COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Synthesis of 35–40 mers of RNA oligomers from unblocked monomers. A simple approach to the RNA world†

Wenhua Huang and James P. Ferris
Department of Chemistry and New York Center for Studies on the Origins of Life, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY, 12180, USA. E-mail: ferrij@rpi.edu

Received (in Corvallis, OR, USA) 18th March 2003, Accepted 24th April 2003
First published as an Advance Article on the web 20th May 2003

RNA oligomers greater than 35–40 mers in length form in one day in the montmorillonite clay-catalyzed reaction of unblocked RNA monomers at 25 °C in aqueous solution.

In the RNA world scenario for the origin of life RNA was the principal biopolymer in the first life on Earth.[1–5] The RNA was formed either from simpler precursors[6] or directly from RNA monomers present on the primitive Earth.[7]

Montmorillonite clay catalyzes the condensation of activated monomers of RNA (ImpN) (Fig. 1a) to form oligomers in aqueous solution.[8] Six to 14 mers are formed in a one step reaction. Longer oligomers are formed when the activated monomer is added daily to a primer over a period of 12–14 days.[9,10] Here we describe a vastly improved synthesis of 35–40 mer homopolymers of A and U and an AU copolymer in a one step reaction that is complete in less than one day without the need of a primer. This facile synthesis provides a more efficient and less tedious route to informational polymers[11] that may have initiated the RNA world as well as a new approach to the chemical synthesis of RNA.

Purines, in particular 1-methyladenine, were proposed as more plausible prebiotic activating groups[12] that may have been present on the primitive Earth.[13] The activated nucleotides formed from 1-methyladenine (1-MeadpN) (Fig. 1b) bind to montmorillonite more rapidly and generate a greater proportion of 3′,5′-phosphodiester bonds than does ImpN.[12] In the present study elongation reactions were carried out on the 0.45 μm filter of a Pall Filtron tube.[10] The shorter oligomers present in the aqueous phase were removed by centrifugation at 14,000 rpm and the longer oligomers bound to montmorillonite were eluted from the clay with 0.1 M pyrophosphate. In some instances the longer oligomers were fractionated further on an anion exchange HPLC column.

The products formed by elongation of uridine with 1-MeadpU were 5′-phosphorylated with γ-^{32}P-ATP and T_4 polynucleotide kinase and then analyzed by gel electrophoresis (Fig. 2a). The elongation reaction proceeds rapidly as shown by the formation of oligomers greater than 30 mers in 2 h (lane 2) and elongation to greater than 40 mers in 8 h. The RNA oligomers did not grow longer on addition of more 1-MeadpU (lanes 5 and 6) but there was additional elongation of the shorter oligomers ($<$ 10 mers). The same reaction performed with 1-MeadpU without a primer also gave oligomers greater than 40 mers (Fig. 2b). Elongation of uridine with 1-MeadpA gave $>$ 35 mers (Fig. 2c) as did the reaction of 1-MeadpA alone (data not shown). Forty mers containing both A and U were prepared by the reaction of a 1 : 1 mixture of 1-MeadpA and 1-MeadpU on montmorillonite (Fig. 3a).

Hydrolysis of the oligomers formed by the elongation of uridine with 1-MeadpU with ribonuclease T_2 resulted in their hydrolysis to monomers together with smaller amounts of shorter oligomers (data not shown). Collection of oligomers with chain lengths 9 mers or greater from the reaction of a 1 : 1 mixture of 1-MeadpA and 1-MeadpU was performed using a Melcor anion exchange HPLC column (HPLC trace not shown). The longer oligomers were characterized by anion exchange HPLC using a Hydrocell column (Fig. 3b). The total hydrolysis of this fraction using phosphodiesterase I[14] resulted in the formation of 5′-AMP and 5′-UMP together with traces of adenosine and uridine. Yields of 1.8 and 2.1% of 5′-AMP and 5′-UMP respectively, were determined by comparison with known amounts of authentic samples by reverse phase HPLC analysis. The overall yield of oligomers longer than 9 mers was 2%. A 1% yield of oligomers longer than 9 mers was obtained in the reaction of 1-MeadpU alone as determined by the previously described procedure demonstrating that copolymerization with 1-MeadpA gives higher yields (data not shown).

This approach to RNA synthesis is diametrically opposite to that used in the conventional chemical synthesis of RNA where strictly anhydrous conditions, a variety of special chemical reagents and fully blocked monomers are used to prepare

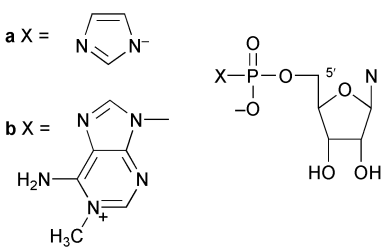

Fig. 1 Structures of the activated nucleotides a. ImpN, N = A, G, U, C and H. b. 1-MeadpN, N = A and U.

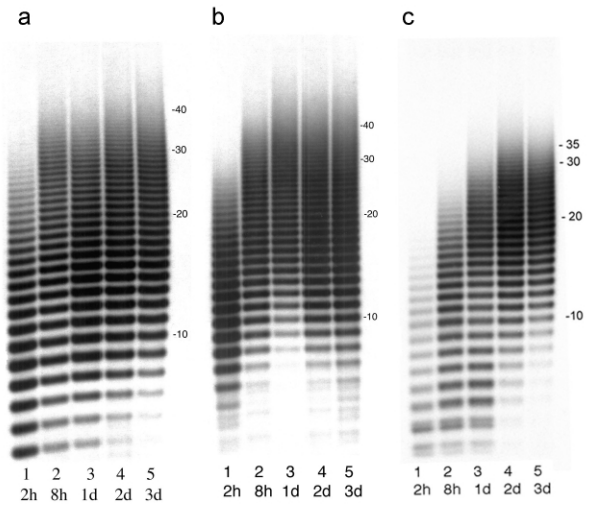

Fig. 2 Gel electrophoresis of the reaction products. a. 15 mM 1-MeadpU with 15 mM uridine for the times listed. In the 2 day reaction (lane 4) the solution phase was removed and replaced with fresh activated monomer. This was repeated for the 3 day reaction in lane 5. b. 15 mM 1-MeadpU. The 5′-phosphates were cleaved with alkaline phosphatase and replaced with ^{32}P-phosphate before analysis. See 2a for the other procedures. c. 15 mM 1-MeadpA with 15 mM uridine. See 2a for the other procedures.

† Electronic supplementary information (ESI) available: experimental procedures. See http://www.rsc.org/suppdata/cc/b3/b303134a/

DOI: 10.1039/b303134a

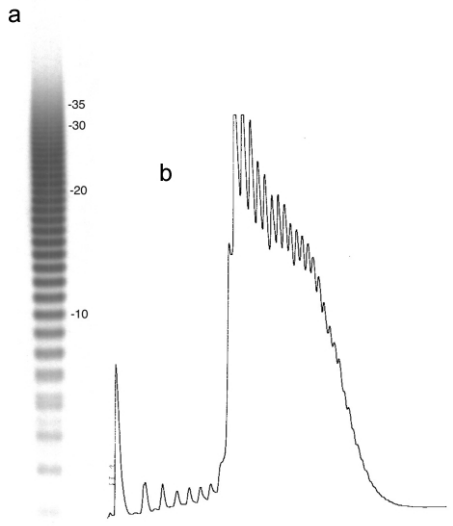

Fig. 3 Analysis of the products of a 3 day reaction of 7.5 mM 1-MeadpU with 7.5 mM 1-MeadpA. a. Gel electrophoresis of the combined pyrophosphate eluates. b. Anion exchange HPLC of the 9 mers and greater oligomers on a Hydrocell anion exchange column.

RNAs. The addition of each monomer to the growing chain is a multi-step process requiring an array of specific reagents. In this synthesis unblocked monomers are used in aqueous solution and there is no need for a primer.[9,10] All that is required is the 1-methyladenine activated 5′-nucleotide, and montmorillonite clay. Changing the mix of nucleotides used and the use of nucleotides containing alternative bases may vary the composition of the RNAs formed. Thus it may be possible to prepare RNAs containing 2, 3, 4 or more possible bases that can be evaluated for catalytic activity[15,16] and replicative properties. The resulting RNAs can be modified by addition of other structures to the hydroxyl groups on the 5′- and 3′- ends of the RNAs. It has already been demonstrated that montmorillonite catalyzes the formation of L-RNA as efficiently as it does D-RNA.[17]

The facile synthesis of relatively large amounts of RNA oligomers provides a convenient route to the proposed RNA world. The 35 ~ 40 mers formed are both sufficiently long to exhibit fidelity in replication as well as catalytic activity.[18,19]

Financial aid was from the National Science Foundation and the NY Center for Studies on the Origins of Life: A NASA Specialized Center for Research and Training.

Notes and references

1 F. H. C. Crick, *J. Mol. Biol.*, 1968, **38**, 367.
2 L. E. Orgel, *J. Mol. Biol.*, 1968, **38**, 381.
3 T. R. Cech, A. Zaug and P. J. Grabowski, *Cell*, 1981, **27**, 487.
4 C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace and S. Altman, *Cell*, 1983, **35**, 849.
5 W. Gilbert, *Nature*, 1986, **319**, 618.
6 L. E. Orgel, *Trends Biochem. Sci.*, 1998, **23**, 491.
7 J. P. Ferris, *Origins Life Evol. Biosphere*, 1993, **23**, 307.
8 J. P. Ferris and G. Ertem, *J. Am. Chem. Soc.*, 1993, **115**, 12270.
9 J. P. Ferris, A. R. Hill, Jr., R. Liu and L. E. Orgel, *Nature*, 1996, **381**, 59.
10 J. P. Ferris, *Origins Life Evol. Biosphere*, 2002, **32**, 311.
11 G. Ertem and J. P. Ferris, *J. Am. Chem. Soc.*, 1997, **119**, 7197.
12 K. J. Prabahar and J. P. Ferris, *J. Am. Chem. Soc.*, 1997, **119**, 4330.
13 M. Levy and S. L. Miller, *J. Mol. Evol.*, 1996, **48**, 631.
14 G. M. Richards, D. J. Tutas, W. J. Wechter and M. Laskowski, *Biochemistry*, 1967, **6**, 2908.
15 J. Rogers and G. F. Joyce, *Nature*, 2001, **402**, 323.
16 J. S. Reader and G. F. Joyce, *Nature*, 2002, **420**, 841.
17 P. C. Joshi, S. Pitsch and J. P. Ferris, *Chem. Commun.*, 2000, 2497.
18 G. F. Joyce and L. E. Orgel, in *Prospects for understanding the origin of the RNA world*, ed. R. F. Gesteland, T. R. Cech and J. F. Atkins, Cold Spring Harbor, New York, 1999.
19 J. W. Szostak and A. D. Ellington, in In vitro *selection of functional RNA sequences*, ed. R. F. Gesteland and J. F. Atkins, Cold Spring Harbor, New York, 1993.