Studies on the reaction of the 5′-phosphorimidazolide of adenosine with Cu(II)-exchanged hectorite

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The role of clay minerals in the prebiotic synthesis of nucleotide oligomers has received considerable attention in recent years. Scanning force microscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry are used to identify oligomers of adenylic acid formed on the clay mineral Cu(II)-exchanged hectorite in simulated prebiotic cycling experiments. Electron-spin resonance and x-ray diffraction data indicate that the monomer (5′-phosphorimidazolide of adenosine, or ImpA) penetrates into the intergallery regions of the mineral substrate, and complexes the gallery Cu(II) cations. It is postulated that polymerization of the monomer is initiated in the clay intergallery regions, producing oligomers of adenylic acid up to 8 units in length or more. © *1999 American Institute of Physics.* [S0003-6951(99)01343-1]

The role of natural clay minerals in the prebiotic evolution of complex biological molecules is the subject of both conjecture and experiment.[1] Clay minerals act to selectively adsorb and concentrate amino acids and nucleotides.[2–4] Clay minerals may also provide a medium on which polymerization of these molecules occurs.[5] Recently, studies have focused on clay-processed RNA-like molecules which may have formed on the primitive earth and, subsequently, condensed to form the first oligonucleotides. For example, a mixture of an unblocked, activated monomer (phosphorimidazolide of adenosine, or ImpA) and montmorillonite produce oligomers of adenylic acid up to 11 mer in length.[6,7] Here, the extent of oligomer formation is highly dependent on the type of exchangeable metal cation present in the clay. The oligomers produced contain a large majority of 3′,5′-phosphodiester bonds (over the 2′,5′ linkage), rendering them amendable to further chain elongation by ribozymes or template-directed replication.[8,9] The synthesis of polyadenylates up to 50 mer in length was recently accomplished through the elongation of the decanucleotide $[^{32}\mathrm{P}]dA(pdA)_8pA$ in the presence of Na^+ montmorillonite via periodic ''feedings'' of the activated nucleotide ImpA.[10]

The role of clay minerals in the formation of viable biomolecules is many-fold. Clay surfaces or intergallery regions act to adsorb and/or concentrate and order the reacting species.[2,3,11] The chemical nature of specific sites on or within the clay mineral (including sites where exchangeable metal cations are present) may be sufficient to initiate the reactions in which biomonomers are joined or biopolymers are elongated.[2,12–16] Catalytic properties of certain clay minerals related to their structure and local chemical properties may act to control the regiospecificity of the reaction, leading to more biologically viable molecules (the particular phosphate activating group used also controls the relative percentage of 3′,5′ linkages).[17] A role for clay minerals in the evolution of the genetic code was recently postulated.[18]

In this letter we focus on the interactions of the activated nucleotide ImpA with Cu^{2+}-exchanged hectorite. Previously, scanning force microscopy (SFM) of the amino acid glycine reacted with Cu^{2+} hectorite provided direct evidence of peptide formation at surface step edges and micropore sites on the clay.[11] These sites are where the exchanged cation (Cu^{2+}) is directly accessible to the monomer or growing chain. Here, the reaction of ImpA with Cu^{2+}-exchanged hectorite is studied using the techniques of SFM, x-ray diffraction (XRD), electron-spin resonance spectroscopy (ESR), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI).

Cu^{2+}-exchanged hectorite was prepared by stirring the Na^+-exchanged hectorite (Rheox Corp.) in a 0.5 M solution of $CuSO_4$ for at least 24 h. The resulting material was subjected to a series of cycles in which the suspension was centrifuged and the centrifugate resuspended in distilled water. This process was continued until the water above the centrifugate tested negative for SO_4^{2-}. ImpA solution (15 mM) was prepared in 0.2 M NaCl and 0.075 M $MgCl_2$, adjusted to PH 8. Small hectorite film samples were exposed to the solution until saturated, and then covered. At 24 h intervals, the samples were resaturated with the ImpA solution. At the end of 72 h, the films were allowed to dry completely. Small sections of intact, reacted clay were mounted for SFM imaging, XRD, and ESR analysis. The remainder of the clay samples were mixed with 0.1 M ammonium acetate, vortexed, and allowed to stand for 24 h. This mixture was centrifuged, and the supernatant removed for commercial MALDI analysis.

Figure 1 shows a negative-ion MALDI spectrum from the reacted sample. This spectrum was acquired using a 355 nm ultraviolet laser with a total of 10 shots. The matrix used was 3-hydroxypicolinic acid (3-HPA). The presence of doubly charged polyadenylates up to 8 units in length is clearly demonstrated, with longer oligomers (9, 10, etc.) possibly

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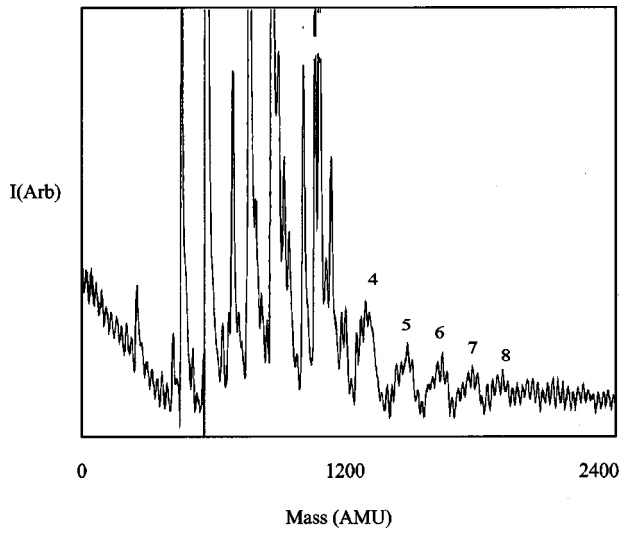

FIG. 1. Negative-ion MALDI spectrum from the reacted sample. This spectrum was acquired using a 355 nm ultraviolet laser with a total of 10 shots. The matrix used was 3-hydroxypicolinic acid (3-HPA). The presence of doubly charged polyadenylates up to 8 in length is clearly demonstrated, with longer oligomers (9, 10, etc.) possibly present.

present. This result is consistent with the results of Ferris,[6,7,12] on similar samples. The use of MALDI as an analytical tool for the detection (and possible sequencing) of nucleotide oligomers has received much attention in the past few years. While the analysis of long proteins using the MALDI technique is well established, precise information on the structure of nucleotide oligomers via MALDI analysis remains somewhat elusive. MALDI spectra of nucleotide oligomers are strongly dependent on base composition, choice of matrix, and choice of positive- or negative-ion detection.[19–21] Base fragmentation and multiple charging can make analysis of all but the simplest oligonucleotides very difficult. The use of 3-HPA as a matrix in the analysis of oligonucleotides has lessened the effects of fragmentation for certain species, but at the same time greatly enhances the multiple charging of the oligonucleotides being analyzed.[19,21]

In Fig. 2, a noncontact $0.55\,\mu\text{m}\times 0.55\,\mu\text{m}$ SFM image of the hectorite surface reacted with ImpA is shown. We note

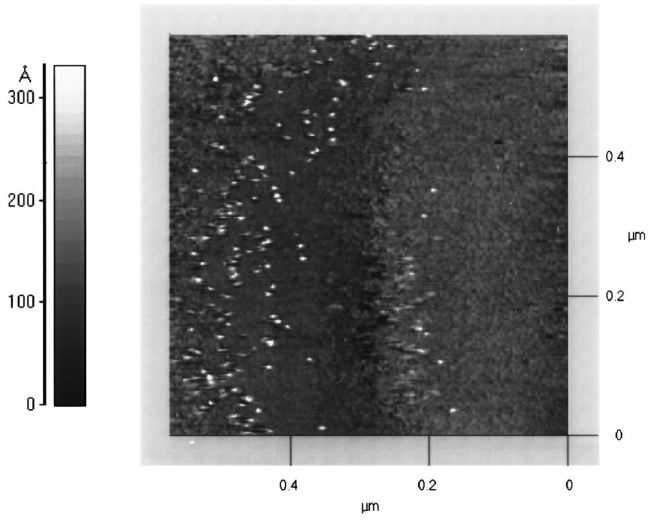

FIG. 2. Noncontact SFM image of the hectorite surface reacted with ImpA. The dimensions of this image are $0.55\,\mu\text{m}\times 0.55\,\mu\text{m}$.

that these clay samples were gently washed with ultrapure water prior to imaging in order to eliminate the presence of any salts on the surface. Most of the topmost surface regions imaged using SFM appeared to be free of any nucleotide oligomers, including step edges and micropore sites. Certain regions, however, exhibiting large concentrations of surface defects such as cracks and faults, contain numerous apparent nucleotide oligomers. In Fig. 2, two large fault regions running the length of the image from top to bottom are present near the left side of the image. These faults or cracks are extensive, with heights corresponding to several layers of the clay structure. SFM images of control samples (prepared and processed identically, but no initial ImpA exposure) showed no evidence of this type of surface moiety.

The many polyadenylate oligomers present do not appear to be attached or adsorbed to the fault edges, in contrast to peptide oligomers imaged on similar hectorite surfaces.[11] Approximately 75% of the observed oligomers are concentrated in surface regions exhibiting these structural features. It has been proposed that for oligonucleotides to form in aqueous solution in the presence of certain clay minerals, the nitrogen-containing activation groups of the monomer must contain either a positively charged nitrogen attached to the phosphorous or a nitrogen that is sufficiently basic so that it is protonated by acidic sites on the clay.[17] The positive charge binds the activated nucleotide to local negative sites on the clay. Protonated ImpA does not bind as strongly to negative sites on the clay as other positively charged nitrogen-containing activating groups.[17] We must also consider the role of the exchangeable cation in the polymerization reaction. The exchangeable Cu(II) cations present in hectorite are firmly located in the intergallery regions of the clay layers. Only a small fraction of these cations may be present on the topmost clay surface. With the presence of extensive cracks or faults in the clay surface, however, some cations may wash onto the clay surface where they might initiate polymerization of bound ImpA.

A second possibility is that polymerization of the activated nucleotide is taking place within the intergallery regions of the clay, and the oligonucleotides thus formed have simply washed out of these regions through cracks or faults onto the clay surface where they are observed by SFM. Previous evidence for this second scenario using montmorillonites has been obtained by ''blocking'' clay edge sites.[16] In this study, the blocked step edges had minimal effect on the formation of subsequent RNA oligomers. Our XRD and ESR data support this scenario. Using XRD, we compared the (001) spacings of samples of Cu(II)-exchanged hectorite thin films. For a ''dry'' film of Cu(II)-exchanged hectorite with no ImpA exposure, at an ambient humidity of 20%–40%, the XRD measured basal spacing is $12.6\pm 0.4\,\text{Å}$. For an identical film, exposed to the ImpA and subsequently analyzed by XRD at the same relative humidity level $\pm 10\%$, the measured basal spacing increases to $14.9\pm 0.4\,\text{Å}$.

In the case of the unexposed film, the intergallery regions contain the Cu(II) cations and water molecules. If all of the intergallery water were removed, the basal spacing for this type of clay is 9.4–9.5 Å. Incorporation of the activated nucleotide into the intergallery regions of the hectorite films invariably involves some degree of replacement of the water

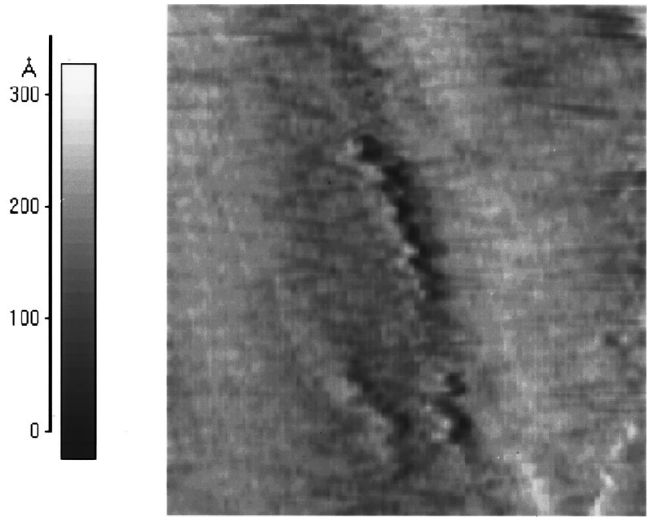

FIG. 3. 110×110 Å SFM image containing three adenylic acid oligomers. The largest oligomer measures approximately 35 Å in length, while the smaller two are on the order of 10–12 Å in dimension. The length of the largest oligomer suggests that it consists of at least 8 monomer units.

molecules present. For this reason, there is a maximum ''available'' spacing of about 5.5 Å to accommodate the intercalated nucleotide molecules. Comparing the dimensions of an ImpA molecule with these spacings suggests that the ring structures of the ImpA may be oriented in a variety of conformations within the clay intergallery regions. While the (approximately 10 Å long) molecule probably cannot ''stand on end'' within these regions, the ring structures of the molecule may be oriented from parallel to perpendicular to the planar silica sheets forming the galleries.

Information on the interaction of ImpA with the Cu^{2+} ions in the interlayer can be obtained using electron-spin resonance. Samples of Cu^{2+}-exchanged hectorite were exposed to buffered ImpA and allowed to air dry. ESR of these films at 77 K yield axially symmetric magnetic parameters for Cu^{2+}: $A_{\|}=196$ G, $g_{\|}=2.24$, and $g_{\perp}=2.077$. Axial symmetry was also observed in the ESR of untreated Cu^{2+}-exchanged hectorite, however, the magnetic parameters ($A_{\|}=164$ G, $g_{\|}=2.337$, and $g_{\perp}=2.076$) differed substantially from the treated material. Both sets of spectral parameters are consistent with octahedrally coordinated Cu^{2+}.[21–23] We also note that the Cu^{2+}-exchanged hectorite treated with imidazole, adenine, and adenine in HEPES buffer solution yields substantially different spectra than those films treated with the full ImpA solution. At present, we do not know the precise role of the Cu^{2+}/ImpA complex in the oligomerization reaction.

Large-area SFM scans of the reacted clay surface (such as Fig. 2) seem to show the adenylic acid oligomers to be in a generally ''globular,'' or highly folded morphological state. High-resolution SFM images of these oligomers, however, indicate that a small percentage are in an elongated state on the hectorite surface. We note that these small elongated oligomers may have been sectioned from larger molecules during processing. In Fig. 3, a 110×110 Å SFM image containing three oligomers is shown. The largest oligomer measures approximately 35 Å in length, while the smaller two are on the order of 10–12 Å in dimension. High-resolution scans of the globular oligomers do not reveal any substructure.

DNA in its dehydrated state might be expected to form an *A*-type helical structure, where the phosphate groups bind fewer water molecules than when the molecule is in other conformations. In this *A*-type conformation, the strand is both wider and shorter than when in the *B*-type helical structure. This effect may explain why many of the surface oligomers appear to be in a more globular than elongated conformation. For example, an eight-unit oligomer of adenylic acid in an *A*-type helical conformation should have a length on the order of 25 Å. In the *B*-type helix, that same molecule would be expected to have a length of over 30 Å. We note that a *Z*-type helical structure would be even more elongated than the *B*-type helix, but adenine oligomers are not expected to adopt this structure. The interaction of the biological molecule with the clay surface itself may affect the molecular conformation, however, these effects are still largely unknown.

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