

PREBIOTIC POLYMERIZATION: OXIDATIVE POLYMERIZATION OF 2,3-DIMERCAPTO-1-PROPANOL ON THE SURFACE OF IRON(III) HYDROXIDE OXIDE

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Abstract. The oxidation of 2,3-dimercapto-1-propanol by ferric ions on the surface of iron(III) hydroxide oxide (Fe(OH)O) yielded polydisulfide oligomers. This polymerization occurred readily at low dithiol concentration under mild aqueous conditions. Polydisulfide polymers up to the 15-mer were synthesized from 1 mM dithiol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 μ mole Fe) for 3 days under anaerobic conditions at 40 °C and pH 4. About 91% of the dithiol was converted to short soluble oligomers and 9% to insoluble larger oligomers that were isolated with the Fe(OH)O phase. Reactions carried out at the same ratio of dithiol to Fe(OH)O but at higher dithiol concentrations gave higher yields of the larger insoluble oligomers. The relationship of these results to prebiotic polymer synthesis is discussed.

1. Introduction

A major problem in the prebiotic synthesis of anhydride polymers like polypeptides and polynucleotides is the destruction of chemical condensing agents and reactive intermediates by water that prevents the formation of large polymers. Studies of prebiotic peptide and oligonucleotide formation in water using chemical condensing reagents have yielded only small amounts of dimers and trimers (Hulshof and Ponnamperna, 1976; Ferris and Hagan, 1984). As a way to overcome this problem Strehler *et al.* (1982) and Weber (1987a) proposed the oxidative synthesis of peptides and polyesters, respectively. Schwartz and Orgel (1985) also suggested that nucleoside dithiol derivatives might undergo template-directed oxidative polymerization. As a prebiotic model of oxidative polymer synthesis we here describe the oxidative polymerization of 2,3-dimercapto-1-propanol by ferric ions on the surface of iron(III) hydroxide oxide that yields polydisulfide oligomers.

Iron(III) hydroxide oxide was selected as the oxidant because it has been shown to readily oxidize aldehydes to carboxylic acids (Weber, 1992); and ferric ions in solution have been shown to oxidize thiols to disulfides (Wallace, 1966). Ferric ions needed for the formation of Fe(OH)O on the early Earth could have been continually generated by photooxidation of dissolved ferrous ions (Getoff *et al.*, 1960; Braterman *et al.*, 1983; Mauzerall *et al.*, 1993). The prebiotic synthesis of 2,3-dimercapto-1-propanol has not been studied; however, related thiols have been synthesized under prebiotic conditions. In general hydrogen sulfide is readily incorporated into organic compounds synthesized under prebiotic conditions (Raulin, 1978 and ref. therein). The oxidative formation of polydisulfides from dithiols

has been reviewed by Spassky *et al.* (1992). Recently, polydisulfide polymers have been used in batteries as solid redox polymerization electrodes (Liu *et al.*, 1991; Doeff *et al.*, 1992).

2. Materials and Methods

A. MATERIALS

Racemic 2,3-dimercapto-1-propanol was obtained from Fluka; ferric chloride and methanesulfonic acid from Sigma; sodium borohydride and Extract-Clean SCX cation exchange columns from Aldrich; synthetic goethite (iron(III) hydroxide, alpha 99%) from Johnson/Matthey; ampules (1–10 ml vacules) from Scientific Products.

Synthetic iron(III) hydroxide oxide (Fe(OH)O) was prepared by adding 0.5 ml aliquots of 2 M sodium hydroxide (13.8 ml total) to a 5 ml solution of 2 M ferric chloride in a 50 ml centrifuge tube agitated on a vortex mixer. When the pH 6 was reached the precipitate was isolated by centrifugation, washed three times with 10 ml water, and dried overnight in a desiccator in vacuo over phosphorus pentoxide. The yield was 945 mg (Weber, 1992). The elemental analysis described in Weber (1992) indicates that the preparation is primarily Fe(OH)O containing a small amount of Fe(OH)O · Cl_n. Electron diffraction of this preparation showed a ring pattern characteristic of a fine grain size polycrystalline specimen; however, we could not identify the crystal type from the ring pattern. The binding isotherm of glycerate on synthetic (Fe(OH)O showed maximal adsorption at 1.0 μmol glycerate per 9.7 μmol Fe(OH)O. If we assume that each glycerate binds to one surface ferric ion, then about 10% of the ferric ions of synthetic Fe(OH)O are on the surface. This conclusion is reinforced by the observation that only 8% of the ferric ions are rapidly reduced by glyceraldehyde (Weber, 1992).

B. CHROMATOGRAPHIC ANALYSIS

The polydisulfide oligomers of 2,3-dimercaptopropanol were separated by high pressure liquid chromatography (HPLC) using an Alltech Carbohydrate OH-100 column (250 × 4.6 mm) eluted with acetonitrile – water (93 : 7 v/v) at a rate of 1.0 ml/min. The absorbance of the eluent at 220 nm and 280 nm was recorded as a function of time. Figure 1 shows a typical chromatogram. The Alltech OH-100 column separates the polydisulfide oligomers by their chain length, because the number of hydroxyl groups increases with the oligomer length, and the affinity of the Alltech column is stronger for substances with a greater number of hydroxyl groups. This hydroxyl group-based separation also applies to alditols and carbohydrates as described in an Alltech technical bulletin on the OH-100 column.

C. OXIDATIVE POLYMERIZATION OF 2,3-DIMERCAPTO-1-PROPANOL BY IRON(III) HYDROXIDE OXIDE

In a typical reaction 20 mg of synthetic iron(III) hydroxide oxide and 4.9 ml of

sterile water was placed in a 10 ml ampule (vacule) that had been sterilized with ethanol. While the ampule was partially immersed in an ultrasonic bath, the air was removed by cycling four times between a vacuum and a source of nitrogen. Then 100 μl of 50 mM 2,3-dimercapto-1-propanol was added to the ampule. The deaeration procedure was repeated and the ampule was sealed in vacuo. The reactions were carried out in the dark at 40 °C at pH 4.0. All reactions used 5 μmoles of dithiol and 160 μmoles of $\text{Fe}(\text{OH})\text{O}$ (20 mg) of which about 8% reacts rapidly as an oxidant (Weber, 1992). This translates to 2.5 μmoles of active iron (III) oxidant per 1.0 μmole of dithiol reductant.

At the end of the reaction the soluble reaction products were separated by centrifugation from insoluble products including $\text{Fe}(\text{OH})\text{O}$. A 60 μl aliquot of the supernatant containing the soluble products was placed on an Extract-Clean cation exchange column. The products were eluted with 1.0 ml of acetonitrile. This column treatment was used to remove iron cations and to prevent further oxidation of thiol groups by acidifying the reaction solution. Finally the eluent was concentrated to 350 μl under a stream of nitrogen, and a 40 μl aliquot was analyzed by HPLC. The insoluble fraction containing $\text{Fe}(\text{OH})\text{O}$ and insoluble oligomers was extracted with 350 μl of acetonitrile three times, and the extract was passed through an Extract-Clean cation exchange column. The eluent was concentrated to 350 μl under a stream of nitrogen, and a 40 μl aliquot was analyzed by HPLC.

D. CHARACTERIZATIONS OF POLYDISULFIDE PRODUCTS

Several types of evidence support the identification of the Peaks in Figure 1 as an ascending series of oligomers from the 1-mer to the 15-mer. First, a time course study of the oxidation of 2,3-dimercaptopropanol by molecular oxygen showed that the disappearance of the dithiol was accompanied by a sequential appearance of peaks identified as the 2-mer, 3-mer, 4-mer and 5-mer. This oxidation was accompanied by an increase in the absorbance at 250 nm where alkyl disulfides have a characteristic absorption peak (Calvin and Barltrop, 1952). Furthermore, chemical reduction of the isolated 3-mer, 4-mer, 5-mer and 6-mer yielded the expected series of shorter oligomers. Polydisulfide oligomers from the $\text{Fe}(\text{OH})\text{O}$ oxidation reaction with retention times of 3.65 min (3-mer), 3.86 min (4-mer), 4.10 min (5-mer), and 4.25 min (6-mer) were each collected from the HPLC column. The volume of each sample was adjusted to 700 μl . A 350 μl aliquot of each solution was acidified by adding 2.0 μl of methanesulfonic acid and 30 μl was analyzed by HPLC as a reduction control. The remaining 350 μl of each solution was chemically reduced by reacting for 30 min with 1.2 μl of freshly prepared aqueous sodium borohydride (3 mg/ml). This solution was then acidified with 2.0 μl of methanesulfonic acid and 30 μl was analyzed by HPLC. No change was observed in the controls. Sodium borohydride reduction of each oligomer generated only oligomers of smaller size, including the 2-mer and monomer with retention times of 3.47 min and 3.31 min, respectively. The oligomers are presumed to contain both D- and L-enantiomers of 2,3-dimercaptopropanol because racemic 2,3-dimercaptopropanol was used in

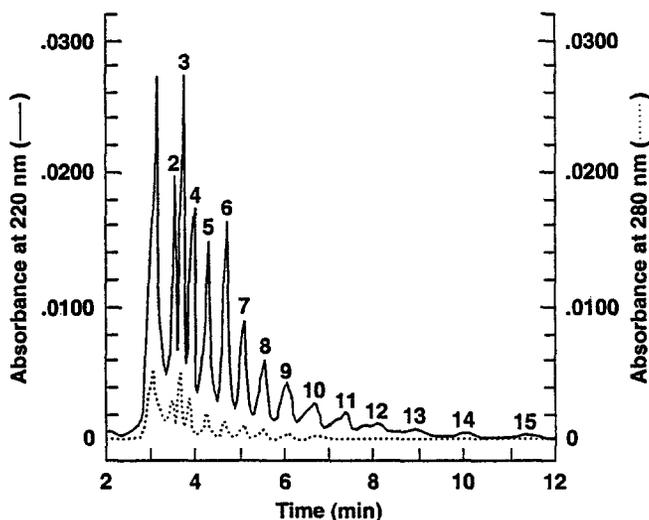


Fig. 1. High pressure liquid chromatographic separation of the insoluble polydisulfide oligomers from the oxidation of 1mM 2,3-dimercaptopropanol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 μ mole Fe) for 3 days under anaerobic conditions at 40 °C and pH 4. The numbers over the peaks indicate the chain length of the oligomers.

the polymerization. The percentage of [2-3]-linked, and [3-3]-linked disulfide isomers present in the oligomeric product was not measured.

3. Results and Discussion

Figure 1 shows the HPLC separation of the insoluble polydisulfide polymers from the oxidation of 1mM 2,3-dimercaptopropanol by ferric ions on the surface of iron(III) hydroxide oxide for 3 days at 40 °C and pH 4. As shown this reaction using 1mM dithiol yielded oligomers up to the 15-mer with the trimer having the highest yield. This insoluble fraction contained about 9% of the total oligomeric products; the remaining 91% of the oligomers were soluble. The soluble fraction was made up of the trimer through hexamer with negligible amounts of shorter or longer oligomers. Controls under nitrogen without Fe(OH)O showed no significant polymer synthesis. Substitution of oxygen for Fe(OH)O yielded oligomers above pH 6, but not at pH 4. This pH effect is consistent with the general stability of thiols towards oxidation under acidic conditions (Capozzi and Modena, 1974).

Figure 2 compares the percent yield of polydisulfide oligomers from 1mM and 10mM dithiol. All reactions were carried out at 40 °C, pH 4, and used 5 μ moles of dithiol and 160 μ moles of Fe(OH)O (20 mg). About 8% of the ferric ions in Fe(OH)O were previously shown to react rapidly as an oxidant (Weber, 1992). This translates to roughly 2.5 μ moles of active iron(III) oxidant per μ mole of dithiol reductant – a ratio that is slightly higher than the 2.0 iron(III) ions needed to

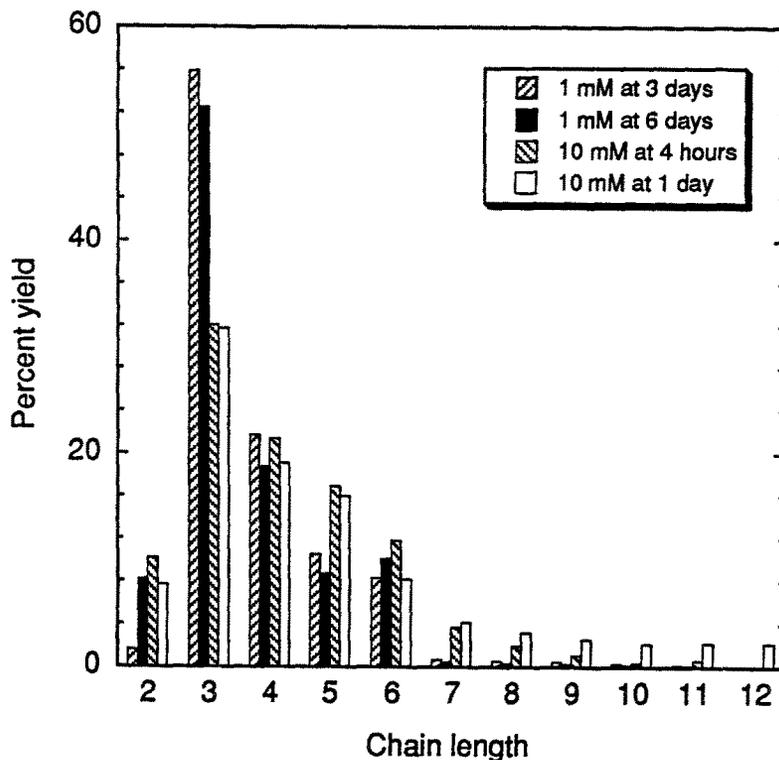


Fig. 2. Percent yield of polydisulfide oligomers from 1 and 10 mM solutions of 2,3-dimercaptopropanol in 5 ml and 0.5 ml of water, respectively, oxidized by iron(III) hydroxide oxide (20 mg, 160 μ mole Fe) under anaerobic conditions at 40 $^{\circ}$ C and pH 4. The percent yields were calculated from the HPLC separation monitored at 220 nm by dividing the area of each oligomer peak by the total of the area of all the soluble and insoluble oligomer peaks. The molar extinction coefficient at 220 nm of the disulfide groups was assumed to be the same for all polydisulfide oligomers.

oxidatively incorporate each dithiol monomer. As shown in Figure 2 the oxidative polymerization of 10 mM dithiol gave a higher yield of the larger oligomers than the 1 mM reaction. The oligomers from the 10 mM reaction were also more insoluble. The yield of insoluble polymer from 1 mM dithiol at 3 days and 6 days, and from 10 mM dithiol at 4 hours and 1 day were 9%, 7%, 45% and 46%, respectively. Figure 2 also shows that the polymerization of 1 mM dithiol was complete at 3 days. However, the 10 mM reaction was not complete at 4 hours, since the yield of the larger oligomers was greater in the 1 day reaction.

The polymerization of 10 mM dithiol was also examined using synthetic goethite (α -Fe(OH)O) as an oxidant at pH 6 for 2 days. Both synthetic goethite and our Fe(OH)O preparation yielded oligomers up to the 13-mer under these conditions, and gave similar distributions of insoluble oligomers (respective yields as a percent of total insoluble product: 2-mer, 2.6(2.3); 3-mer, 15.6(15.2); 4-mer, 21.9(17.1); 5-mer 25.5(22.7); 6-mer 14.0(14.9); 7-mer 8.3(12.0); 8-mer 4.9(5.0); 9-mer 2.7(4.2);

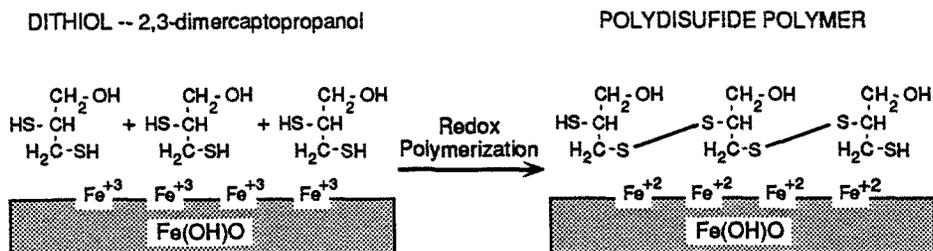


Fig. 3. Oxidative polymerization of 2,3-dimercaptopropanol on the surface of iron(III) hydroxide oxide.

10-mer 2.5(3.9); 11-mer 1.3(1.6); 12-mer 0.7(0.9)). HPLC analysis of insoluble products from other reactions above pH 5 frequently showed an anomalous increase in the relative yield and complexity of products in the 10-12-mer region of the chromatogram. This phenomena requires further study. It could be a result of intrastrand disulfide interchange in large oligomers that generates circular polydisulfide polymers (Burns and Whitesides, 1990).

4. Prebiotic Significance

Figure 3 shows the oxidative formation of polydisulfide oligomers from 2,3-dimercaptopropanol on the surface of iron(III) hydroxide oxide. There are several aspects of this oxidative polymerization of dithiols that make it an attractive model of prebiotic polymerization at the earliest stage of the origin of life. First the chemistry seems favorable for a prebiotic reaction. The polymerization uses a simple three carbon monomer that polymerizes readily at low concentration to give reasonably large oligomers under mild conditions. Moreover, water does not interfere chemically in the reaction. Second, the fact that modern life obtains its energy from redox reactions (de Duve, 1991a) suggests that redox reactions could have provided the energy for the earliest type of polymer synthesis involved in the origin of life. In this context it is important to stress that phosphoanhydrides are not a biological energy source, they are simply an energy carrier that is cyclically used to distribute energy from redox reactions to other cellular reactions.

The photocatalytic oxidation of hydrogen sulfide by colloidal semiconductor particles suggests that similar semiconductor particles could have photooxidized dithiols to polydisulfide polymers under prebiotic conditions (Fox and Dulay, 1993; Kamat, 1993; Reber and Meier, 1984). Furthermore, the photoreduction of protons to hydrogen (and carbon dioxide to organic carbon) that accompanies the oxidation of hydrogen sulfide by semiconductor particles suggests that similar substances could have photoreduced carbon dioxide to give organic dithiols under prebiotic conditions using electrons from hydrogen sulfide. Possible photocatalysts that could have been involved in prebiotic dithiol synthesis and polymerization are α -iron(III) hydroxide oxide, partially reduced α -iron(III) hydroxide oxide, iron(III) zinc oxide, zinc oxide, and zinc sulfide - substances that have been shown to photoreduce protons to

hydrogen, and carbon dioxide to organic compounds using electrons from water or hydrogen sulfide (Lu and Li, 1992; Khader *et al.*, 1987; Reber and Meier, 1984; Inoue *et al.*, 1979).

The photocatalytic model described above is similar to earlier proposals describing the photoreduction of carbon dioxide (Granick, 1957, 1965; Halmann *et al.*, 1981), except that it uses hydrogen sulfide as a source of electrons instead of water, and proposes a direct coupling of prebiotic polymer synthesis to the photocatalytic process. The use of hydrogen sulfide instead of water as a source of electrons dramatically lowers energy requirement for the reduction of carbon dioxide to formaldehyde (standard free energy is 118 kcal/mol for water, and 18 kcal/mol for hydrogen sulfide, Thauer *et al.*, 1977). Furthermore, the redox chemistry of the model allows it to be easily adapted to oxidize aldehyde hemithioacetals to thioesters – an advance that would open the door to group transfer reactions required for the synthesis of phosphoanhydrides, polypeptides, and polynucleotides (Weber, 1981, 1984a, b, 1987b; de Duve, 1991b). Since very little is known about the structural requirements of molecular self-replication, there is no reason to exclude the possibility that polydisulfide polymers could have functioned as early autocatalysts capable of rudimentary self-replication.

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