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 precursors6 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 mers of RNA monomers greater than 30 mers in 2 h (lane 2) with chain lengths 9 mers or greater from the reaction of a 1 : 1 mixture of 1-MeadpA and 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 T2 resulted in their hydrolysis to monomers 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 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 T2 resulted in their hydrolysis to monomers 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 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 T2 resulted in their hydrolysis to monomers 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 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 T2 resulted in their hydrolysis to monomers 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 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 T2 resulted in their hydrolysis to monomers 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 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 T2 resulted in their hydrolysis to monomers 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
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 activity15,16 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 t-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