CHEMISTRY LETTERS, pp. 495-498, 1980.

HYDROGEN-DEUTERIUM EXCHANGE KINETICS IN A TRIPLE-HELICAL POLYNUCLEOTIDE

Yuzuru HAYASHI, Mamoru NAKANISHI, and Masamichi TSUBOI
Faculty of Pharmaceutical Sciences, University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113

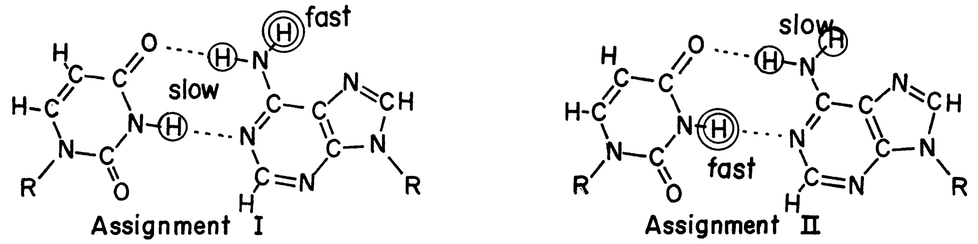
The kinetics of the hydrogen-deuterium exchange reaction has been followed by stopped flow ultraviolet absorption spectroscopy of a triple-helical polynucleotide, composed of 1 mole polyriboadenylic acid and 2 moles of polyribouridylic acid, poly(rU)·poly(rA)·poly(rU).

The rates of the hydrogen exchange in double-helical polynucleotides have been examined in aqueous solutions by several investigators, of a few different samples, by two different methods, and at various temperatures and pHs.1-10) For every double-helix so far examined, two rate classes have been found, faster and slower classes. To these classes two different sets of assignments have been proposed:

Assignment I --- The faster class to the amino hydrogen that is not involved in the hydrogen bonding in the base-pair, and the slower class to the amino and imide hydrogens involved in the base-pair.1-3), 6), 8), 9)

Assignment II --- The faster class to the imide hydrogen of uracil or thymine, and the slower class to the amino hydrogens of adenine or cytosine.4), 5), 7) 10)

The assignments I and II are illustrated in Fig. 1 for the case of the adenine·uracil base-pair which is involved in double-helical poly(rA)·poly(rU), for example.



Assignment I

Assignment II

Fig. 1. Base-pair in the double-helical poly(rA)·poly(rU).

We have made a hydrogen exchange study of a triple-helical polynucleotide with an expectation that the assignment problem may be fixed by it. The triple-helical poly(rU)·poly(rA)·poly(rU) is considered to have11) an inter-base hydrogen bonding shown in Fig. 2. Therefore, one can expect no hydrogen atom with a greater rate constant and four with a lower one in the assignment I, whereas two fast and two slow hydrogens in the assignment II.

Polyriboadenylic acid, poly(rA), and polyribouridylic acid, poly(rU), were purchased from Boehringer and some from Yamasa. For preparing pure double-helical and triple-helical samples, a reference was made to previous works[12-15] on the equilibrium among the double-helical poly(rA)·poly(rU), triple-helical poly(rU)·poly(rA)·poly(rU), and single-stranded poly(rA) and poly(rU). It has been found that an equimolar mixture of poly(rA) and poly(rU) in a solvent with 0.09 M Na^+ often forms a small amount of metastable triple-helix. If a solvent with 0.01 M Na^+ is used at 30°C, only double helix is always formed, and even when the polymer concentration is suddenly lowered (by a stopped-flow device) no strand disproportionation is detected. The sample of the triple-helical poly(rU)·poly(rA)·poly(rU) was prepared by dissolving the 1 mole to 2 mole mixture into a solvent with 0.3 M Na^+ + 0.005 M phosphate buffer, pH 7.0 (at 30°C).

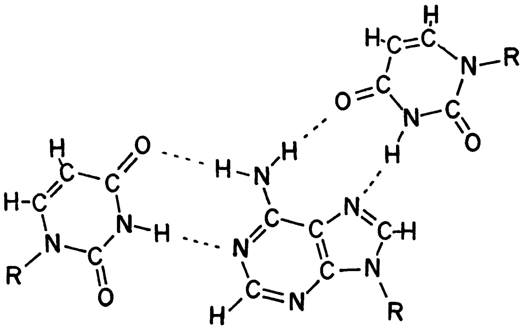
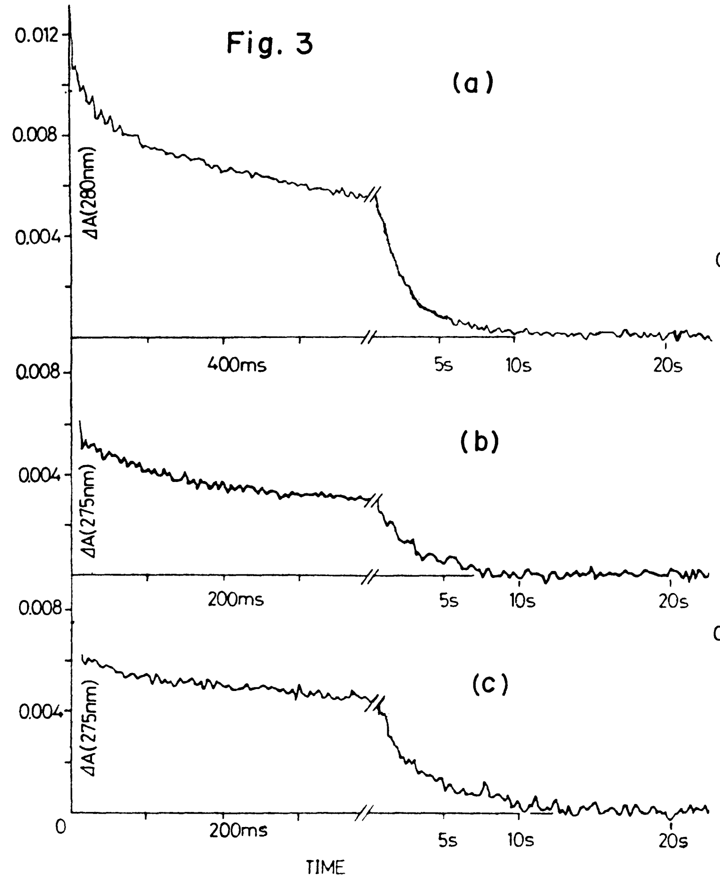


Fig.2. Inter-base hydrogen bonding in the triple-helical poly(rU)·poly(rA)·poly(rU).[11]

The hydrogen-deuterium exchange reactions were traced with a Union Giken stopped-flow spectrophotometer RA-401. By the use of this instrument, poly(rA)·poly(rU) double helix in ^1H_2O was rapidly mixed with the same volume of ^2H_2O, and then the time dependent decrease in absorbance at 280 nm was observed. The result is illustrated in Fig. 3 (a). Such an absorbance-time curve is taken as representing the time-course of deuteration of the double helix; the deuteration causes a slight blue shift of the absorption bands of the base residues, and therefore an appreciable intensity drop at the long-wavelength side of the band, at 280 nm for example. As may readily be seen here, rather sharp absorbance drop takes place not only around 5s (slower process) but also around 100 ms (faster process). Our similar experiment on the triple helix poly(rU)·poly(rA)·poly(rU), on the other hand, indicated that the recorded curve is always lacking in the sharp absorbance drop around 100 ms.

Figure 3(b) and (c) are the results of our comparative experiments of the double helix, poly(rA)·poly(rU) and the triple helix poly(rU)·poly(rA)·poly(rU), made under the experimental conditions (polymer concentration, wavelength, pH, and temperature) as similar as possible to each other. As may be seen here, the triple helix shows greater absorbance drop at about 5s than the double helix does, while at about 100 ms the former shows much smaller (if any) drop than the latter. Replots of these data with the logarithmic scale along the ordinate are shown in Fig. 4. In the period of 1-5s, the absorbance decrease (deuteration) proceeds as a simple first order process (see the straight lines in the left half of Fig. 4) both for the double helix and triple helix. The rate constants are 0.35 s^{-1} and 0.30 s^{-1}, respectively (at 32°C). The extrapolation of such a straight line (see the right half of Fig. 4) indicates that the double helix has another first order deuteration process with the rate constant 6.9 s^{-1}, while the triple helix has practically none.



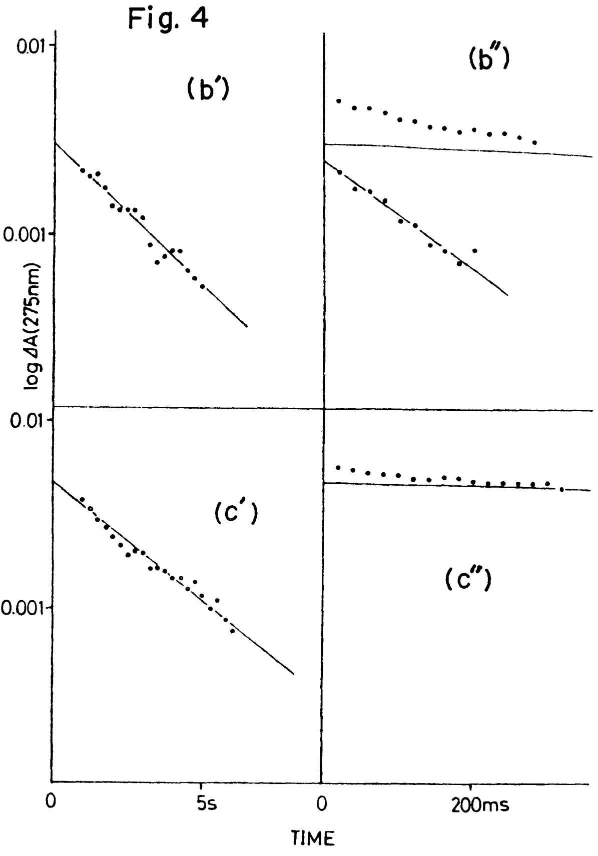


Fig. 3. Recorded curves in a stopped flow ultraviolet absorption study of the $^{1}H \rightarrow {}^{2}H$ exchange reactions. (a) Double-helical poly(rA)·poly(rU) 2×10^{-4} M in $^{1}H_2O$ (0.01 M Na^++5 mM phosphate buffer, pH 7.0) was rapidly mixed with $^{2}H_2O$ (0.01 M Na^+ + 5 mM phosphate buffer, pH 7.0) in 1:1 in volume, so that the final concentration of poly(rA)·poly(rU) was 1×10^{-4} M. Then, a time-dependent decrease in absorbance at 280 nm was recorded at 32.5°C. (b) The same experiment of double-helical poly(rA)·poly(rU) was made except that the absorbance at 275 nm was recorded. (c) Triple-helical poly(rU)·poly(rA)·poly(rU) 2×10^{-4} M in $^{1}H_2O$ (0.3 M Na^+ + 5 mM phosphate buffer, pH 7.0) was rapidly mixed with $^{2}H_2O$ (0.3 M Na^+ + 5 mM phosphate buffer, pH 7.0) in 1:1 in volume, so that the final concentration of poly(rU)·poly(rA)·poly(rU) was 1×10^{-4} M. Then, time-dependent decrease in absorbance at 275 nm was recorded at 32.5°C.

Fig. 4. (b') (b") Semilogarithmic plot of the absorbance against time data obtained in Fig. 3 (b), for double-helical poly(rA)·poly(rU). (c') (c") Semilogarithmic plot of the data obtained in Fig. 3 (c), for triple-helical poly(rU)·poly(rA)·poly(rU). The straight lines obtained in the range of longer incubation time (b' and c') are extrapolated into the range of shorter incubation time (b" and c"). Then, the deviation of every plotted point (b", upper), in the ΔA value, from the straight line just mentioned is determined and replotted (b", lower). In (c"), such a deviation is found to be very small.

Thus, it may be concluded that the triple helix, poly(rU)·poly(rA)·poly(rU), has almost no "fast-deuterated" hydrogen atom and a greater number of slowly deuterated hydrogen atoms than the double helix, poly(rA)·poly(rU). As long as this conclusion is concerned, the assignment I is supported.

We thank Professor S. W. Englander (Philadelphia) for his sending us the preprint of reference 10).

References

1) M. P. Printz and P. H. von Hippel, Proc. Nat. Acad. Sci. (USA), 53, 363 (1965).
2) M. P. Printz and P. H. von Hippel, Biochemistry, 7, 3194 (1968).
3) C. V. Hanson, J. Mol. Biol., 58, 847 (1971).
4) H. Teitelbaum and S. W. Englander, J. Mol. Biol., 92, 55 (1975).
5) H. Teitelbaum and S. W. Englander, J. Mol. Biol., 92, 79 (1975).
6) M. Nakanishi, M. Tsuboi, Y. Saijo, and T. Nagamura, FEBS Letters, 81, 61 (1977).
7) M. Nakanishi, Y. Hayashi, and M. Tsuboi, "Proc. IIrd International Symposium, Division of Biophys., The Taniguchi Foundation", ed by M. Kotani and A. Wada, (1977), p.189.
8) M. Nakanishi and M. Tsuboi, J. Mol. Biol., 124, 61 (1978).
9) Y. Hayashi, Master Thesis, 1979, University of Tokyo.
10) C. Mandel, N. R. Kallenbach, and S. W. Englander, J. Mol. Biol. in press.
11) S. Arnott and P. T. Bond, Nature (New Biology), 244, 99 (1973).
12) C. L. Stevenx and G. Felsenfeld, Biopolymers, 2, 293 (1964).
13) R. D. Blake and T. R. Fresco, J. Mol. Biol., 19, 145 (1966).
14) S. Higuchi and M. Tsuboi, Bull. Chem. Soc. Japan, 39, 1886 (1966).
15) J. R. Fresco, "Informational Macromolecules", ed by H. J. Vogel, V. Bryson, and J. O. Lampen, Academic Press, New York, (1963), p.121.

(Received February 22, 1980)