Efficient and Homochiral Selective Oligomerization of Racemic Ribonucleotides on Mineral Surface

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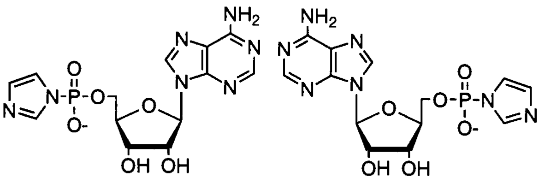
We have found that activated racemic mononucleotides oligomerize with comparable efficiency to chiral D-monomers on Na^+-montmorillonite, and that the reaction proceeds in a homochiral selective manner to preferentially give optically homogeneous D- and L-oligomers. Mineral surfaces might play important roles in an early stage of chemical evolution of RNAs.

The RNA world hypothesis[1–3] has several major problems associated with chemical evolution of RNAs on the primitive earth. Although template-directed syntheses of polynucleotides[4,5] are a possible candidate for the theories of prebiotic chemical evolution of RNAs, these reactions are limited by "enantiomeric cross-inhibition", whereby the reaction is strongly inhibited by addition of monomers having unnatural L-chirality,[6,7] Because prebiotic syntheses of biomolecules such as amino acids and nucleos(t)ides would yield racemic mixtures, "enantiomeric cross-inhibition" has been thought as a serious hurdle for prebiotic chemical evolution of RNAs. It should be thus necessary to devise some processes, which efficiently produce RNA oligomers from a racemic mixture of mononucleotides. Here, we show that montmorillonite clay is an effective catalyst for the oligomerization of activated racemic mononucleotides to preferentially produce homochiral oligomers.

L-Adenosine was synthesized according to the procedure of Visser et al.[8] L-Adenosine 5'-phosphorimidazolide (L-ImpA, Figure 1) was synthesized according to the literature procedure[5] for D-ImpA after phosphorylation[9] of L-adenosine.

It is well known that montmorillonite is a very effective catalyst for oligomerization of D-ImpA, and then we carried out oligomerization reactions in a similar manner as reported.[10] The reaction of D-ImpA on Na^+-montmorillonite[11] gave oligomers efficiently up to decamers (Figure 2a).[12] The reaction of L-ImpA showed very similar chromatographic profile to that of the reaction of D-ImpA, and both reactions of D- and L-ImpAs provided similar yields for all products (Table 1). Na^+-montmorillonite is thus equally effective for oligomerization of D- and L-ImpAs as expected. On the other hand, the racemic reaction on the clay also gave oligomers up to octamers (Figure 2b).[13] Although the racemic reaction gave somewhat lower yields for longer chain products, it should be noted that the yield of dimers in the racemic reaction are significantly higher than those of two other reactions (Table 1). Since amounts of remaining monomers are similar in the three reactions, it is thus very likely that in the racemic system, condensation between monomers proceeds efficiently, however subsequent elongation of dimers is less efficient. Figure 3a shows the expanded chromatographic profiles of the dimer region. A large amount of a specific product is observed at about 7 min only in the racemic reaction. The extensive formation of this product might be related to some decrease of the efficiency of racemic reaction.



D-ImpA L-ImpA

Figure 1. Enantiomeric structures of ImpA.

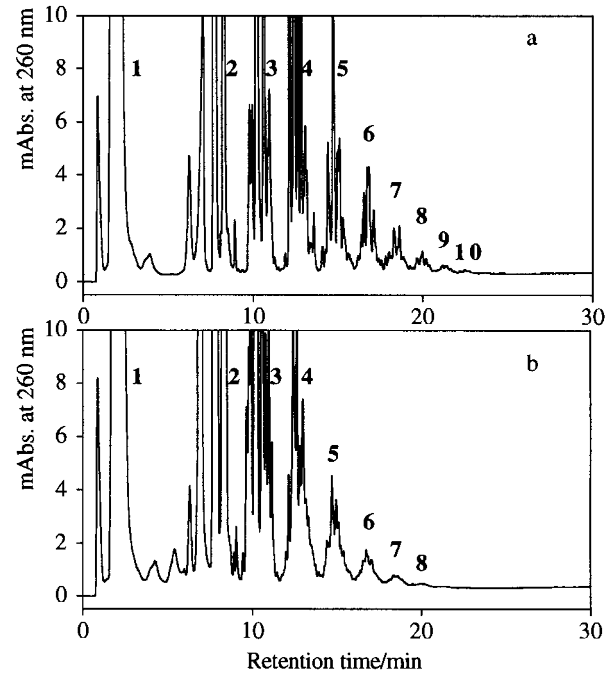


Figure 2. The anion-exchange HPLC profiles of reaction mixtures of (a) D-ImpA, (b) racemic ImpA. Reactions carried out by adding 50 mg of Na^+-motmorillonite to a solution (1 mL) containing 14 mmol dm^{-3} D- or D,L-ImpA, 0.2 mol dm^{-3} NaCl, 75 mmol dm^{-3} $MgCl_2$, 0.1 mol dm^{-3} HEPES (pH 8.0) for 7 days at ambient temperature. Aliquots of the supernatants were analyzed by an anion exchange HPLC on a column of DNA-NPR (TOSOH) with a linear gradient of $NaClO_4$ (0–0.15 mol dm^{-3}, 30 min) in 20 mmol dm^{-3} Tris-HCl, pH 9.0.

The major product in the trimer region for the racemic reaction coincided with those for the D- and L-reactions, which are exactly homochiral trimers (Figure 3b). The major product in the trimers for the racemic reaction would be thus an enantiomeric mixture of D- and L-homochiral trimers. In racemic reactions, the formation of eight diastereomeric isomers (2^3) for trimers is possible and the theoretically predicted content of the homochiral trimers (D-D-D and L-L-L) in the trimer region is 25%, if D- and L-ImpAs react with each other nonselectively. However, in the

Table 1. Product distribution of oligomerization reaction[a]

Reactants	Oligomer/% 1 mer	2 mer	3 mer	4 mer	5 mer	6 mer	7 mer	8 mer	9 mer	10 mer
D-ImpA	34.4	25.2	19.7	10.6	5.24	2.59	1.31	0.65	0.25	0.08
L-ImpA	34.7	24.3	20.1	10.7	5.26	2.63	1.33	0.68	0.26	0.09
D,L-ImpA[b]	34.8	37.6	16.9	6.57	2.60	1.07	0.40	0.10	—	—

[a] Reactions contained 14 mmol dm^{-3} ImpA, 0.2 mol dm^{-3} NaCl, 75 mmol dm^{-3} $MgCl_2$ and 0.1 mol dm^{-3} HEPES, pH 8.0. Percentages listed are the uncorrected HPLC absorbance readings because the composition of each fraction has not been determined. [b] Each enantiomer concentration is 7 mmol dm^{-3}.

racemic reaction, the content of the major product in the trimer region is 48%. Because other homochiral oligomers might be included in the trimer region as minor products, the actual content of homochiral products in the trimer region is more than 48%, which is substantially higher than the theoretical value (25%). It is thus very likely that racemic ImpA on Na^+-montmorillonite reacts preferentially with the ImpA enantiomer having the same chirality to produce homochiral oligomers.[14]

Although our racemic system affords a complex mixture of homochiral, heterochiral oligomers and phosphodiester linkage isomers, this reaction would result in somewhat preferential formation of the enantiomeric homochiral oligomers among diastereomeric isomers. Such homochiral oligomers would be able to serve as a template for the replication. It was found that oligomerization of activated D-mononucleotides on mineral surfaces affords oligomers up to 55 mers by successive feedings with the monomers.[16] Although the chain length of the oligomers produced in this work is not long enough to have catalytic activities, the successive feedings of racemic ImpA might make oligomers possible to elongate up to 40–50 mers.

In conclusion, we have demonstrated the efficient oligomerization of racemic mononucleotides on montmorillonite clay, whereby homochiral oligomers are preferentially produced. Thus, oligomerization on mineral surfaces should shed new lights on the mechanisms for the formation of optically homogeneous RNAs from a primordial soup containing racemic mononucleotides on the primitive earth leading to emergence of RNAs with catalytic or template activities.

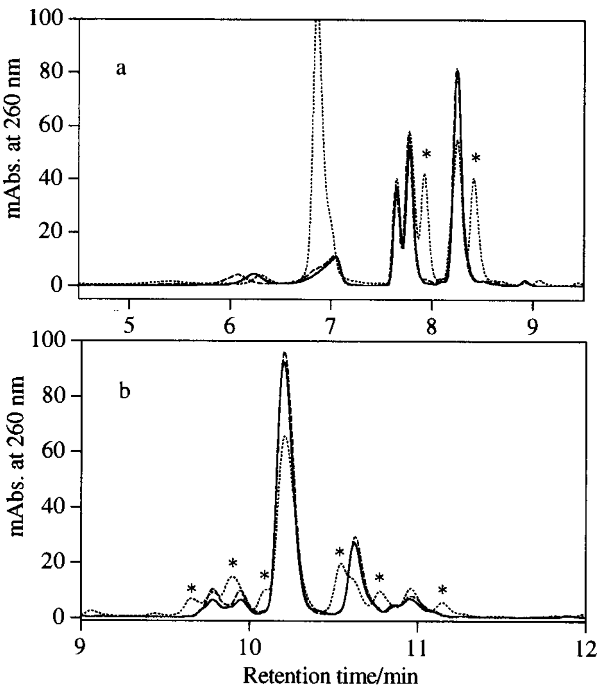


Figure 3. The expanded anion-exchange HPLC chromatographic profiles of the dimer (a) and trimer (b) regions for the reaction of D- (solid line), L- (broken line) and racemic ImpAs (dotted line). Chromatographic conditions are the same as shown in the Figure 2 legend. Asterisks indicate peaks observed only in the racemic reaction, and thus correspond to heterochiral oligomers.

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12 The negative control in the absence of the clay gave oligomers up to only trimers and trace amounts of tetramers. Chain length of each oligomer was estimated by comparison with an authentic sample of $r(pA)_{2-6}$.
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14 Very recently, Ferris et al. reported the homochiral selectivity for the dimer formation of racemic ImpA on montmorillonite.[15] In order to evaluated the selectivity, they used low concentration conditions of racemic ImpA to minimize the formation of trimers and longer oligomers. Therefore, their results might not reflect the selectivity in the reactions producing longer oligomers. Indeed, they focused on the product ratio of only four dimers for evaluation of the selectivity, although we observed six dimers in the racemic reaction.
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