Synthesis of long prebiotic oligomers on mineral surfaces

James P. Ferris*, Aubrey R. Hill Jr†, Rihe Liu† & Leslie E. Orgel†

* Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180, USA
† The Salk Institute for Biological Studies, PO Box 85800, San Diego, California 92186, USA

Most theories of the origin of biological organization assume that polymers with lengths in the range of 30–60 monomers are needed to make a genetic system viable[1]. But it has not proved possible to synthesize plausibly prebiotic polymers this long by condensation in aqueous solution, because hydrolysis competes with polymerization. The potential of mineral surfaces to facilitate prebiotic polymerization was pointed out long ago[2]. Here we describe a system that models prebiotic polymerization by the oligomerization of activated monomers—both nucleotides and amino acids. We find that whereas the reactions in solution produce only short oligomers (the longest typically being a 10-mer), the presence of mineral surfaces (montmorillonite for nucleotides, illite and hydroxylapatite for amino acids) induces the formation of oligomers up to 55 monomers long. These are formed by successive 'feedings' with the monomers; polymerization takes place on the mineral surfaces in a manner akin to solid-phase synthesis of biopolymers[3,4].

Solid-phase synthesis depends on the ease with which growing chains are separated from spent reagents in solution by washing. The attachment to the resin must be irreversible, or nearly so. If the strength of adsorption of a polymer to a surface increases with length, sufficiently-long polymers will be adsorbed almost irreversibly and can then be extended indefinitely by the prebiotic equivalent of solid-phase synthesis. Our experimental approach is simple. A mineral is incubated with an activated monomer until a family of short oligomers are formed. The solid is separated by centrifugation and the activated monomers are replaced. This process is repeated as often as necessary for the production of oligomers of length 40 or more. Finally, the solid is eluted with a solution of sodium pyrophosphate and the eluate is analysed.

Montmorillonite is a very effective catalyst for the oligomerization of nucleoside 5′-phosphorimidazolides[5,6]. We therefore studied the elongation of the decanucleotide [^{32}P]dA(pdA)$_8$pA adsorbed on Na^+-montmorillonite by daily additions of ImpA (Fig. 1). Polyadenylates containing more than 20 monomers were formed after 'feeding' twice with ImpA, with the main products being 11–14-mers (Fig. 2). Polynucleotides containing more than 50 monomers were formed after 14 feedings, with the principal oligomeric products containing 20–40 monomer units (Fig. 2). A small amount of the pyrophosphate-capped primer was the only product observed after a 12-day reaction in the absence of montmorillonite (data not shown).

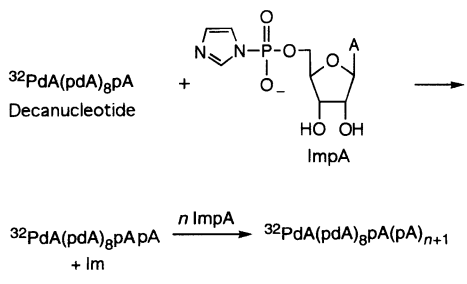

FIG. 1 Elongation of a decanucleotide by reaction with ImpA: Im is imidazole, pA is adenosine-5′-phosphate, pdA is 3′-deoxyadenosine-5′-phosphate, and ^{32}P indicates a radioactively labelled phosphate group.

The oligomerization of glutamic acid in the presence of the condensing agent carbonyl diimidazole is a very efficient reaction that proceeds via an *N*-carboxy anhydride intermediate[7]. The products of a reaction using a relatively dilute solution of the amino acid are illustrated in Fig. 3*a*. Oligomers up to the 10-mer can be detected, but the great majority of the products are shorter than the 5-mer. If the reaction is carried out in the presence of illite, the shorter oligomers remain in the supernatant and longer oligomers adsorb to the mineral. There is no evidence for catalysis by the mineral. The products eluted from the mineral after 10, 25 and 50 feedings are illustrated in Fig. 3*b*, *c* and *d*, respectively. After 50 feedings oligomers up to at least the 55-mer can be detected, and the bulk of the adsorbed product is in the size range 30–50.

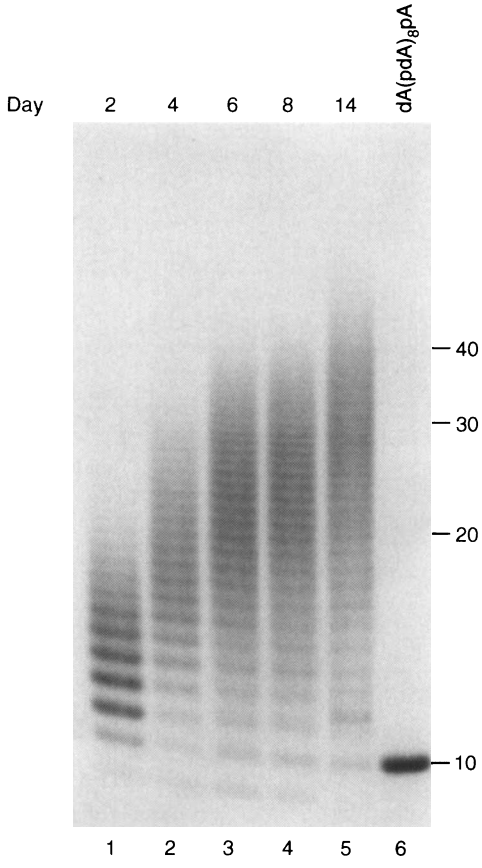

FIG. 2 Gel electrophoresis of the polyadenylates formed by the elongation of [^{32}P]dA(pdA)$_8$pA. Migration positions of size markers (for 10-, 20-, 30- and 40-mers) are shown on the right. dA(pdA)$_8$pA is prepared on an Applied Biosystems 391 DNA synthesizer initiated by adenosine attached to porous glass beads (Milligen). The deblocked oligomer is phosphorylated with a mixture of T4-polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (Amersham) and the product is deionized and purified on a Nensorb (duPont) column. The primer migrated as one band on gel electrophoresis in a 20% polyacrylamide denaturing gel using a buffer of 0.09 M Tris–borate and 0.002 M EDTA (TBE) buffer, pH 8. The primer (~500,000 c.p.m.) is dissolved in 40 μl buffer (0.1 M HEPES, 0.1 M NaCl, 0.075 M $MgCl_2$, pH 8) and added to 2 mg Na^+-montmorillonite in a Gelman 4937 Z-spin filtration unit containing a 0.45-μm filter. To this is added sufficient ImpA to give a 15 mM solution. The reaction mixture is vortexed and allowed to stand for one day at 25 °C. The tube is then centrifuged to remove the aqueous phase from the montmorillonite and then washed with 40 μl of the buffer reaction solution. Fresh ImpA and buffer salt (total volume 40 μl) are added to the clay–oligomer complex in the filtration tube and the reaction allowed to proceed for another day. Six reaction tubes are prepared and, after appropriate numbers of incubations, gel products are eluted from the montmorillonite by 3 × 40 μl washes of 0.1 M pyrophosphate (pH 9). The combined washes are deionized on a Nensorb column and analysed by gel electrophoresis on a 20% gel using TBE buffer.

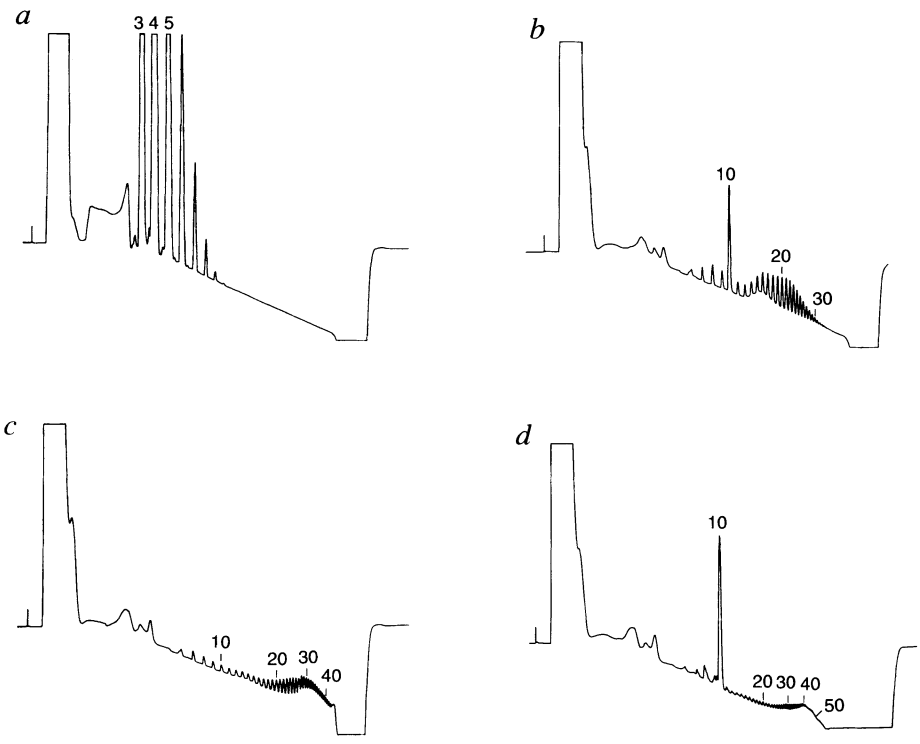

FIG. 3 Polymerization of glutamic acid (Glu) on illite. *a*, Control reaction in the absence of illite; *b*, eluate after 10 feedings; *c*, after 25 feedings; *d*, after 50 feedings. A marker of authentic $(Glu)_{10}$ is added to the samples in *b* and *d*. Illite (10 mg or 20 mg) is weighed in a microfuge tube. As illite cannot be divided into aliquots accurately, a separate tube is prepared for each reaction sequence. A solution of activated monomer is prepared by adding 20 mM L-Glu (pH 8.0) to a 2.5-fold excess of dry, solid carbonyl diimidazole at 0 °C. The solution is kept at 0 °C for 1 min, after which 50 μl aliquots are pipetted into the illite-containing reaction tubes. The tubes are vortexed to suspend the illite and then tumbled at room temperature. After 6 h the tubes are spun for 1 min, the supernatant is removed, and an aliquot of freshly prepared activated glutamic acid is added. The tubes are vortexed to resuspend the illite and tumbled for 18 h. The tubes are then spun again for 1 min, the supernatant removed, and activated glutamic acid is again added. The sequence is repeated, with alternating incubations of 6 and 18 h until the illite has been 'fed' an appropriate number of times. The reaction tubes are then spun for 1 min, and the supernatant set aside. The solid is washed four times with 250 μl water. It is then eluted with each of three 500-μl aliquots of 20 mM $K_4P_2O_7$ for 15 min. The eluates are combined and analysed by HPLC on RPC-5 using a perchlorate gradient (pH 8; 0–0.03 M in 40 min).

We have also used a water-soluble carbodiimide, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDAC) as a condensing agent for the polymerization of aspartic acid on the surface of hydroxylapatite. In this system both of the carboxyl groups of the amino acid are activated, so branching is possible. Because this leads to a complex family of isomers for each molecular weight, individual peaks corresponding to longer oligomers are poorly resolved in the high performance liquid chromatography (HPLC) traces. Figure 4 shows elution profiles of the products formed in homogeneous solution, and the compositions of the material eluted from the mineral after different numbers of 'feedings'. Clearly, very large amounts of material of high molecular weight are accumulated. After 30 feedings, most of the product is retained by the mineral under our standard elution conditions.

The 'feeding' protocol that we have outlined is a plausible model of prebiotic polymerization. The repeated incubations with low concentrations of activated monomers simulate conditions in environments where rocks are in constant contact with low concentrations of activated substrates or are episodically washed with higher concentrations. The model is robust in the sense that the presence of a large excess of unactivated substrate does not inhibit chain elongation. Thus a solution containing 1 μM of activated monomer in the presence of 100 μM unactivated monomer could yield only dimers in homogeneous solution but, on mineral surfaces, would extend a primer almost as efficiently as a pure 1 μM solution of the activated substrate.

The model as presented so far could lead to the covering of mineral surfaces by polymers of enormous length. In most relevant polymerizations, however, hydrolysis and chain termination would intervene. It is hard to discuss chain termination in a systematic way, as it depends on the idiosyncracies of the system under investigation and on the assumptions made about the prebiotic environment. The consequences of hydrolysis can be treated more systematically.

A simple calculation shows that the mean chain length $\bar{n} \approx \{[t_h(1/2)]/[t_c(1/2)]\}^{0.5}$ where $t_e(1/2)$ is the half-time for chain elongation and $t_h(1/2)$ is the half-time for hydrolysis of each of the bonds in a polymer. It follows that, even if as much as one residue were added each day, the bonds in the resulting polymer would have to be stable for two or three years to achieve a mean chain length of 30. As elongation on the primitive Earth

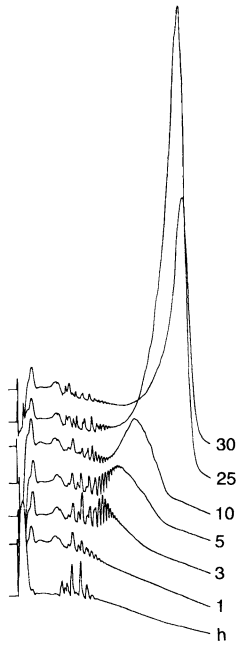

FIG. 4 Polymerization of aspartic acid on hydroxylapatite using *N*-ethyl-*N'*-dimethylaminopropyl-carbodiimide (EDAC) as condensing agent. The elution profile of a reaction mixture after a single incubation in the absence of mineral is labelled 'h'; the other traces correspond to products eluted after the designated numbers of cycles. Reactions were carried out at room temperature; each reaction tube contained 40 mg hydroxylapatite; the reaction solution contained 50 mM aspartic acid (pH 6.0) and 50 mM EDAC. To start the experiment, an aliquot (100 μl) of a freshly prepared solution of aspartic acid and EDAC is added to 40 mg hydroxylapatite in an 0.7 ml Eppendorf tube at room temperature. After 3.5 h, the tube is centrifuged, the supernatant is removed, and an aliquot of freshly prepared reaction mixture is added to begin the next cycle. After three cycles, the tubes are stored overnight at 4 °C in the absence of supernatant. After an appropriate number of cycles, the solid is washed 4 times with 250 μl water, and then eluted with each of two 250-μl aliquots of 20 mM $K_4P_2O_7$ for 15 minutes. An aliquot of the combined eluate (25 μl) is mixed with 2 ml starting buffer (pH 8) and analysed by HPLC on RPC-5; the reaction products are eluted from the column using a linear gradient of $NaClO_4$ (pH 8; 0–0.06 M, 80 minutes).

cannot have been rapid, only stable polymers such as peptides and nucleic acids could have accumulated from aqueous solution. Esters, for example, would have hydrolysed too quickly.

We have described experiments with negatively charged polymers, but the model is not restricted to these compounds. In principle it should apply, for example, to the synthesis of sulphydryl-containing polymers on metal sulphides or to the synthesis of positively charged polymers on cation-exchanging minerals. We have extended the method to the synthesis of copolymers in an obvious way by incubating with a mixture of activated amino acids or by incubating successively with different activated amino acids.

The arguments presented above suggest that the minerals on the primitive Earth would have provided a 'library' of surfaces for the exploration of molecular evolution. Substrate–mineral combinations that lead to surface catalysis would be advantageous as sites for the development of a genetic system, but catalysis, although beneficial, is not essential. The feature lacking in our experimental systems is the ability of adsorbed molecules to replicate. In this regard, studies of template-directed reactions of nucleotides on surfaces[3,8,9] and experiments related to those described above but involving template-directed ligation, are encouraging[4]. The possibility of simpler surface-bound replicating systems remains to be explored. □

Received 8 February; accepted 19 March 1996.

1. Szostak, J. W. & Ellington, A. D. in *The RNA World* (eds Gesteland, R. F. & Atkins, J. F.) 511–533 (Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY, 1993).
2. Bernal, J. D. *The Physical Basis of Life* (Routledge & Kegan Paul, London, 1951).
3. Gibbs, D., Lohrmann, R. & Orgel, L. E. *J. Molec. Evol.* **15,** 347–354 (1980).
4. Acevedo, O. L. & Orgel, L. E. *Nature* **321,** 790–792 (1986).
5. Ferris, J. P. & Ertem, G. *J. Am. chem. Soc.* **115,** 12270–12275 (1993).
6. Kawamura, K. & Ferris, J. P. *J. Am. chem. Soc.* **116,** 7564–7572 (1994).
7. Ehler, K. W. & Orgel, L. E. *Biochim. biophys. Acta* **434,** 233–243 (1976).
8. Schwartz, A. W. & Orgel, L. E. *J. molec. Evol.* **21,** 299–300 (1985).
9. Holm, N. G., Ertem, G. & Ferris, J. P. *Orig. Life Evol. Biosphere* **23,** 195–215 (1993).

ACKNOWLEDGEMENTS. We thank the American Colloid Co. for a gift of volclay, the mineral from which the Na^+-montmorillonite used in this study was prepared, and G. Arrhenius for a gift of illite. We also thank S. Bailey for manuscript preparation. Research at RPI was supported by the US NSF, and at The Salk Institute by NSCORT/EXOBIOLOGY and NASA.

Rapid braincase evolution between *Panderichthys* and the earliest tetrapods

Per E. Ahlberg*, Jennifer A. Clack† & Ervīns Lukševičs‡

* Department of Palaeontology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
† University Museum of Zoology, Downing Street, Cambridge CB2 3EJ, UK
‡ Latvian Museum of Natural History, 4 Krišjāna Barona Street, Riga, LV1050, Latvia

The panderichthyids (or elpistostegids) are the most tetrapod-like fishes that still retain paired fins rather than limbs. During the transition from fish to tetrapod, the braincase, previously subdivided by a joint, was remodelled into a solid structure[4]. Here we present the complete braincase of the fish *Panderichthys rhombolepis*, a Middle Devonian[1] member of the tetrapod stemgroup[2,3]. *Panderichthys* has an externally tetrapod-like skull[2], but we show that the braincase retained the intracranial joint, conforming wholly to the generalized pattern of lobe-finned fish, and sharing no obvious derived features with tetrapods. This places the braincase transformation between *Panderichthys* and the earliest tetrapods exemplified by *Acanthostega*[4]. The timing of the braincase transformation closely matches that of the limbs. There are also striking similarities with the braincase transformation in the lungfish lineage. Both phenomena may reflect developmental linkages and canalization.

The braincase of *Panderichthys* (Figs 1, 2 and 3*c*, *d*) resembles the osteolepiform *Eusthenopteron*[5] (Fig. 3*a*, *b*), a less crownward (more primative) member of the tetrapod stem group, but also compares well with the living coelacanth *Latimeria*[6]. Like these taxa it has an intracranial joint, which runs between the basisphenoid and basioccipital ventrally, and immediately anterior to the otic capsule dorsally. Anteriorly, the basioccipital divides into left and right 'otic shelves' either side of a basicranial fenestra, which contains an arcual plate and would in life have accommodated the anteriormost part of the notochord. A lateral commissure (Fig. 2*a*, *b*) straddles the jugular canal and carries the hyomandibular articulations. The hyomandibula and basipterygoid process resemble those of *Eusthenopteron*. Two apparently unique characters of *Panderichthys* (or panderichthyids) are the vestibular fontanelle, which is rather small and anteriorly placed for a sarcopterygian (compare Fig. 3*b*, *d*, *j*), and the dorsally displaced optic tract, which reflects the position of the orbit. However, the general resemblance to *Eusthenopteron* is very close.

By contrast there are profound differences between the braincases of *Panderichthys* and the more crownward (less primative) stem tetrapod *Acanthostega*[4] (Fig. 3*e*, *f*). In the latter there is no lateral commissure, jugular groove, basicranial fenestra, arcual plate or intracranial joint. The anterior parts of the basioccipital and otic capsule have been drastically shortened, the hyomandibula has become a stapes with its footplate lodged in the enlarged and dorsally displaced vestibular fontanelle[4,7], and the basipterygoid process has changed shape.

This character distribution contrasts with the external morphology of the head. *Panderichthys* displays external tetrapod-like characters such as frontal bones, flattened snout, posteriorly

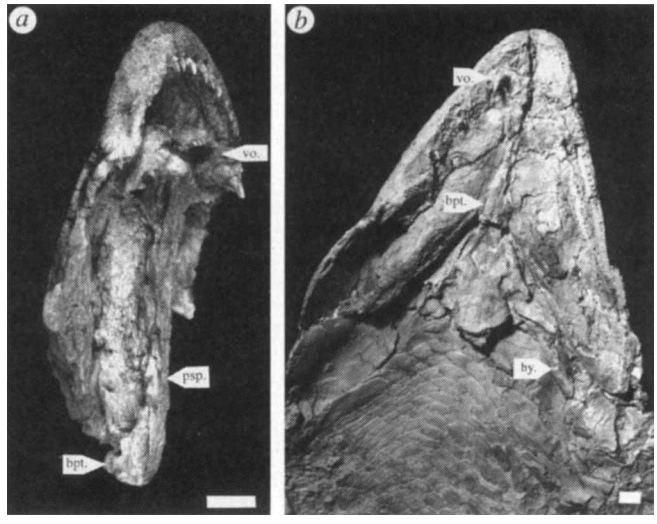

FIG. 1 *a*, Specimen LDM 43/4002, anterior (ethmosphenoid) portion of the braincase of *Panderichthys rhombolepis* together with partial skull roof, vomers (vo.) and parasphenoid (psp.). Note well-preserved optic nerve foramen (II) and basipterygoid process (bpt.). Right ventrolateral view. A left ventrolateral view of this specimen was presented by Worobjewa[28]; we agree with her interpertation of the basipterygoid process and associated arterial foramina, but the 'nerve foramina' she identified appear to be artefacts of preparation. *b*, Specimen LDM 60/123, dorsoventrally flattened head and anterior part of the body in ventral view. Uniquely, this specimen preserves the whole braincase, as well as a crushed hyomandibular (hy.), but the anterior part is less well exposed than in 43/4002; it also includes the anterior end of the vertebral column, but this was removed before the photograph was taken. Scale bars, 10 mm. Both specimens were collected from Lode Quarry in Latvia (Gauja Formation, probably Upper Givetian[1]) by L. Lyarskaya, and belong to the Latvian Museum of Natural History (Latvijas Dabas Muzejs) in Riga.