Biochemistry **1985**, *24*, 4823–4825

Determination of the Kinetics of Deuteration of DNA·RNA Hybrids by Ultraviolet Spectroscopy†

Hiroyuki Takashima, Mamoru Nakanishi, and Masamichi Tsuboi*
Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Received November 28, 1984

ABSTRACT: The kinetics of the hydrogen–deuterium exchange reactions of poly(dA)·poly(rU) and poly(rA)·poly(dT) has been examined, at pH 7.0 and at various temperatures in the 15–35 °C range, by stopped-flow ultraviolet spectrophotometry. For comparison, the deuteration kinetics of poly[d(A-T)]·poly[d(A-T)] and poly(rA)·poly(rU) has been reexamined. At 20 °C, the imino deuteration (NH → ND) rates of the two hybrid duplexes were found to be 1.5 and 1.8 s^{-1}, respectively. These are nearly equal to the imino deuteration rates of poly[d(A-T)]·poly[d(A-T)] (1.1 s^{-1}) and poly(rA)·poly(rU) (1.5 s^{-1}) but appreciably higher than that of poly(dA)·poly(dT) (0.35 s^{-1}). It has been suggested that a DNA·RNA hybrid, an RNA duplex, and the AT-alternating DNA duplex have in general higher base-pair-opening reaction rates than the ordinary DNA duplex. The amino deuteration ($NH_2 \rightarrow ND_2$) rates, on the other hand, have been found to be 0.25, 0.28, and 0.33 s^{-1}, respectively, for poly(dA)·poly(rU), poly(rA)·poly(dT), and poly[d(A-T)]·poly[d(A-T)], at 20 °C. These are appreciably higher than that for poly(rA)·poly(rU) (0.10 s^{-1}). In general, the equilibrium constants (K) of the base-pair opening are considered to be greatest for the DNA·RNA hybrid duplex (0.05 at 20 °C), second greatest for the RNA duplex (0.02 at 20 °C), and smallest for the DNA duplex (0.005 at 20 °C), although the AT-alternating DNA duplex has an exceptionally great K (0.07 at 20 °C). From the temperature effect on the K value, the enthalpy of the base-pair opening was estimated to be 3.0 kcal/mol for the DNA·RNA hybrid duplex.

In continuation of our kinetic studies of hydrogen–deuterium exchange reactions of DNA duplexes (Nakanishi et al., 1977, 1984, 1985) and RNA duplexes (Nakanishi & Tsuboi, 1978; Hayashi et al., 1981), DNA·RNA hybrid duplexes have now been subjected to our similar study. A DNA·RNA hybrid duplex is known to have an A conformation (Milman et al., 1967; Higuchi et al., 1969; Wang et al., 1982), and it is considered to be involved in an initiating complex of the synthesis of DNA Okazaki fragment during DNA replication. It is therefore significant to learn something of the dynamical aspect of its structure. We attempted, for example, to elucidate whether its base-pair-opening reaction takes place with a higher rate than that of a DNA or RNA duplex.

MATERIALS AND METHODS

Poly(rA)·poly(dT), poly(dA)·poly(rU), poly[d(A-T)]·poly[d(A-T)], and poly(dA)·poly(dT) were purchased from P-L Biochemicals. Poly(rA) and poly(rU) were purchased from Yamasa Shōyu Co. The double-helical poly(rA)·poly(rU) was prepared by mixing their aqueous solutions in a 1:1 mole ratio and monitored by ultraviolet absorption measurement.

The hydrogen–deuterium exchange reactions were traced with a Union-Giken stopped-flow spectrophotometer (RA-401). This is equipped with a rapid mixing device with a dead time of 0.5 ms. Its ultraviolet spectrophotometer has a focal length of 25 cm, a photomultiplier tube (Hamamatsu R374), and a D_2 discharge lamp. Its response time is 0.1 ms. The optical path length of the cell is 10 mm. This equipment was connected to a Union-Giken data processor (RA-450), a monitorscope, and an *xy* plotter.

RESULTS AND DISCUSSION

When poly(dA)·poly(rU) solution in H_2O + 0.3 M NaCl + 5 mM sodium phosphate buffer, pH 7.0, was rapidly mixed with D_2O (with the same amounts of buffer and salt), so that the final concentration of nucleotides is 1 OD/cm^3, a time-dependent decrease of the absorbance at 285 nm was observed. Figure 1 is a reproduction of the recorded curve in such an experiment, for which the abscissa is the time (t) and the ordinate is the absorbance (A). Such data were subjected to a computer analysis in which the best fit was achieved with $A(t \rightarrow \infty)$ (the height of base line) and rate constants as parameters. By use of a proper value of the base line height, the data were replotted with a logarithmic scale along the ordinate. Such a semilogarithmic plot against time (t) is illustrated in Figure 2a. As may be seen here, the absorbance (A) decrease does not take place along a single exponential decay curve. The log A vs. t plot seems to consist of two straight lines. Such an observed kinetic plot was reproduced by the equation

$$A = \sum_{j=1}^{2} A_j \exp(-k_j t) \qquad (1)$$

where $j = 1$ for the faster process and $j = 2$ for the slower process. The rate constant for the faster process was found to be 0.74 s^{-1} at 15 °C and is assignable to the deuteration of rU-NH (Mandal et al., 1979; Hayashi et al., 1981), while the rate constant for the slower process was found to be 0.17 s^{-1} (15 °C) and is attributed to dA-NH_2 deuteration. The experiment was repeated at different temperatures in the 15–35 °C range. The observed rate constants are plotted in a logarithmic scale against reciprocal absolute temperature in Figure 3.

Similar experiments were done with poly(rA)·poly(dT), poly(rA)·poly(rU), poly[d(A-T)]·poly[d(A-T)], and poly(dA)·poly(dT). Some of the recorded kinetic curves are illustrated in Figure 2b–d, and the rate constants determined are given in Table I and in Figure 3. As may be seen in the figure, the hydrogen exchange reaction rates of poly(rA)·poly(dT) are nearly equal to those of poly(dA)·poly(rU), at every temperature, both for fast (imino) and slow (amino)

---

† This work was supported by a grant (57060004) from the Ministry of Education, Science, and Culture of Japan.

0006-2960/85/0424-4823$01.50/0

Table I: Kinetic Parameters for Hydrogen Exchange of Polynucleotide Duplexes

	polynucleotide duplex	process	rate const k (s^{-1}) at 20 °C, pH 7.0	assignment	$\Delta H^{\ddagger}$ (kcal)[a]	$\Delta S^{\ddagger}$ (eu)[a]
DNA·RNA hybrid	poly(dA)·poly(rU)	fast	1.45	rU-NH	24.2 (5.6)	12.5 (9.6)
		slow	0.25	dA-NH_2	14.9 (2.4)	-5.3 (4.1)
	poly(rA)·poly(dT)	fast	1.82	dT-NH	25.8 (7.2)	15.5 (12.3)
		slow	0.28	rA-NH_2	15.7 (2.5)	-3.8 (4.3)
RNA	poly(rA)·poly(rU)	fast	1.47	rU-NH	26.0 (9.2)	15.7 (15.8)
		slow	0.10	rA-NH_2	17.3 (1.4)	-1.9 (2.4)
DNA	poly[d(A-T)]·poly[d(A-T)]	fast	1.05	dT-NH	29.4 (6.6)	21.0 (11.2)
		slow	0.33	dA-NH_2	14.9 (2.2)	-5.0 (3.7)
	poly(dA)·poly(dT)[b]	fast	0.35	dT-NH	17.0 (0.9)	-2.6 (3.1)
		slow	0.024	dA-NH_2	18.8 (0.8)	-2.0 (2.8)

[a] Activation energy and activation entropy. Standard deviations are given in parentheses. [b] The parameters of this DNA duplex were given by Nakanishi et al. (1984). Our reexamination of poly(dA)·poly(dT) has been done only at 25 and 29 °C, and the results confirmed what Nakanishi et al. (1984) found.

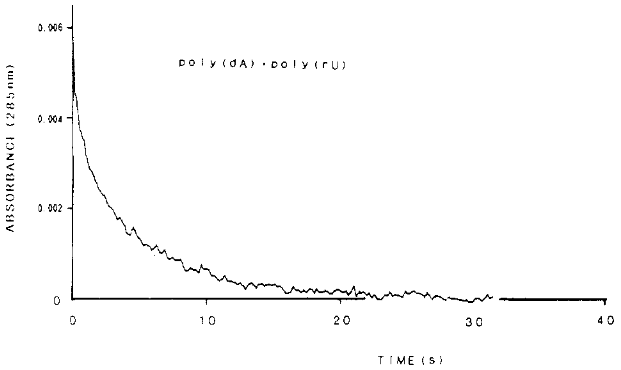

FIGURE 1: The time dependence of the decrease in absorbance at 285 nm observed when poly(dA)·poly(rU) dissolved in H_2O is mixed with D_2O (1:1 in volume; final concentration = 1 OD/cm^3). The solvent is 0.3 M NaCl + 5 mM sodium phosphate buffer, pH 7.0; the temperature is 15 °C.

reactions. The rate constants for the NH deuteration (faster process) of poly[d(A-T)]·poly[d(A-T)] are now found to be somewhat higher than those we gave previously (Nakanishi et al., 1984), by our present more proper analysis. For the rate constants of the slower process (NH_2 deuteration), however, the newly estimated values have been found to be equal to our previous values. For both the fast and the slow processes, the rate constants obtained for poly(rA)·poly(rU) are in agreement with those reported previously by Mandal et al. (1979). For poly(dA)·poly(dT), our present experiment was done only at 25 and 29 °C to confirm our previous results (Nakanishi et al., 1984). It has now become clear that fast (imino) exchange rates are not greatly different from one another among the four duplexes: poly[d(A-T)]·poly[d(A-T)], the two hybrids, and poly(rA)·poly(rU). However, the slow (amino) exchange rates are different: highest for poly[d(A-T)]·poly[d(A-T)], second highest for the hybrids, and lowest for poly(rA)·poly(rU) (see Figure 3). One should be reminded, however, that poly[d(A-T)]·poly[d(A-T)] was found to be exceptional (Nakanishi et al., 1984, 1985) among DNAs and that ordinary DNA [including poly(dA)·poly(dT)] has a much lower amino deuteration rate. It is interesting that this extraordinary DNA has an even higher amino exchange rate than the DNA·RNA hybrid.

If the imino and amino hydrogen exchange reactions are assumed to take place only during a transient state, in which the base pair is open, the pathway for such a reaction is given (Teitelbaum & Englander, 1975; Mandal et al., 1979; Nakanishi et al., 1984) as

$$\text{closed} \underset{k_{cl}}{\overset{k_{op}}{\rightleftharpoons}} \text{open} \xrightarrow{k_e} \text{exchanged} \qquad (2)$$

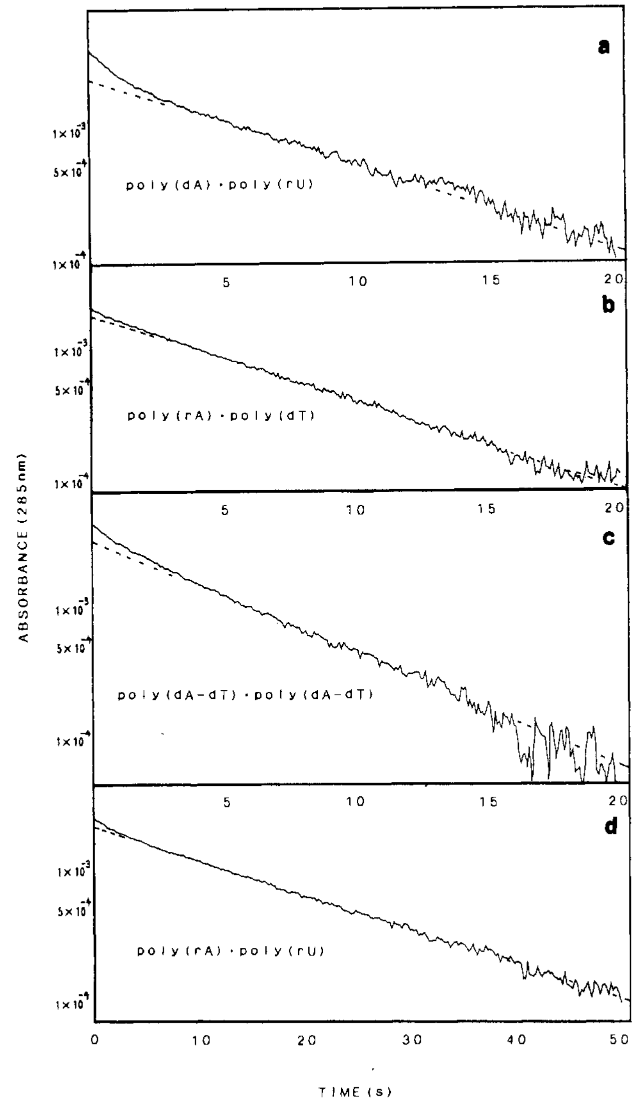

FIGURE 2: (a) A recorded curve, with a logarithmic scale along the ordinate, for the same experiment as that given in Figure 1, for poly(dA)·poly(rU). (b) Time dependence of the decrease in absorbance at 285 nm observed when poly(rA)·poly(dT) dissolved in H_2O is mixed with D_2O. (c) The same experimental result for poly[d(A-T)]·poly[d(A-T)]. (d) The same experimental result for poly(rA)·poly(rU). For all of the above (panels a–d), the solvent is 0.3 M NaCl + 5 mM sodium phosphate buffer, pH 7.0, and the temperature is 15 °C. For determining the rate constants of faster processes, recordings were made with a small range of time (0–1 s or 0–5 s) and an enlarged ordinate.

Thus, the overall exchange rates k(imino) and k(amino) should then be functions of the rates of the base-pair opening and

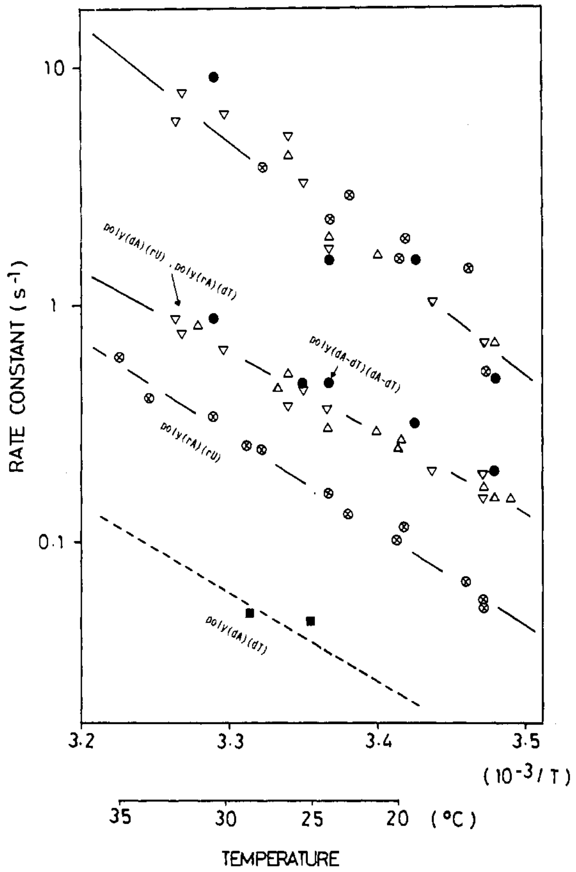

FIGURE 3: Arrhenius plots for the rate constants of the hydrogen exchange reactions: (▽) poly(dA)·poly(rU), (△) poly(rA)·poly(dT), (⊗) poly(rA)·poly(rU), (●) poly[d(A-T)]·poly[d(A-T)], and (■) poly(dA)·poly(dT). The dashed line for poly(dA)·poly(dT) is what was given by Nakanishi et al. (1984), on the basis of their experimental results for a greater range of temperature.

closing processes (k_{op} and k_{cl}, respectively) as well as their intrinsic exchange rate constants k_e(imino) and k_e(amino):

$$k(\text{imino}) = k_{op}k_e(\text{imino})/[k_{cl} + k_e(\text{imino})] \quad (3)$$

$$k(\text{amino}) = k_{op}k_e(\text{amino})/[k_{cl} + k_e(\text{amino})] \quad (4)$$

It should be pointed out here that our use of eq 3 and 4 implies two further assumptions. First, a measured mononucleotide exchange rate (Mandel et al., 1979; Nakanishi et al., 1984) is assumed to be equal to the intrinsic exchange rate (k_e) of the "open form". One might suspect that the "open" imino or amino exchange rate could vary depending upon the base sequence and structural nature of the polynucleotide strand in question. Because any adjacent two bases are separated with many single bonds, there would practically be no through-bond base–base interaction. So, k_e would not be sequence dependent. The assumption that k_e is independent even of the secondary structure should be taken as relating to the definition of the open form. It is defined to be a form in which a base residue is exposed to the solvent as completely as in a monomeric nucleotide. Secondly, we assume below that such an open form is the same for the imino proton exchange process as it is for the amino proton exchange process. The transient states may certainly be different for these two processes; for an imino exchange, the imino group itself is required to be exposed to the solvent, whereas for an adenine amino exchange its position-1 nitrogen is required to be exposed to the solvent (Mandel et al., 1979). For simplicity, however, we neglect any possibility of a "partially open form", so that our brief discussion below is based upon a two-state model in which only a "completely" closed form and a "completely" open from are taken into consideration.

Such k_e(imino) and k_e(amino) values are given by Nakanishi et al. (1984) as well as by Mandal et al. (1979). Therefore, k_{op} and k_{cl} can now be calculated from the observed k(imino) and k(amino) values by eq 3 and 4. At 20 °C, for example, $k(\text{imino}) = 1.4\ \text{s}^{-1}$ and $k(\text{amino}) = 0.27\ \text{s}^{-1}$ for the DNA·RNA hybrid, and $k_e(\text{imino}) = 80\ \text{s}^{-1}$ and $k_e(\text{amino}) = 4.9\ \text{s}^{-1}$ (Nakanishi et al., 1984), and hence it is concluded that $k_{op} = 1.9\ \text{s}^{-1}$ and $k_{cl} = 30\ \text{s}^{-1}$. From these, the equilibrium constant $K = k_{op}/k_{cl}$ of the base-pair-opening reaction is calculated to be 0.06 at 20 °C. The equilibrium constant K can be obtained at different temperatures in the 15–35 °C range from the present kinetic data at different temperatures. From a van't Hoff plot, the enthalpy and entropy of the base-pair-opening reaction are given as $\Delta H = 3.0$ kcal/mol and $\Delta S = 4.8$ eu for the DNA·RNA hybrid.

In conclusion, a DNA·RNA hybrid has been found to have the lowest base-pair-opening enthalpy ($\Delta H = 3.0$ kcal/mol) and the smallest base-pair-opening entropy ($\Delta S = 4.8$ eu) among the polynucleotide duplexes. This is due to its low base-pair-closing rate. As far as the base-pair-opening rates are concerned, those for the hybrids are nearly equal to those for poly(rA)·poly(rU) (RNA) and poly[d(A-T)]·poly[d(A-T)] (extraordinary DNA), although ordinary DNA [including poly(dA)·poly(dT)] has an appreciably lower rate. Such a general trend found for polynucleotide duplexes seems to be in agreement with what Pardi & Tinoco (1982) found for oligonucleotide helices. The base-pairing dynamics of the hybrid does not seem to depend upon whether the pairing is formed between deoxyribopurine and ribopyrimidine or vice versa.

Registry No. Poly(dA)·poly(rU), 33572-75-7; poly(rA)·poly(dT), 27156-07-6; poly(rA)·poly(rU), 24936-38-7; poly[d(A-T)], 26966-61-0; hydrogen, 1333-74-0.

REFERENCES

Hayashi, Y., Nakanishi, M., Tsuboi, M., Tazawa, I., & Inoue, Y. (1981) *Biochim. Biophys. Acta 656*, 120–122.

Higuchi, S., Tsuboi, M., & Iitaka, Y. (1969) *Biopolymers 7*, 909–916.

Mandal, C., Kallenbach, N. R., & Englander, S. W. (1979) *J. Mol. Biol. 135*, 391–411.

Milman, G., Langridge, R., & Chamberlin, M. J. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 1804–1810.

Nakanishi, M., & Tsuboi, M. (1978) *J. Mol. Biol. 124*, 61–71.

Nakanishi, M., Tsuboi, M., Saijo, Y., & Nagamura, T. (1977) *FEBS Lett. 81*, 61–64.

Nakanishi, M., Mitane, Y., & Tsuboi, M. (1984) *Biochim. Biophys. Acta 798*, 46–52.

Nakanishi, M., Tsuneda, S., Takahashi, S., & Tsuboi, M. (1985) *J. Biochem. (Tokyo) 97*, 483–491.

Pardi, A., & Tinoco, I., Jr. (1982) *Biochemistry 21*, 4686–4693.

Teitelbaum, H., & Englander, S. W. (1975) *J. Mol. Biol. 92*, 55–78.

Wang, A. H. J., Fujii, S., Van Boom, J. H., & Rich, A. (1982) *Cold Spring Harbor Symp. Quant. Biol. 47*, 37–44.