Kinetic analysis of hydrolytic reaction of homo- and heterochiral adenylyl(3′–5′)adenosine isomers: breaking homochirality reduces hydrolytic stability of RNA†

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The hydrolytic stability of the diastereomeric isomers of ApA was compared and the results show that heterochiral ApAs are more rapidly hydrolyzed than homochiral ApAs at low temperatures, suggesting that hydrolytic selection in cold environments in conjunction with selective polymerization may have been effective in enriching the homochirality of RNA.

There is still no generally accepted explanation for the intriguing question of the origin of the homochirality of biomolecules.[1] The polymerization of racemic monomers in achiral solution results in the formation of random polymers composed of D- and L-repeating units. Therefore, the homochirality of biopolymers must have been achieved by polymerization after strict chiral selection of monomers, or more preferably by amplification of a small chiral imbalance during the formation of biopolymers from racemic monomers. One possible process for the latter case is the enantio-selective condensation of racemic or low enantio excess monomers.[2–8] However, the chiral selectivity of such condensation reactions is not sufficient for producing *completely* homochiral polymers. As polymerization often competes with degradation,[9–11] the degradation processes of polymers are expected to be another possible process of enriching the homochirality of polymers.[12] In fact, selective hydrolysis of heterochiral peptides over homochiral ones has been reported.[13] RNA is a polymer that is intrinsically sensitive to hydrolysis even under very weak alkaline conditions,[14] and the selective hydrolysis of heterochiral RNA over homochiral RNA is predicted,[7] based on the expectation of the formation of less stable secondary and tertiary structures for heterochiral RNAs. However, such hydrolytic selectivity for heterochiral RNA oligomers has not yet been reported, and the first attempt to detect the difference between the hydrolysis rates of the homo- and heterochiral isomers of uridylyl(3′–5′)uridine (UpU) under relatively strong alkaline and acidic conditions was unsuccessful.[15] Here, we report a careful kinetic analysis of the hydrolytic reactions of the diastereomeric isomers of adenylyl(3′–5′)adenosine (ApA, Fig. 1) under prebiotically plausible conditions.

To carefully compare the hydrolytic stabilities of homo- and heterochiral RNAs, we carried out the hydrolysis of the diastereomeric isomers of ApA at different temperatures and the diminishing rates of the dimers were determined by reversed phase HPLC. Rate constants for the hydrolysis were obtained by the first-order rate plots (Fig. S1), and are summarized in Table 1. At high temperatures (70 and 80 °C), no significant difference in the rate constant between the homo- and heterochiral isomers was observed, as reported by Mikkola *et al.*[15] However, a small yet significant difference in the rate constant was observed at low temperatures. In fact, the $k_{homochiral}/k_{heterochiral}$ ratios are decreased as the temperature is decreased (Table 1). It is thus very likely that the heterochiral ApAs are more easily hydrolyzed than the homochiral ApAs at low temperatures. The half-lives at 0 °C for D-(ApA) and L-(ApA) estimated from the Arrhenius plots (Fig. S2) are 104.9 ± 0.9 and 105.4 ± 1.7 y, whereas those for ADpAL and ALpAD are 95.9 ± 1.5 and 92.5 ± 2.6 y, respectively. Although the difference in half-life between the homo- and heterochiral ApAs is small, even such an extent of differential susceptibility of the oligomers to the hydrolysis may have been effective in enriching the homochiral RNA oligomers on the time scale of the Earth. On the other hand, the half-lives at 100 °C of the dimers are very similar (≈1.6 h), therefore, the contribution of the hydrolysis to the enrichment of the homochiral oligomers would be effective at low temperatures rather than at high temperatures.

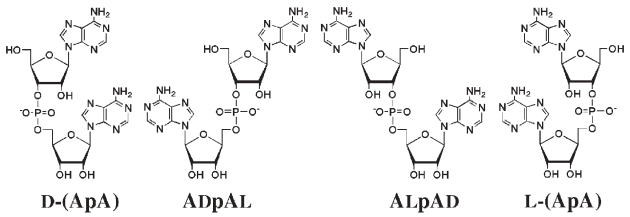

Fig. 1 Structures of diastereomeric ApAs.

Table 2 shows the activation parameters for the hydrolysis of ApAs. The values of the activation enthalpy ($\Delta H^{\ddagger}$) of the homochiral ApAs are slightly larger (≈1 kJ mol^{-1}) than those of the heterochiral ApAs, indicating that the strength of the phosphodiester bond of the homochiral ApAs is intrinsically higher than that of the heterochiral ones. Besides, the homochiral dimers showed more positive activation entropies ($\Delta S^{\ddagger}$) compared with the heterochiral dimers. Therefore, the homochiral ApAs have lesser constraint in the activation process to the transition state than the heterochiral ApAs, and thus the preferential hydrolysis of the heterochiral ApAs at low temperatures is due to the enthalpic contribution. This means that in the transition

† Electronic supplementary information (ESI) available: experimental details and Figures showing the hydrolytic kinetics, the Arrhenius and Eyring plots. See http://www.rsc.org/suppdata/cc/b5/b500673b/

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Table 1 Pseudo-first order rate constants for hydrolysis of ApAs[a]

Temp. (°C)	k_{obs} (s^{-1}) D-(ApA)	L-(ApA)	ADpAL	ALpAD	$k_{[D\text{-}(ApA)]}/k_{[ALpAD]}$	$k_{[L\text{-}(ApA)]}/k_{[ADpAL]}$
80	$(1.49 \pm 0.01) \times 10^{-5}$	$(1.48 \pm 0.02) \times 10^{-5}$	$(1.50 \pm 0.01) \times 10^{-5}$	$(1.49 \pm 0.01) \times 10^{-5}$	1.00	0.99
70	$(5.00 \pm 0.02) \times 10^{-6}$	$(5.00 \pm 0.01) \times 10^{-6}$	$(5.07 \pm 0.01) \times 10^{-6}$	$(5.07 \pm 0.04) \times 10^{-6}$	0.99	0.99
60	$(1.57 \pm 0.00) \times 10^{-6}$	$(1.56 \pm 0.01) \times 10^{-6}$	$(1.60 \pm 0.02) \times 10^{-6}$	$(1.61 \pm 0.01) \times 10^{-6}$	0.98	0.98
50	$(4.44 \pm 0.04) \times 10^{-7}$	$(4.44 \pm 0.01) \times 10^{-7}$	$(4.58 \pm 0.09) \times 10^{-7}$	$(4.64 \pm 0.06) \times 10^{-7}$	0.96	0.97
40	$(1.12 \pm 0.01) \times 10^{-7}$	$(1.12 \pm 0.01) \times 10^{-7}$	$(1.18 \pm 0.02) \times 10^{-7}$	$(1.19 \pm 0.01) \times 10^{-7}$	0.94	0.95

[a] Each reaction contained 40 μM dimer, 0.2 M NaCl, 75 mM $MgCl_2$, and 0.1 M HEPES (pH 8.0). Rate constants are means ± standard deviation in triplicate experiments.

Table 2 Activation parameters for hydrolysis of ApAs[a]

Dimers	E_a (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})
D-(ApA)	112.2 ± 0.1	109.4 ± 0.1	−28.74 ± 0.26
L-(ApA)	112.2 ± 0.2	109.4 ± 0.2	−28.76 ± 0.66
ADpAL	111.3 ± 0.2	108.6 ± 0.2	−31.06 ± 0.58
ALpAD	110.9 ± 0.3	108.2 ± 0.3	−32.26 ± 0.87

[a] Reaction conditions are the same as those in Table 1.

state, the cleavage of the P–O(5′) bond of a pentacoordinated intermediate[16] of the heterochiral ApAs is easier than that of the homochiral ones. The heterochiral ApAs have been shown to have a more flexible stacked helical conformation than the homochiral ApAs in solution.[17] Therefore, the increase in activation entropy ($\Delta S^{\ddagger}$) of the homochiral ApAs relative to the heterochiral ApAs can be explained by the more rigid stacked conformation of the homochiral ApAs at the ground state.

Two theories for the chemical evolution of biomolecules have been proposed: one states that the chemical evolution of biomolecules has proceeded in a hot environment[18,19] and the other postulates the cold origin of life.[20] The hot environment, such as hydrothermal vents, may be more suitable for accelerating the monomer condensation,[18] although it has been reported that the nonenzymatic polymerization of activated RNA monomers proceeds effectively even in ice eutectic phases.[21] The present results suggest that the cold environment has a better hydrolytic selection pressure for homochiral RNA than the hot environment. It has been predicted that heterochiral polymers are more susceptible to hydrolytic degradation than homochiral ones, based on the expectation that heterochiral RNA forms less stable secondary and tertiary structures.[7] Indeed, the duplex stability of heterochiral RNA is considerably lower than that of homochiral RNA,[22] and double-stranded RNA is more resistant to hydrolysis than single-stranded RNA.[11,23] However, the present results demonstrate that even in the case of simple single-stranded oligomers such as ApA, heterochiral oligomers are preferentially hydrolyzed relative to homochiral ones, and the selective hydrolysis of heterochiral RNA does not necessarily require the formation of higher order structures. Although the hydrolytic selectivity is low, repeated cycles of polymerization and hydrolysis may have resulted in the enrichment of the homochirality of RNA.

Orgel and coworkers reported the nonenzymatic template-directed polymerization of activated mononucleotides. This reaction was strongly inhibited when racemic nucleotides were employed as the monomer. This phenomenon is called "enantiomeric cross-inhibition", whereby unnatural L-nucleotides serve as the chain terminator,[6] and is considered to be a serious hurdle for the theories of the prebiotic chemical evolution of RNA. The present results suggest that terminated (heterochiral) oligomers can be transformed into non-terminated (homochiral) oligomers by the preferential hydrolysis of terminated oligomers.

In conclusion, we found that the homochiral ApAs are intrinsically more stable than the heterochiral ApAs under very weak alkaline conditions. The results suggest that the hydrolytic instability of RNA and the combined action of polymerization and hydrolysis during the chemical evolution of RNA may have been effective in the establishment of the homochirality of RNA. However, it remains unknown whether RNAs containing other bases show a similar behavior or not. Studies of the effects of the other bases and the length of RNA oligomers on the hydrolysis are currently under way.

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