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PREBIOTIC CHEMISTRY

Nucleic Acids Bind to Nanoparticulate iron (II) Monosulphide in Aqueous Solutions

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Abstract In the hydrothermal FeS-world origin of life scenarios nucleic acids are suggested to bind to iron (II) monosulphide precipitated from the reaction between hydrothermal sulphidic vent solutions and iron-bearing oceanic water. In lower temperature systems, the first precipitate from this process is nanoparticulate, metastable FeS_m with a mackinawite structure. Although the interactions between bulk crystalline iron sulphide minerals and nucleic acids have been reported, their reaction with nanoparticulate FeS_m has not previously been investigated. We investigated the binding of different nucleic acids, and their constituents, to freshly precipitated, nanoparticulate FeS_m. The degree to which the organic molecules interacted with FeS_m is chromosomal DNA > RNA > oligomeric DNA > deoxadenosine monophosphate ≈ deoxyadenosine ≈ adenine. Although we found that FeS_m does not fluoresce within the visible spectrum and there is no quantum confinement effect seen in the absorption, the mechanism of linkage of the FeS_m to these biomolecules appears to be primarily electrostatic and similar to that found for the attachment of ZnS quantum dots. The results of a preliminary study of similar reactions with nanoparticulate CuS further supported the suggestion that the interaction mechanism was generic for nanoparticulate transition metal sulphides. In terms of the FeS-world hypothesis, the results of this study further support the idea that sulphide minerals precipitated at hydrothermal vents interact with biomolecules and could have assisted in the formation and polymerisation of nucleic acids.

Keywords DNA · Nanoparticles · Origin of life · Adsorption · Iron sulphide · UV/Vis spectroscopy

Introduction

Adsorption of organic molecules onto mineral surfaces has long been considered to have been involved in the origin of life (Bernal 1951). Adsorption on to minerals can facilitate

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polymerisation of nucleotides (Ertem and Ferris 1996) and amino acids (Brack 1993; Huber and Wächtershäuser 1998), catalyse metabolic reactions (Cody et al. 2000), discriminate between chiral enantiomers (Hazen et al. 2001) protect molecules from hydrolysis (Shapiro 1995; Sowerby et al. 2001b) and provide a mechanism for concentrating molecules. The concentration of adenine in the prebiotic ocean has been estimated at 30 μM. Therefore, a mechanism to concentrate it, such as adsorption to a mineral surface, may have been required (Miller 1987). Adsorption is a critical process in the FeS-world origin of life hypotheses of both Wächtershäuser (1988, 1990, 1992, 1994, 1997, 1998, 2003, 2006) and Russell and co-workers (Russell and Arndt 2005; Russell and Hall 1997, 2002, 2006; Russell et al. 1988) and could have played an important part in other hypotheses such as the older prebiotic soup theory (Haldane 1929; Miller and Urey 1959; Oparin 1938) by promoting reactions between molecules which then desorb from the mineral.

Considerable work has been done on the adsorption and modification of nucleic acid bases and short oligomers onto clays (Ertem and Ferris 1993; Ferris et al. 1989; Franchi et al. 2003; Huang and Ferris 2003; Winter and Zubay 1995),and graphite (Sowerby et al. 2001a). Cohn et al. (2001) also reported the adsorption of the nucleobase adenine onto pyrite, pyrrhotite and quartz. All these studies used ground, natural minerals. In aqueous solutions at low temperatures, however, the initial precipitates are often nanoparticulate, metastable phases with properties considerably different to the stable, bulk crystalline equivalents. In particular, the size of the nanoparticles (e.g. 2–10 nm) overlaps the sizes of these large organic molecules. Therefore, the terms adsorption and desorption may not be strictly relevant and the non-specific terms “coupling” or “binding” better describe the attachment processes in these cases.

In the hydrothermal FeS world hypothesis the inorganic substructure on which organic evolution takes place is a precipitate formed when sulphide-bearing hydrothermal vent fluid comes in contact with oceanic waters high in Fe(II) (Russell and Hall 1997; Wächtershäuser 2006). At lower temperatures, this reaction produces stoichiometric iron (II) monosulphide similar to the mineral mackinawite (FeS_m) (Ohfuji and Rickard 2006; Russell et al. 1988). No study has been reported which has examined the binding of nucleotides or polynucleotides to natural mackinawite or precipitated FeS_m.

FeS_m may have been capable of acting as a primitive ribosome by attaching to RNA which then grips amino acids and catalyses the formation of bonds between them (Russell and Hall 1997, 2006; Wächtershäuser 2006). Wächtershäuser (1990, 1992) hypothesises that phosphorylation in the sugars of nucleic acids and in other biochemical roles emerged to enable these molecules to bind to positively charged surface sites on bulk mineral surfaces via electrostatic interactions. In the nanoworld, this process is more likely to be chemical bonding between the molecules and the nanoparticles. An analogous system has been described in modern natural aqueous environments where large organic molecules may act to stabilise metal sulphide clusters (Rozan et al. 2000). This paper reports the results of an experimental investigation into the extent to which a variety of nucleic acids interact with nanoparticulate FeS_m. The following polymers were examined: chromosomal DNA (cDNA), oligomeric DNA (oDNA) and RNA, as well as the DNA nucleotide deoxyadenosine monophosphate (dAMP), the non-phosphorylated version of this molecule deoxyadenosine and the purine nucleobase adenine from which they are derived. Adenine has been selected for study as it is likely to have been the first nucleobase formed, through HCN polymerisation (Ferris et al. 1978) and also has other key roles in biochemistry particularly in energy transfer molecules.

There has been considerable interest in the attachment of semiconductor nanoparticles to organic molecules because of their use as fluorophorescent tags to track biochemical

processes [see Jamieson et al. (2007) for a recent review]. These particles are called quantum dots since they have physical dimensions (2–6 nm) which are less than the exciton Bohr radius and quantum confinement effects then result in the emission of light at specific wavelengths. Of the simple sulfides, ZnS is widely employed in these structures. The methods of attachment of ZnS to biomolecules has been widely studied and we compare the effects of ZnS on DNA to FeS_m on DNA in this study. Nanoparticulate CuS has no known fluorophorescent effect and its relationship with nucleic polymers and oligonucleotides is unknown. We report the results of a preliminary study on nanoparticlulate CuS in this paper.

Materials and Methods

Different nucleic acids were added to freshly precipitated FeS_m and centrifuged to remove the solid precipitate and any adsorbed nucleic acids. The supernatant was removed and analysed by UV-Vis spectroscopy to determine the concentration of the nucleic acid remaining in solution. The initial concentration of the nucleic acid was determined by preparing a solution from the same stock, diluted to the same concentration, centrifuged and analysed by UV-Vis spectroscopy.

Sample Preparation

Iron (II) monosulphide was precipitated in sterile 1.5 ml Eppendorf tubes or sterile 15 ml conical centrifuge tubes from 50 mM stock solutions of sodium sulphide nonahydrate ($Na_2S.9H_2O$) and ammonium iron sulphate [$Fe(NH_4)_2(SO_4)_2.6H_2O$]. Pre-prepared aqueous solutions of cDNA, pDNA, oDNA, RNA, adenine, deoxyadenosine or deoxyadenosine monophosphate were added after the precipitates were formed. Stock solutions of nucleic acid, sulphide and metal sulphate were made by weighing salts into the sterile containers in a mass balance wiped with ethanol prior to use, nitrile gloves were worn the entire time. The solids were dissolved and diluted in sterile dH2O in an anoxic chamber. Nitrile gloves were used on top of the chamber's rubber gloves when handling samples in the anoxic chamber.

Adenine, 2′deoxyadenosine, 2′deoxyadenosine 5′monophosphate and RNA (R1753, *E. coli* Strain W, Type XX) were purchased from *Sigma* as powders and dissolved in sterile dH_2O. The solutions were centrifuged to remove any undissolved matter and then diluted to a concentration which on a 1:5 dilution gave an A260 nm of ~1. Oligomeric DNA (oDNA) was a 20-mer single stranded DNA molecule purchased from MWG-biotech. Chromosomal DNA (cDNA) was extracted from an *E. coli* culture grown in LB broth using Bio 101 Systems Fast DNA® Spin Kit according to manufacturer's instructions. Electrophoretic analysis determined the number of base pairs in this cDNA to vary between approximately 1,000 and 10,000.

All reagents were of analytical quality. Water was of MilliQ quality with a resistance of 18 MΩ, sterilized through autoclaving and deoxygenated by bubbling O_2 free N_2 through it for at least 1 h with glass pipette which was sterilised by autoclaving11H1r. Micro cuvettes and 15 ml centrifuge tubes were sterile on purchase; 1.5 ml microfuge tubes were sterilized through autoclaving for 2 h at 2 atm. All pipette tips were either sterile on purchase or autoclaved for 2 h at 2 atm.

Plastic bottles containing stock solutions were sterilised by washing with Decon 90® and then with ethanol. All samples were prepared and analysed at 20±3°C. The pH of a 10 mM,

50 ml solution of FeS_m in dH_2O was measured on a Jenway 2030™ digital pH meter and found to be 7.4. The effect of the organic molecules on this pH is thought to be negligible so a pH of 7.4 is assumed for all the FeS_m samples in this study.

The nanoparticulate metal sulphides prepared as reactants in this study have been characterized in previous reports. The FeS_m used has been characterized in some detail by Ohfuji and Rickard (2006), Rickard (2006) and Rickard et al. (2006). FeS_m is stoichiometric nanoparticulate FeS with a mackinawite structure. The particles are between 2 and 5.7 nm thick and between 3 and 10.8 nm in length with a mean size of 3.6×5.8 nm. The specific surface area is 380±10 m^2 g^{-1}. The length of a nucleic acid molecule is 0.34 nm per base. This gives lengths of 6.8 nm, ~ 27.2 nm and 340–3,400 nm for the oDNA, RNA and cDNA respectively.

The FeS_m reaction was compared with reactions with nanoparticulate CuS and ZnS. These were prepared in a similar manner to FeS_m except that the metal reactants were copper (II) sulphate ($CuSO_4.5H_2O$) or zinc sulphate ($ZnSO_4.7H_2O$).

The nature of nanoparticulate ZnS we used has been discussed by Luther et al. (1999), Zhang and Banfield (2004) and Luther and Rickard (2005). The CuS precipitates we used were originally described by Rickard (1973) and subsequently defined by Luther and Rickard (2005).

Analysis

Metal sulphide+nucleic acid preparations were incubated in a Labmaster 130™ anoxic cabinet. Samples were either removed from the cabinet, kept sealed and centrifuged in the tubes they were prepared in or 1 ml was subsampled in the anoxic cabinet and pipetted into a sterile microfuge tube which was sealed and removed. Centrifugation was performed in an Eppendorf 54515D microfuge at 5,000 rpm or until the supernatant had cleared; 0.5 ml of the cleared supernatant was then pipetted into a UV-permeable micro cuvette (Fisher). A Cary 50 Probe UV-Vis spectrophotometer was used to measure the absorbance of the samples from 220 to 600 nm in the experiments with adenine, adenosine, dAMP and RNA. The experiments with cDNA and oDNA were performed with a Perkin-Elmer Lambda 2 which took a single reading at 260 nm.

One milliliter samples of 10 mM FeS_m, CuS, ZnS or $FeSO_4$ with 44.9 μg/ml chromosomal DNA were mixed and left for 1 h in an anoxic cabinet. The solutions were centrifuged at 5,000 rpm for 5 min or until the supernatant had cleared; 0.5 ml of the supernatant was pipetted into a UV cuvette and the absorbance at 260 nm was measured in the spectrophotometer. Inorganic blanks of 10 mM FeS, CuS, ZnS and $FeSO_4$ without DNA were performed simultaneously and the A260 reading of these negative controls was subtracted. In all cases A260 nm of the blanks was <0.1. For both the DNA samples and the blanks two samples were prepared and the mean A260 nm reading was calculated. cDNA was quantified from the A260 nm reading using an extinction coefficient of 1 A260=50 μg/ml cDNA (Gallagher and Desjardins 2006). In all experiments a positive control was prepared from the same nucleic acid stock solutions, diluted to the same extent as the experimental samples, to ascertain the initial concentration of the nucleic acids in the experimental samples.

Experiments with RNA, oDNA and adenine derivatives were performed over a longer time-period and with only FeS_m. Solutions of 10 mM FeS_m plus the nucleic acid were made up to 5 ml in 15 ml conical centrifuge tubes. FeS_m-free nucleic acid controls were also made up from the same stock solution and to the same concentration as in the experimental preparations. Solutions were stored in an anoxic cabinet. At the given time intervals 1 ml

was subsampled into a 1.5 ml Eppendorf microfuge tube which was removed from the anoxic cabinet and centrifuged at 5,000 rpm for 5 min or until the supernatant had cleared. Subsequently 0.5 ml of the clear supernatant was pipetted into a UV cuvette and an absorbance scan from 220 to 600 nm was performed.

To correct for background absorbance due to aqueous sulphide and aqueous FeS_m, absorbance readings were taken at 320 nm (nucleotides) or 600 nm (RNA). Nucleic acids do not absorb radiation at these wavelengths so the absorbencies are solely the result of the centrifuged FeS_m solutions. For the experiments with RNA 600 nm was used as it was found that centrifuged FeS_m+RNA solutions absorbed at 320 nm to a degree which was not entirely attributable to FeS_m. FeS_m blanks determined the A260/A320 of centrifuged 10 mM FeS_m to be 1.13 and the A600 nm to be 0.95. To determine the amount of the A260 nm which was due to sulphide the A320 nm or A600 nm in each sample was multiplied by the correction factor of 1.13 or 0.95 respectively. This value was subtracted from the A260 nm of the sample and the resultant value was multiplied by its extinction coefficient (Gallagher and Desjardins 2006) to determine the concentration of nucleic acid in solution.

Results

Chromosomal DNA

Mean A260 nm for the two centrifuged FeS_m+cDNA preparations after 1 h was 0.013 (Fig. 1) which equates to 0.65 μg/ml cDNA remaining in solution (Fig. 1), using an extinction coefficient of 50 μg/ml per A260. Therefore, a total of 44.3 μg of cDNA bound to 10 μmol (880 μg) FeS_m. To ensure that these results are not due to metal ions binding to the DNA and causing it to precipitate, $FeSO_4$ was incubated with cDNA, centrifuged and analysed in the same manner. A cDNA concentration of 40.4 μg/ml was detected in this sample (Fig. 1), constituting a total loss of 4.5 μg/ml, approximately 10% of the reduction in cDNA concentration observed with FeS_m. A loss in cDNA solution concentration, similar to FeS_m, was observed with ZnS and CuS, 44.2 and 42.2 μg/ml respectively (Fig. 1).

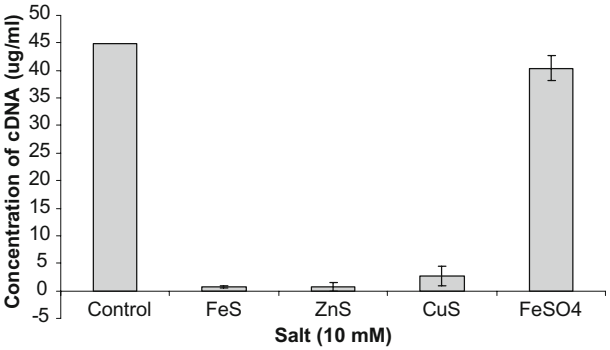

Fig. 1 The amount of cDNA remaining in solution after treatment with 10 mM FeS_m, CuS and ZnS for 1 h and centrifuged. The control shows the amount of cDNA added to each sample. In all three cases <5% of the cDNA remained in solution. The $FeSO_4$ caused a much smaller reduction in the concentration of cDNA in solution confirming that the results are not due to precipitation of cDNA with aqueous metal ions. Chart shows the mean of two samples error bars show ± standard deviation

Oligomeric DNA

After 1 h the reduction in oDNA solution concentration was 6.8 μg/ml from a starting concentration of 26.2 μg/ml (Fig. 2) representing a reduction of 26.0%. The amount of oDNA in solution decreased over time with the maximum at 24 h at which the concentration of oDNA in solution was 6.0 μg/ml; 22.9% of the original concentration.

RNA

The reduction in RNA concentration occurred in the first 24 h and progressed slowly over the next 120 reaching a maximum at 144 h when the solution concentration was approximately 24.1% (8.8 μg/ml) of the original 36.5 μg/ml (Fig. 3). In the experiments with RNA it was necessary to correct for background absorbance at 600 nm rather than 320 nm due to an unexpected absorption at ~ 300–400 nm (Fig. 4). This absorbance is greater than the combined absorbance of RNA and an FeS_m blank which are both low and approximately constant over this region whilst the absorbance of the FeS+RNA supernatant decreases from 300 nm to 400 nm by ~0.2 Abs.

Adenine and Derivatives

The DNA monomers adenine, adenosine and dAMP all combined with FeS_m to a much lower extent than the three polymers in this study. The reduction in concentration of the monomers occurred in the first 24 h to 67.9%, 66.5% and 84.7% of their original concentrations for adenine, deoxyadenosine and dAMP respectively (Fig. 5). The concentration of adenine and adenosine in solution rose slightly over time whilst the concentration of dAMP dropped slightly between 24 and 48 h. After 360 h 82.9% of the dAMP remained in solution compared to 74.3% and 76.2% for adenine and adenosine respectively.

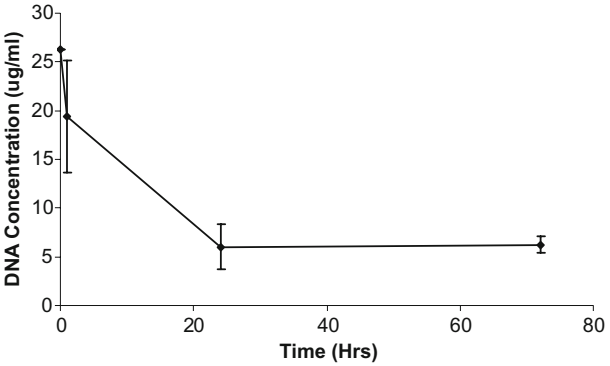

Fig. 2 Decrease in oDNA concentration on treatment with 10 mM FeS_m over a 72 h period. The amount of oDNA in solution decreased over time, reaching a minimum at 24 h. The concentration of oligomeric DNA remaining in solution was determined using an extinction coefficient of 37 μg/ml per A260 nm (Gallagher and Desjardins 2006). The results represent the mean of two oDNA samples corrected for sulphide absorption at A260 nm through the use of 10 mM FeS blanks. Four 10 mM blanks were prepared, centrifuged and analysed, the average A260 nm×37 of these samples was 2.92 with a standard deviation of 1.64; 2.92 was subtracted from both of the A260 nm readings for each time interval. The *graph* displays the mean and the *error bars* show the standard deviation of these two figures

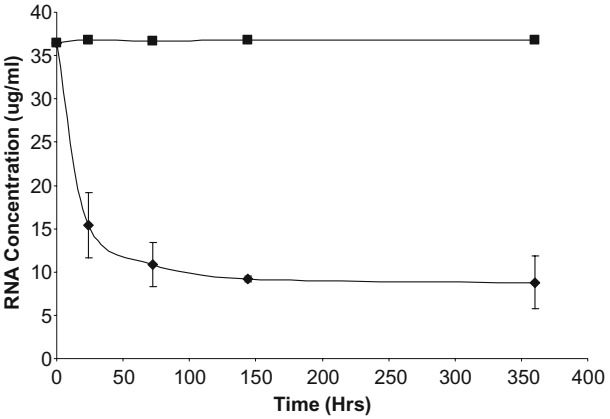

Fig. 3 Decrease in RNA concentration on treatment with 10 mM FeS_m over a 144 h period. The amount of RNA in solution decreased over time in a logarithmic fashion, A260 nm was reduced to less than 50% within 1 h. Very little reduction in RNA concentration occurred between 72 and 144 h. The results represent the mean of two RNA+10 mM FeS_m samples corrected for sulphide absorbance by subtracting background absorbance at 600 nm multiplied by a correction factor of 0.95. The concentration of RNA was determined using an extinction coefficient of 1 A260 nm=40 μg/ml RNA (Gallagher and Desjardins 2006)

Discussion

All the nucleic acids examined in this study interacted with FeS_m to some extent. To determine if other metal sulphides interacted with DNA in a similar manner, CuS and ZnS were also mixed with cDNA, centrifuged and analysed. The results were similar to those with FeS_m. However, further investigation into these materials was not pursued because Zn^{2+} causes DNA sedimentation (Kejnovsky and Kypr 1998) and because CuS did not consistently centrifuge to a clear supernatant but occasionally left a suspension which absorbed substantially at 260 nm. However, the preliminary results with ZnS are interesting since it suggests that the similar results obtained with FeS_m suggest a similar general mechanism for the binding with the biomolecules. The further similarities with nanoparticulate CuS further suggest that the overarching processes are common to sulfide nanoparticles and not metal-specific.

Chromosomal DNA exhibited a greater tendency to bind with FeS_m than either RNA or oDNA which both bound substantially more than any of the monomers. This suggests that length is a significant factor in determining the interactions between nucleic acids and FeS_m. However, even small polymers (oDNA, 20 base pairs) experienced a reduction in concentration of more than 50% given sufficient time which did not occur with the monomers. The reduction in RNA concentration (~80 base pairs) was slightly greater than that of oDNA which could be attributed to the difference in length or the differences in chemistry of the two molecules.

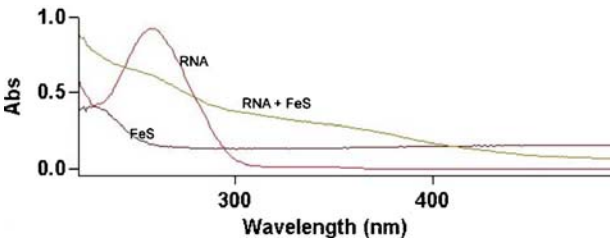

Fig. 4 Absorption of RNA+ FeS_m samples from 220 nm–550 nm with RNA and FeS_m controls after 24 h incubation. The absorption between ~300 and 380 nm is greater than the combined absorption of FeS_m and RNA

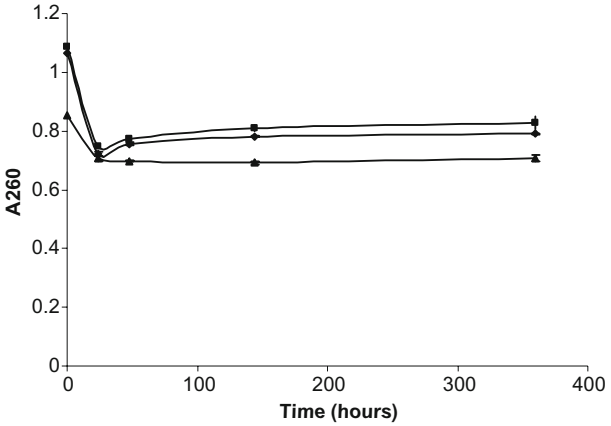

Fig. 5 The decrease in adenine (*diamond*), deoxyadenosine (*square*) and deoxyadenosine monophosphate (*triangle*) concentration on treatment with 10 mM FeS_m. Only a small fraction of the molecules were attached, in all three cases. Maximum attachment occurred within the first 24 h and did not significantly change after this time. Deoxyadenosine monophosphate experienced the smallest reduction in A260 nm. Each result represents the mean of two RNA+FeS samples corrected for background absorbance at 320 nm multiplied by a correction factor of 1.13. *Error bars* are included and show the standard deviation of the two samples but lie within the symbol size in most cases

Length has been found to be a significant factor in the binding of oligonucleotides to bulk minerals. Holm et al. (1993) found that polynucleotides adsorb onto the iron oxide hydroxide minerals goethite and akaganéite more readily than monomers. Gibbs et al. (1980) reported that hydroxyapatite selectively adsorbs higher molecular weight oligoadenylates from a mixture of oligomers.

Adenine, deoxyadenosine and deoxyadenosine monophosphate all exhibited similar binding capacity to FeS_m. This indicates that adenine itself is capable of interacting with FeS_m and this is not enhanced by the presence of the ribose-sugar group or, critically, the charged phosphate group. This is compatible with the view that RNA bound more than oDNA due to its greater length and not to differences in its sugar because the sugar does not seem to be involved in binding. This finding is contrary to the adsorption of nucleic acids onto clays which was found to require the phosphate group (Franchi et al. 2003) and that double stranded DNA had a lower affinity for the mineral than single stranded DNA. In that case divalent cations promoted adsorption by intercalating between the DNA and the clay, neutralising the negative charges on both.

FeS_m nanoparticles have a mean size of 3.6×5.8 nm (Ohfuji and Rickard 2006), substantially larger than nucleic acid monomers but small compared to the lengths of some of the polymers used in this study: 27.2 and 340–3400 nm for RNA and cDNA respectively. Interactions between bulk minerals and small organic molecules are usually discussed in terms of adsorption. Although the adenine derivatives are small enough to have adsorbed onto a single nanoparticle, the polymers are not. The surface area of the average FeS_m nanoparticle is around 150 nm^2. This is large enough for linking to multiple biomolecules. It is estimated that 50 or more small molecules (such as oligonucleotides or peptides) may be conjugated to a single FeS_m nanoparticle (Chan et al. 2002).

It is envisaged that the FeS_m nanoparticles, and possibly aqueous FeS clusters, attach to the nucleic acid polymers at multiple locations. The resultant FeS_m-nucleic acid complexes are of sufficient weight that they centrifuge out of the solution. It should be noted that at the concentrations used in this study the FeS_m flocculated; the majority of the FeS_m would not

pass through a 0.45 μm filter. These loosely aggregated flocs may provide larger masses for attachment although they are still comprised of nanoparticles.

Chan et al. (2002) reported five different processes for linking quantum dots, such as ZnS, to biomolecules. These are (1) through a bifunctional ligand, (2) by hydrophobic attraction, (3) with mercaptosilicones, (4) by electrostatic attraction and (5) via nanobeads. Of these processes, only (6) does not involve the intervention of a specific capping molecule. The p*K*a of adenine is about 3.7 (Dawson et al. 1986) and therefore it is uncharged at pH 7.4. FeS_m has no net charge at this pH either: Wolthers et al. (2005) reported that the point of zero charge (PZC) of disordered mackinawite is ~7.5. Bebie et al. (1998) reported that all metal sulphides have an isoelectric point between pH 0.6 and 3.3 (1998) although they did not examine FeS_m. More alkaline PZC's have been reported which are thought to be the result of slight oxidation (Bebie et al. 1998). Wolthers et al. (2005) dismisses the effect of oxygen because of careful anoxic conditions and because continuous dissolution of the FeS_m surface increases aqueous S(−II) concentration which is more reactive towards oxygen. Although it should be noted that in this experiment, as in Wolthers et al. (2005), the FeS_m is formed by precipitation of iron sulphate and sodium sulphide and no attempt was made to remove the sodium or sulphate ions. Sorption of these onto the surface can affect the surface charges (Bebie et al. 1998). Linkage of adenine with FeS_m may be an electrostatic process arising through induced inter-molecular forces. Uncharged adenine can bind to graphite (Cohn et al. 2001), and other uncharged mineral surfaces through van der Waals forces. Adenine has a planar shape which allows it maximum contact with the mineral surface which enhances the van der Waals forces.

The interactions between pyrite and organic molecules were found to be independent of overall surface charges and governed by interaction with specific surface sites (Bebie and Schoonen 2000). Plekan et al. (2007) found that adenine adsorbs onto pyrite through both physisorption and chemisorption. Unlike the flat monolayer which adenine forms on the surface of graphite, it adsorbs at an angle on pyrite, suggested to be the result of a chemical bond formed between Fe atoms in pyrite and N atoms in adenine (Plekan et al. 2007). However, interactions between nucleotides and other minerals have been found to be governed by electrostatic interactions between the negative charge on the phosphate group and positive charges on the surfaces of bulk iron oxide hydroxide minerals (Holm et al. 1993). This was evidenced by phosphate-free adenosine's lack of interaction and by a reduction in binding as pH increased.

Although the pH in this study is approximately equal to the PZC of FeS_m this does not mean that there are no surface charges; mackinawite has a variety of both negatively and positively charged surface sites. Wolthers et al. (2005) described a surface complexation model for disordered mackinawite. Two major surface functional groups are described; a hydroxylated iron group $FeOH^0$ and an acidic sulphide group SH^0. The acidic sulphur group is dominant in conditions where FeS is saturated and pH<~10 as is the case in the samples in this study. Thus, the Fe-SH bond is expected to be favoured over the Fe-OH bond so the surface is described through monocoordinated and tricoordinated sulphur sites: FeSH and Fe_3SH respectively. These can produce charged species through protonation or deprotonation. At pH 7.4 the most abundant groups are FeS^- and $Fe_3SH_2^+$ which both occur at a concentration of $\sim 1.5 \times 10^{-3}$ g mol^{-1}.

The abundance of thiol sites provides both positive and negative sites for potential reaction. However, surface sulphur atoms in pyrite did not interact with adenine and the iron atoms which did are less prevalent on the surface of FeS_m (Plekan et al. 2007). The dominance of thiol groups might explain why adenosine monophosphate did not bind more readily than adenosine. Negatively charged phosphate would be more likely to bind with

the Fe–OH group. Ertem and Ferris (1998) hypothesised that adenine adsorption onto clays occurs at negatively charged sites through the protonation of adenine. Although adenine was not protonated in bulk solution on entering the acidic interlayer protons could be donated to the molecule, probably at one of the NH_2 groups, giving it a positive charge. A similar reaction could be occurring here in which adenine, deoxyadenosine and dAMP are protonated through interaction with the acidic sulphur sites.

FeS_m is considerably more soluble than pyrite. Substantial Fe^{2+}, S(−II) and $FeS_{(aq)}$ exist in solution (Rickard and Luther 2007). This is relevant because aqueous ions can mediate the adsorption of nucleic acids onto mineral surfaces by intercalating between them. Franchi et al. (2003) found that divalent cations were able to enhance the adsorption of nucleic acids onto negative sites on clays (Franchi et al. 2003) and the adsorption of 5′AMP onto pyrite (Pontes-Buarques et al. 2001). Aqueous iron or iron sulphide complexes interact with the organic molecules in solution, forming nucleic acid-Fe(S) complexes which, via the iron group could form a bond with a surface thiol group. This suggestion is supported by the results with RNA. RNA not only adsorbed onto the FeS_m surface but as this absorbance between ~300 and 400 nm is greater than the combined absorbance of RNA and an FeS_m blank, it suggests the existence of an aqueous RNA-Fe(S) complex. This might arise from RNA interacting with aqueous FeS clusters. These clusters form spontaneously in the presence of iron and sulphide (Luther and Rickard 2005) and are also key electron-transfer agents in some enzymes including the ancient ferredoxins.

The nucleobases of double stranded cDNA are orientated within the double helix and the phosphates are orientated externally. The extent of the interaction between cDNA and FeS is then unexpected given the theory that it is the nucleobase which is responsible the interaction. At greater lengths, van der Waals forces may be the dominating factor which is enhanced with larger molecules. Also, the molecule's greater length may have resulted in more FeS_m particles binding to it resulting in a greater increase in mass and a greater tendency to centrifuge out of solution.

Finally, we note in passing that we did investigate the fluorophosphorence properties of FeS_m. Steady state optical measurements were made on FeS_m using a UV/Vis/NIR Jasco V570 and Cary Eclipse. FeS_m was dispersed into saline phosphate buffer at pH 7 and excited at 450 and 550 nm. The results were negative. FeS_m does not fluoresce within the visible spectrum and there is no quantum confinement effect seen in the absorption. Therefore, it does not exhibit the characteristics of fluorescent nanocrystals. Even though it is a nanoparticulate semiconductor with a size range less than the exciton Bohr radius it appears that the band gap is insufficient for a significant quantum confinement effect.

Conclusions

All the nucleic acids studied interacted with nanoparticulate FeS_m. Nanoparticulate CuS and ZnS appear to act similarly but details of the reactions of these materials with nucleic acids were not pursued for technical reasons. However, the reactions of these transition metal sulphides is interesting because the similar results suggest that the overarching processes involved in the binding of these nanoparticulate transition metal sulfide are similar.

Adenine couples with FeS_m as rapidly as or faster than dAMP so it can be inferred that the moiety which allows for interaction with the transition metal sulfides is not the phosphate as would be expected nor the ribose sugar but the nucleobase itself. This is interesting as it has been speculated that phosphorylation of sugars emerged to facilitate binding to pyrite/FeS minerals (Wächtershäuser 1988, 1990). Non-phosphorylated purine

nucleobases (such as adenine) have been noted to bind to pyrite and this study demonstrates that the same is true for nanoparticulate FeS_m.

It appears that the most likely mechanism for the interaction is electrostatic attraction, which is consistent with known processes with ZnS quantum dots. Spectroscopic work is required to determine the sites of interaction and the orientation of the molecules. This would be a challenge because of the nanoparticulate nature of the material. Nanoparticulate FeS_m does not appear to show any significant fluorophorescent properties, again reducing the range of techniques available for more detailed investigations of the binding mechanism.

It is not possible at this stage to determine if the increased attachment of FeS_m to cDNA over RNA or oDNA was because of its larger size or its double stranded structure. However, since it appears that it is the nucleobases which bind to FeS_m which are orientated within the double helix it would be expected that, if the double stranded nature had any effect, it would be to reduce interaction. Therefore, it would appear that length of the molecule is a significant factor. This could promote elongation of nucleic acids on an FeS_m surface.

This study demonstrates that double and single stranded nucleic acid polymers as well as adenine and the nucleoside and nucleotide derived from it bind to copper, iron and zinc sulphides.

Although iron sulphides are the more relevant minerals in these scenarios, all of these minerals could be present in a hydrothermally precipitated mineral assemblage (Russell et al. 2005). Wächtershäuser's hypothesis focuses entirely on mackinawite and pyrite as the mineral substrates for organic evolution. A hypothesis that realises the potential for a greater variety of minerals to promote a greater range of reactions would be preferable.

In Russell's hypothesis the incubator for life is an assemblage of mostly iron, but also copper, zinc and nickel sulphides, oxides and hydroxides and magnesium-rich clays (Russell et al. 2005). Due to the proposed compartmentalised structure of the mineral, detached organics are not necessarily lost but are able to react in solution, reattach to other sites or diffuse into other compartments. It has been proposed that the prebiotic soup could have been supplied by compounds which had formed on mineral surfaces (Bada and Lazcano 2002). This theory also makes use of a range of minerals as does the proposal of Cockell (2006) that life may have originated in a hydrothermal system within a meteoritic impact crater.

Adsorption of nucleotides onto these minerals could have facilitated their polymerisation (Ertem and Ferris 1996) and protected these polymers from hydrolysis (Shapiro 1995; Sowerby et al. 2001b). Also, the binding of RNA to FeS has been proposed to have been instrumental in the evolution of the genetic coding for peptides. Mackinawite acted in a similar manner to ribosomes in extant cells. Rows of RNA triplets are said to have bound to the mineral and oriented in a manner that allowed them to grip amino acid monomers, with some specificity, and promote peptide bonds between them (Russell and Hall 1997, 2006; Wächtershäuser 2006). However, this relies on the assumption that the phosphate group on the nucleotides would bind to the mineral orientating the nucleobase away from the mineral. This study indicates that nucleotides do not bind to mackinawite through the phosphate group, although conditions such as pH may affect this.

The role of nucleotides in extant biochemistry is not limited to the storage and transmission of information and it should not be assumed that this was their first role in biology. Wächtershäuser (1988, 2006) hypothesises that nucleotides and sugars, long before the advent of base pairing, first served as ligands for metal catalysts. Adenine, thought to be the most ancient nucleobase (Orgel 2000; Oro 1961), is life's universal molecule of energy transport. Adenosine diphosphate is phosphorylised using energy derived from metabolism, the resultant triphosphate dephosphorylises with a concomitant release of energy. Nucleotides may have operated as energy transduction molecules by phosphorylising on an FeS surface. This would be driven by either the oxidative conversion of mackinawite to

pyrite in Wächtershäuser's hypothesis or the electrochemical gradient that Russell compares to the proton motive force which generates ATP in extant metabolism. The energised nucleotide would have been able to detach and diffuse to another location such as the cytoplasm or lipid membrane of Wächtershäuser's semi-cellular structures or the interior of Russell's FeS cells.

Not only would nucleic acids bind to bulk FeS minerals they would also interact with aqueous FeS clusters and FeS nanoparticles in solution. Again the extent of this latter effect is crucial. Nucleic acid polymers entirely coated in FeS particles would not be able to interact with other nucleic acids or amino acids. Alternatively DNA molecules combined with moderate quantities of sulphide particles could possess some novel functions.

Catalytic RNA molecules were discovered in the ciliated protozoan *Tetrahymena thermophila* by Kruger et al. (1982) who found an RNA molecule with regions capable of self-splicing by breaking and forming phosphodiester bonds. These catalytic RNA molecules have been subsequently called ribozymes. Ribozymes, which catalyse the cleavage and polymerisation of RNA, have been found in nature and synthesised (DouDNA and Cech 2002; Scott et al. 1995). Ribozymes capable of redox reactions have not been developed, possibly because research in this area has concentrated on catalysis of RNA replication. Iron sulphur clusters are used in electron transfer reactions in biology today in the ancient class of enzymes: ferredoxins. RNA-FeS ribozymes could have been capable of fulfilling this role prior to the existence of protein enzymes.

As well as potentially fulfilling the catalytic role that proteins later took over nucleic acids could have had a structural role. This is particularly relevant in Russell's theories in which FeS-cells would be subject to collapse and dissolution. Nucleic acids, and possibly abiotically generated lipids, could have coated the interior surfaces offering some protection against destruction of the cell. Nucleic acids in the vicinity of pores between the cells could also have had a role in controlling diffusion between the cells. It is essential that diffusion between the cells is fast enough to allow rapid colonisation of newly formed cells but not so rapid as to cause homogenisation between cells (Koonin and Martin 2005).

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