-strand helices with extensive base stacking (Brahms et al., 1966) while in acid solution double-strand helices are formed with hydrogen bonds between N-7 of adenine of one strand and amino groups of adenine of another strand (Brian and Tomita,1961). The two helices however are quite random in terms of long-range conformation but highly ordered in terms of short-range interactions (Holcomb and Tinoco, 1965).

MATERIALS AND METHODS. Preparations of AMP and poly-A were supplied by "Reanal" (Budapest). Poly-A chains were at least then 60 nucleotides in length. The concentrations of AMP and poly-A were determined spectrophotometrically in 0.1M NaCl containing 0.05 acetate buffer pH7. The extinction coefficients were: $E_{259}^{AMP} = 15.0 \times 10^3$ and $E_{257}^{poly\text{-}A} = 10.0 \times 10^3$.

For the separation of AMP from tritiated water (specific radioactivity 100 mC/ml) DEAE Sephadex (in chlorode form) columns (1x3 cm) were emploed. Poly-A was separated from tritiated water by gel filtration through Sephadex (G-50 medium) columns (1x24 cm). Radioactivity was determined by liquid scintillation counting. Efficiency of tritium determination was about 10%; the background of the counter was 120 cpm.

RESULTS AND DISCUSSION. Table 1 presents the average values of specific radioactivity of AMP and poly-A incubated in tritiated water.

TABLE 1

SPECIFIC ACTIVITY (Sp.a.) OF POLY-A AND AMP.

t°C	Time of incubation, hrs	pH	Number	of Sp.a. of AMP (dpm/μM)	Sp.a. of poly-A (dpm/μM)	K_{ret}
20.0	240	7.1	1	3.7×10^4	1.4×10^4	2.6
35.0	240	7.1	1	2.9×10^5	1.3×10^5	2.2
45.0	72	7.1	4	-	2.0×10^5	-
50.0	72	7.1	3	4.4×10^5	3.2×10^5	1.4
60.0	72	7.1	3	10.7×10^5	8.1×10^5	1.3
80.0	40	7.1	1	19.2×10^5	18.6×10^5	1.0
92*)	-	7.1	-	$\tau_{1/2}$ =90 min	$\tau_{1/2}$ =90min	1.0
60.0	72	4.1	3	10.0×10^5	0.1×10^5	100.0

*) From the work of Mc Donald and Phillips (1965).

The values of rate constants were calculated from the first-order equation:

$$k \cdot t = \ln \frac{(Sp.a)_\infty}{(Sp.a.)_\infty - (Sp.a.)_t}$$

assuming that the values of $(Sp.a.)_\infty$ corresponded to isotopic equilibrium is 0.9×10^3 mC/mole of the base (2×10^6 dpm/mole). The dependence of ln k from 1/T is shown in Fig.1.

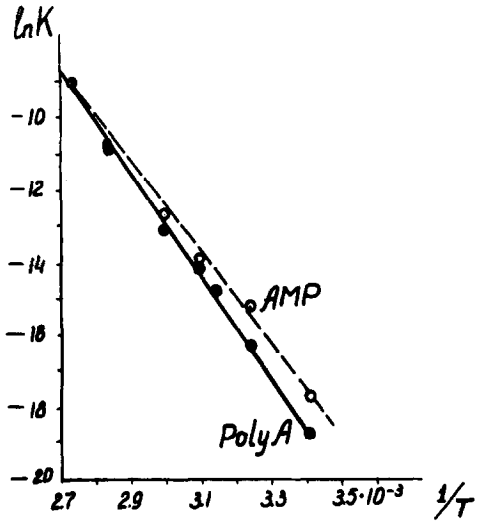

Fig.1. Temperature dependence of $^1H \longrightarrow {}^3H$ exchange rate constants for AMP and poly-A at pH 7.1.

The effective values of activation energy for $^1H \longrightarrow {}^3H$ exchange in AMP and in poly-A calculated from these data amount to 25-26 and 28 kcal/mole of the base respectively. It should be born in mind however that the value of activation energy for poly-A is relative due to the temperature changes in the structure of poly-A.

It can thus be concluded from these data that rate of $^1H \longrightarrow {}^3H$ exchange in AMP under all conditions tested is higher than that in poly-A. The observed difference can be expressed by a constant of retardation:

$$K_{ret} = \frac{\text{Sp.a.of AMP}}{\text{Sp.a. of poly-A}}$$

It can be seen that at pH 7 the value of this constant decreases with the increase in temperature and at 80-90°C it becomes equal approximately to unity. This decrease in K_{ret} correlates with the decrease in the percentage of stacked bases

in poly-A molecules accompanying the rise in temperature. According to the calculations of Poland et al. (1966) the percentage of stacked bases at 35, 50, 60 and 80°C corresponds to 44, 32, 24 and 15% respectively. The proportionality between K_{ret} and the percentage of stacked bases in poly-A might indicate that the stacking retards the hydrogen exchange in CH groups of adenine residues. If the correlation between K_{ret} and the percentage of stakced bases in poly-A holds also for lower temperatures than at 0°C, the rate of hydrogen exchange in poly-A should be about 4 times less than in AMP.

A much stronger retardation of $^1H \longrightarrow {}^3H$ exchange in poly-A as compared with that in AMP is observed in acid solutions. At pH 4 and 60°C the specific radioactivity of poly-A is about 100 times less than that of AMP (see Table 1). Such a strong decrease in the exchange rate in double-strand "acid" form of poly-A cannot be due to the protonation of $N_{(1)}$ of purine ring since the rate of exchange for AMP is the same at pH 7 and pH 4.

It might be suggested that this strong retardation is due to the shift of electron density at $C_{(8)}$ of adenine due to the participation on $N_{(7)}$ in hydrogen bonding in double-strand helices of poly-A.

SUMMARY. The rate of slow isotope exchange of hydrogen atoms in poly-A depends on the secondary structure of the polymer. The stacking of purines in poly-A retards the exchange several times as compared to that in AMP. The formation of double-strand helices in acid solutions (pH 4) results in much stronger retardation of the exchange (about 100 times). This effect might be due to considerable alterations of electron

density at $C_{(8)}$ accompanying the formation of double-strand helices of poly-A.

ACKNOWLEDGEMENT. The authors wish to thank Miss S.Shulak for the technical assistance.

REFERENCES

Abaturov L.V., Varshavsky Ya.M.Dokladi AN SSSR, 160,464 (1965).
Bradbury E.M., Price W., Wilkinson G.R., J.Mol.Biol.,3,301(1961).
Brahms J.,Michelson A.,Van Holde K.E.,J.Mol.Biol.,15,467(1966).
Bryan R.F.,Tomita K.,Nature 192,812(1961).
Fritzsche H.,Biochem.Biophys.Acta,149,173 (1967).
Holcomb D.N.,Tinoko J.R., Biopolymers 3, 121 (1965).
McDonald C.C., Phillips W.D., Biopolymers 3, 609 (1965).
Osterman L., Adler V.V., Bibilashvily R., Varshavsky Ya.M., Biochimia 31, 398 (1966).
Poland D., Vournakis J.N., Scheraga H.A., Biopolymers 4,223 (1966).
Printz M.P., Von Hippel P.H., Proc.Nat.Acad.Sci.USA,53, 363 (1965).
Steiner R.F., Beers R.F., Biochem.Biophys.Acta 32, 166 (1959).
Sutherland G., Tsuboi M., Proc.Roy.Soc. A, 239, 446 (1957).