THERMAL SYNTHESIS AND HYDROLYSIS OF POLYGLYCERIC ACID

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(Received 10 November, 1987)

Abstract. Polyglyceric acid was synthesized by thermal condensation of glyceric acid at 80° in the presence and absence of two mole percent of sulfuric acid catalyst. The acid catalyst accelerated the polymerization over 100-fold and made possible the synthesis of insoluble polymers of both L- and DL-glyceric acid by heating for less than 1 day. Racemization of L-glyceric acid yielded less than 1% D-glyceric acid in condensations carried out at 80°C with catalyst for 1 day and without catalyst for 12 days. The condensation of L-glyceric acid yielded an insoluble polymer much more readily than condensation of DL-glyceric acid. Studies of the hydrolysis of poly-DL-glyceric acid revealed that it was considerably more stable under mild acidic conditions compared to neutral pH. The relationship of this study to the origin of life is discussed.

1. Introduction

In an effort to understand the origin of metabolism, we have studied for the past several years chemical models of glycolysis (Weber 1981, 1982, 1984ab, 1985). These model studies showed the conversion of glyceraldehyde and a thiol to activated monomers – lactoyl, glyceroyl and alanyl thioesters – which have the energy needed for condensation to polyesters and polypeptides (Weber and Orgel, 1979; Weber 1987a). This ability of glyceraldehyde to act as a source of activated monomers, which possess both the energy and matter needed for polymer synthesis, provided the central idea of a new hypothesis of the origin of life – called the triose model (Weber 1987b). In its simplest form this model proposes that formaldehyde in the prebiotic environment was converted into glyceraldehyde which then formed hemiacetal adducts with itself that were oxidized to polyglyceric acid. Polyglyceric acid, in turn, acted on the reactions involved in its synthesis as an autocatalyst with a rudimentary replicating ability. We now report the thermal synthesis and hydrolysis of polyglyceric acid as a first step in assessing the proposed role of this polyester in the origin of life (Weber, 1987b). Thermal treatment of glyceric acid had been reported previously to yield a water insoluble glyceric acid 'anhydride' (Wagner, 1878).

2. Experimental

2.1. MATERIALS

Glyoxylate reductase (D-glycerate dehydrogenase activity – 525 units/ml), glutathione, ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)-aminomethane (Tris), β-

Origins of Life and Evolution of the Biosphere **19** (1989) 7–19.

nicotinamide adenine dinucleotide (β–NAD grade V-C), p-nitrophenol, tetraethylammonium hydroxide (20% aqueous solution), Dowex 50-X4 resin (hydrogen form), L-glyceric acid (hemicalcium salt•$2H_2O$), D-glyceric acid (hemicalcium salt•$2H_2O$), and DL-glyceric acid (hemicalcium salt•$2H_2O$) were purchased from Sigma Chemical Co.; L-[^{14}C(U)]serine from New England Nuclear; sodium nitrite and dimethyl sulfoxide (anhydrous) from Aldrich Chemical Co.; Bio-Rad AG-1-X8 resin from Bio-Rad Laboratories; methyl red from Matheson Coleman and Bell, and centrex microfiltration units (nylon filter, 0.2μm pore size) form Schleicher and Schuell.

L-Glyceric acid (hemicalcium salt–$2H_2O$) was recrystallized (Baer *et al.*, 1939) three times from water-ethanol ($[\alpha]_D^{20}$ - 14.5°, C = 1.25, H_2O; lit. $[\alpha]_D^{20}$ – 14.6°, C = 5, Windholz and Budavari, 1983). D-Glyceric acid (hemicalcium salt•$2H_2O$) was recrystallized once from water-ethanol ($[\alpha]_D^{20}$–14.7°, C = 1.25, H_2O; lit. $[\alpha]_D^{20}$–14.6°, C = 5, Windholz and Budavari, 1983). DL-Glyceric acid (hemicalcium salt•$2H_2O$) was not recrystallized ($[\alpha]_D^{20}$ 0.2°, C = 1.25, H_2O). The hemicalcium salts of L-, D-, and DL-glyceric acid were converted to the free acid form by passing the salts through a column containing the hydrogen form of Dowex 50W-X4 resin (Weber, 1987a). L-[^{14}C(U)]Glyceric acid (25 mCi/mmole) was prepared from L-[^{14}C(U)]serine by a modified version of the method of Lok *et al.* (1976) as described in Weber (1987a). Since impurities in this preparation inhibited the sulfuric acid-catalyzed polymerization of L-glyceric acid, it was further purified on a column containing Bio-Rad AG-1-X8 resin (formate) which was eluted with water, 0.01 M formic acid and 0.10 M formic acid. This preparation was then passed through a column of Dowex 50-X4 resin (hydrogen form) eluted with water to insure that the glyceric acid was in the acid form.

2.2. CHROMATOGRAPHY AND ELECTROPHORESIS

Paper chromatography was carried out by descending elution on Whatman 3MM paper with n-butanol-formic acid-water (8:1:1, v/v/v). Mobilities relative to glyceric acid (Glc) were diglyceric acid $(Glc)_2$, 0.74; triglyceric acid $(Glc)_3$, 0.50; tetraglyceric acid $(Glc)_4$, 0.32; larger than tetraglyceric acid $(Glc)_{>4}$, 0–0.48; glyceric acid hydroxamate, 0.55. High-voltage paper electrophoresis on Whatman 3MM paper used a buffer of 0.03 M potassium phosphate (pH 7.1). Mobilities relative to Glc were $(Glc)_2$, 0.77; $(Glc)_3$, 0.62;

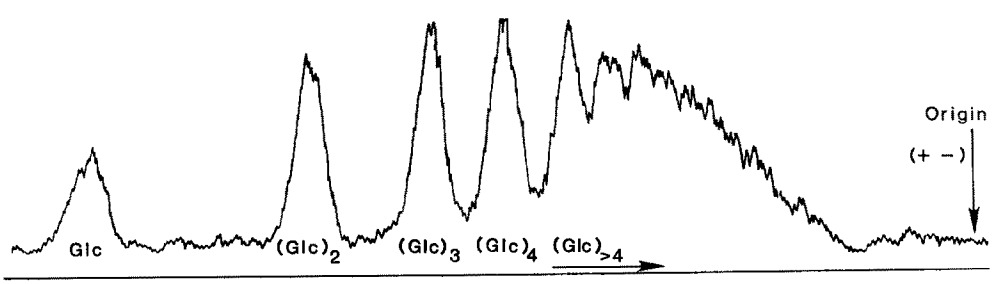

Fig. 1. Radioscan of the electrophoretogram of the soluble oligomers produced by heating desiccated L-[^{14}C]glyceric acid. Section 2.3 describes experimental procedure.

$(Glc)_4$, 0.52; $(Glc)_{>4}$, 0–0.48; glyceric acid hydroxamate, 0.10. Products derived from L-[^{14}C]glyceric acid were estimated by running the chromatograms and electrophoretograms through a Baird RSC-363 radiochromatographic scanner with integrator. Figure 1 shows a typical radioscan of the electrophoretogram of oligoglyceric acids synthesized by heating glyceric acid. Radioactive products were identified by co-chromatography with commercially available standards, whenever possible. Organic acids were detected on chromatograms by spraying with methyl red (Lederer and Lederer, 1957).

2.3. THERMAL SYNTHESIS OF POLYGLYCERIC ACID

Ten microliter aliquots of a solution of 0.25 M L-glyceric acid and 8 μCi/ml L-[^{14}C]glyceric acid with and without 0.005 M sulfuric acid as a catalyst were added to 6 × 50 mm tubes that had been cut in half. The reaction solutions were dried 24 h over phosphorus pentoxide in a desiccator which was evacuated with a water aspirator. These tubes were then placed in larger 10×70 mm tubes in a heating block at 80°C. The tubes were swept with nitrogen during the thermal condensation and stored at -80°C until analyzed. Polymerization of DL-glyceric was carried out in similar manner with labeled L-[^{14}C]glyceric acid. For analysis, water and 0.05 M sodium bicarbonate were added to each tube to give a final volume of 60 μl and a pH of 5.5–6.0. After vigorous mixing, the solids were centrifuged to the bottom of each tube. A 35 μl aliquot of the supernatant which contained soluble oligoglyceric acids was subjected to high voltage electrophoresis. The amount of insoluble polyglyceric acid present in each tube was calculated from the radioactivity of an aliquot of this supernatant and of a second aliquot of the solution after alkaline hydrolysis of the insoluble polymer.

2.4. CHARACTERIZATION OF POLYGLYCERIC ACID

The infrared spectrum of polyglyceric acid by the KBr method exhibited absorption maxima characteristic of a polyester possessing free hydroxyl groups (IR maxima: C = 0 stretching at 1750 cm^{-1}, C–O stretching at 1205 cm^{-1} and 1125 cm^{-1}, O–H stretching at 3400 cm^{-1} (broad band) (Nakanishi, 1962). The stability of the polymer in weak acid and its rapid alkaline hydrolysis to give only glyceric acid indicates a polyester structure composed of glyceric acid residues (Euranto, 1969). This view is reinforced by the pattern of products of regularly decreasing mobility seen in electrophoretograms of glyceric acid condensation reactions (see Figure 1). This pattern is consistent with that of a series of glyceric acid oligomers whose mobility decreases with each incremental increase in chain length, since the oligomers have only one negative charge at the carboxylate terminus. This interpretation is supported by a) the continuous conversion of labeled glyceric acid into increasingly larger oligomers during the thermal condensation (see Figures 2 and 3), and b) the reverse movement of label into smaller oligomers that occurs during hydrolytic degradation of polymer at pH 7. Also the chain length of $(Glc)_2$ was confirmed by reaction of its ester bond with alkaline hydroxylamine (Hestrin, 1949; Jencks *et al.*, 1960) that gave a glyceric acid hydroxamate/glyceric acid ratio of 0.82

(theory 1.00). Similar cleavage of $(Glc)_3$ and $(Glc)_4$ yielded glyceric acid hydroxamate/glyceric acid ratios respectively of 1.7 (theory 2.0) and 2.8 (theory 3.0) when calculated assuming the hydroxylaminolysis efficiency of 82% of the $(Glc)_2$ reaction.

2.5. DETERMINATION OF THE AVERAGE CHAIN LENGTH OF POLYGLYCERIC ACID BY SPECTROPHOTOMETRIC TITRATION OF ITS CARBOXYLIC ACID END GROUP

Polyglyceric acid was titrated in 80% DMSO-20% water because the homochiral polymer was much more soluble in this solvent compared to water. p-Nitrophenol was used as the indicator in the spectrophotometric titration, since its pK_a of 8.52 in the DMSO-water solvent is high enough to permit the titration of carboxylic acid end groups that are estimated to have a pK_a of about 6 in the DMSO-water solvent (Georgieva *et al.* 1977; Georgieva *et al.*, 1980; Goddu and Hume, 1954).

Polyglyceric acid was prepared as described in Section 2.3 in reaction tubes that contained 2.5 μmoles of radioactive [^{14}C]glyceric acid. The polymeric residue in each of two reaction tubes was dissolved in 70 μl of DMSO and the solutions were combined. Eighty micoliters of DMSO and 55 μl of water were added to this solution to give a final 80% DMSO-20% water solution (275 μl). A 250 μl aliquot of this solution was added to a cuvette containing 250 μl of 0.0025 M p-nitrophenol in 80% DMSO-20% water. The absorbance of this solution was recorded at 470 nm against a reference cuvette that contained 500 μl of 80% DMSO-20% water after each addition of the titrant – 0.002 M tetraethylammonium hydroxide in 80% DMSO-20% water. The solution was mixed with a magnetic stirrer after each addition. The end point of each titration was graphically determined from a plot of the absorbance at 470 nm (corrected for dilution error) as a function of the amount of titrant (Goddu and Hume, 1954). Titration of standard amounts of L-glyceric acid (0.15–0.75 μmole) was used to construct a standard curve of L-glyceric acid (μmoles) versus titrant (μmoles). This plot was linear with a slope (glyceric acid/titrant) of 0.91 that intersected at 0.06 μmoles on the glyceric acid axis. Likewise, titration of glyceric acid in the presence of 0.1 μmole sulfuric acid gave a linear standard curve with a slope (glyceric acid/titrant) of 0.87 that intersected at –0.075 μmoles on the glyceric acid axis. These standard curves were used to convert the amount of base at a titration end point into the corresponding amount of carboxylic acid end groups in the titrated polymer. The average chain length of the polymer was then calculated by dividing this carboxylic acid end group value into the total amount of [^{14}C]glyceric acid residues measured by their radioactivity.

2.6. RACEMIZATION ASSAY

Forty microliter aliquots of a solution of 0.5 M L-glyceric acid and 8 μCi/ml L-[^{14}C]-glyceric acid with and without 0.01 M sulfuric acid as a catalyst were dried and heated at 80°C as described in Section 2.3. Heating was carried out in 10×70 mm tubes for 1 day with catalyst and 12 days without catalyst. Then thirty-six microliters of water and 25 μl

of 1.0 M NaOH were added and the polymeric residue hydrolyzed for 2 h at ambient temperature with ultrasonic agitation. Hydrolysis was ended by addition of 8 µl of 1.0 M HCl. A 65 µl aliquot of this solution was assayed for D-glycerate.

A modified version of the D-glycerate dehydrogenase assay described by Sallach (1966) was used to measure D-glycerate. In this assay D-glycerate was measured by the increase in absorbance at 340 nm that is due to the formation of NADH from the oxidation of D-glycerate by NAD^+ catalyzed by glyoxylate reductase (D-glycerate dehydrogenase). The assay was performed by adding to a microcuvette – (a) 220 µl of stock buffer that contained 0.50 M Tris-chloride (pH 9.0), 0.40 M hydrazine sulfate (pH 9.0) and 0.025 M EDTA (pH 9.0), (b) 20 µl of 0.05 M glutathione, (c) 65 µl of the solution being assayed, (d) 105 µl of water, (e) 20 µl of enzyme (5.0 µl of glyoxylate reductase in 100 µl of sodium phosphate (pH 7)), and (f) 20 µl of 0.40 M β-NAD. The solution was mixed by inverting the cuvette and the absorbance was recorded against a water blank at one minute intervals over 9 minutes with zero time considered the time addition of β-NAD. The change in absorbance from 1 to 9 min at 340 nm in condensations with sulfuric acid was: 0.045 for the unheated but desiccated control, 0.048 for the reaction heated at 80°C (24 h), 0.066 for a similarly heated reaction containing 1% added D-glyceric acid, and 0.084 for a similarly heated reaction containing 2% added D-glyceric acid. Likewise, the absorbance change from 1 to 9 min in condensations without sulfuric acid was: 0.048 for the unheated but desiccated control, 0.054 for the reaction heated at 80°C (12 days), and 0.074 for a similarly heated reaction with 1% added D-glyceric acid. These measurements indicate that racemization from heating yielded less than 1% D-glyceric acid, since the absorbance difference between the heated reactions and their unheated controls (0.003 with H_2SO_4, and 0.006 without H_2SO_4) is less than the absorbance increase brought about by adding 1% D-glyceric acid to the heated reactions (0.018 with H_2SO_4, and 0.020 without H_2SO_4).

2.7. SOLUBILITY OF POLY-L-GLYCERIC ACID

Ten microliter aliquots of a solution of 0.25 M L-glyceric acid, 6 µCi/ml L-[^{14}C]glyceric acid, and 0.005 M sulfuric acid were dried and heated (1h, 5h, 24h) at 80°C as described in Section 2.3. One-hundred and six microliters of each solvent tested was added to the polymeric residue in each tube and vigorously mixed for 15 minutes. The insoluble polymer was centrifuged to the bottom of each tube and the amount of polymer in the supernatant was measured from the radioactivity of a 10 µl aliquot. The percent solubility was calculated by dividing these values for the various solvents by the total amount of polymer which was determined from similar radioactivity measurements after alkaline hydrolysis of the polymer.

2.8. HYDROLYSIS OF POLY-DL-GLYCERIC ACID

Fifty microliter aliquots of a solution of 0.25 M DL-glyceric acid, 6 µCi/ml L-[^{14}C]glyceric acid, and 0.005 M sulfuric acid were added to ten 10×70 mm tubes. These

solutions were dried and heated 5 h at 80°C as described in Section 2.3. The polymeric residue in each tube was dissolved in 50 µl of water and the solutions were combined. The pH of the resultant solution was adjusted to pH 5.5–6.0 with 1.0 M sodium bicarbonate. Fifty microliter aliquots were placed in 10 microdialysis cells (Horowitz and Barnes, 1983) that used Spectrapore #6 dialysis tubing (m.w. cut-off: 1000). Dialysis against water was carried out for 5 h. The retentates were combined, filtered through a centrex filtration unit (nylon filter, 0.2 µm pore size), and adjusted to pH 6.0 with 0.15 M NaOH. After the addition of water the preparation was 0.113 M in glyceric acid residues as measured by its radioactivity. The carboxylic acid end groups of this preparation were measured by titration of a 70.8 µl aliquot with 0.05 M HCl. The polymer preparation was estimated to have an average chain length of 20 residues from the ratio of glyceric acid residues (8 µmoles) to carboxylic acid end groups (0.4 µmoles, estimated pKa = 3.1).

Hydrolysis of 40 mM poly-DL-glyceric acid (200 µl) was carried out at 25°C in 80 mM sodium phosphate (pH 7.0), 80 mM sodium succinate (pH 6.0), 80 mM sodium acetate (pH 5.0), and 40 mM sodium glycerate (pH 4.0). At timed intervals the carboxylic acid end groups released by hydrolysis were measured by titrating the pH back to its original value with 0.1–0.25 M sodium hydroxide. In each reaction the pH was maintained within ± 0.1 pH unit of its initial value. The percent of ester linkages unhydrolyzed was calculated by subtracting the µmoles of carboxylic acid end groups released by hydrolysis from the µmoles of glyceroyl esters initially present in the reaction solution. The initial amount of glyceroyl esters (7.6 µmoles) was calculated by subtracting the one glyceric acid termini (0.4 µmoles) that exists for every 20 glyceric acid residues (8.0 µmoles) in the hydrolysis solution. In Figure 4 the logarithm of the percent ester linkages unhydrolyzed is plotted as a function of time.

3. Results

Figures 2(a) and 2(b) show the thermal condensation of respectively L- and DL-glyceric acid in the dry state at 80°. As depicted, the first products were short glyceric acid oligomers – the dimer through tetramer $(Glc)_{2-4}$. Some of $(Glc)_{2-4}$ was even produced at ambient temperature during the drying process (time 0 to 0′). At 2 days the major condensation products were soluble glyceric acid oligomers larger than the tetramer $(Glc)_{>4}$. From 2–12 days L-glyceric acid was steadily converted to insoluble poly-L-glyceric acid (poly Glc); however, DL-glyceric did not yield an insoluble polymer under these reaction conditions. This solubility difference occurred even though the average chain length of the DL-polymer (7 and 14 residues at respectively 4 and 10 days) is equal to or larger than the length of the L-polymer (6 and 8 residues at respectively 4 and 10 days). In these studies the measurement of insoluble polymer was carried out at pH 5.5–6.0. Alkaline hydrolysis of the polymer from these thermal reactions yielded only glyceric acid, and the yield of D-glyceric acid from racemization of L-glyceric acid at 12 days of reaction was less than 1%.

As shown in Table 1, prior desiccation was not required for thermal condensation of L-glyceric acid. Simply heating solutions of L-glyceric acid at 60°C or 80°C under a stream

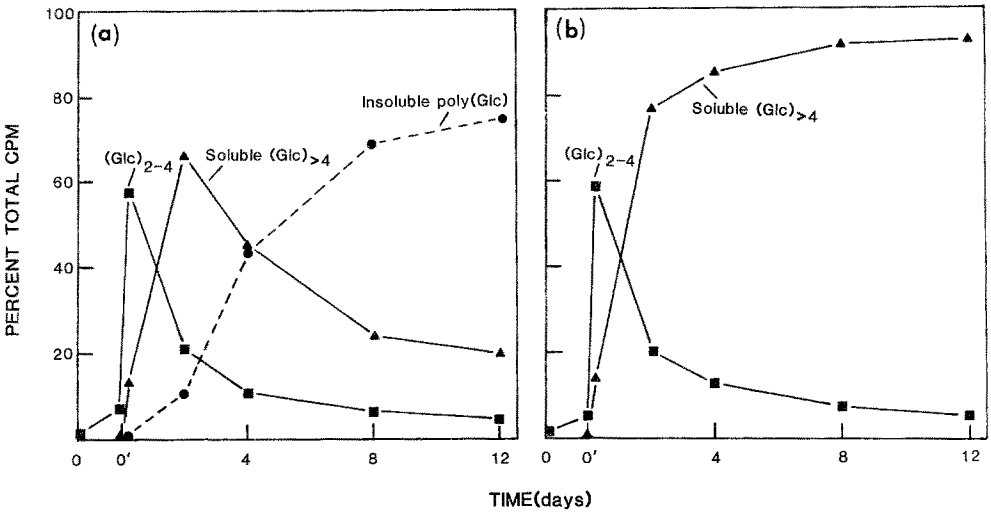

Fig. 2. Thermal polymerization of (a) L-glyceric acid and (b) DL-glyceric acid in the dry state at 80°C. The values at time (0) and time (0') were obtained respectively before and after desiccation at ambient temperature. The 80°C reaction was started at time (0').

TABLE I

Polymerization of L-glyceric acid by heating 0.20 M L-[^{14}C]-glyceric acid under a nitrogen stream.

Temp.	Time (days)	Percentage of total cpm				
		Glc	$(Glc)_2$	$(Glc)_3$	$(Glc)_4$	$(Glc)_{>4}$
60°C	0	100	–	–	–	–
	1	41.1	28.3	16.3	6.9	7.5
	2	23.3	22.0	19.7	14.0	21.0
	4	12.1	13.3	13.5	12.9	48.3
	8	3.8	7.8	5.3	9.1	74.1
80°C	0	100	–	–	–	–
	0.2	57.0	27.1	10.9	3.6	1.3
	1	10.4	17.5	13.2	13.5	45.4
	2	3.2	7.5	8.8	9.4	71.2
	4	0.5	1.8	2.8	3.4	91.4
	8	0.6	1.4	1.7	1.3	95.0

of nitrogen brought about a fairly rapid polymerization of glyceric acid. Similar reactions were also carried out for 1 day at 110°C and 140°C. Although hydrolysis of the residue from the 110°C reaction product yielded only glyceric acid, hydrolysis of the 140°C reaction product yielded about 50% glyceric acid and 50% of an unidentified decomposition product.

Figures 3(a) and 3(b) show the acid-catalyzed thermal condensation of respectively L-

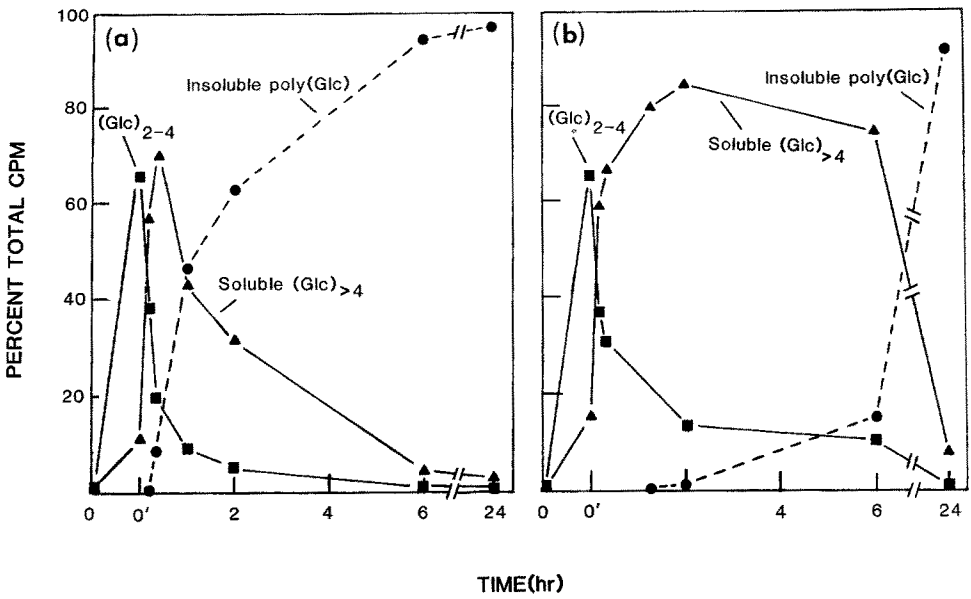

Fig. 3. Acid-catalyzed thermal polymerization of (a) L-glyceric acid and (b) DL-glyceric acid in the dry state at 80°C with 2 mole percent sulfuric acid. The values at time (0) and time (0') were obtained respectively before and after desiccation at ambient temperature. The 80°C reaction was started at time (0').

and DL-glyceric acid in the dry state at 80°C. The rate of these acid-catalyzed reactions was estimated to be about 140 times that of the uncatalyzed reactions shown in Figure 2(a)(b), since the conversion of L-glyceric acid to about 37% $(Glc)_{2-4}$ took 1 day in the uncatalyzed reaction and only 10 min in the acid-catalyzed reaction. Although the acid-catalyzed condensation of DL-glyceric acid yielded insoluble poly-DL-glyceric acid, its rate of formation was much slower than the acid-catalyzed formation of poly-L-glyceric acid. However, the average chain length of the DL-polymer (12 and 25 residues at respectively 2h and 4h) was appreciably larger than the chain length of the L-polymer (10 and 13 residues at respectively 2h and 4h). In this acid-catalyzed condensation the yield of D-glyceric acid from racemization of L-glyceric acid at 1 day of reaction was less than 1%.

We next studied the solubility of thermal polymers prepared at 80°C for 2 h from different ratios of D- and L-glyceric acid with the method described in Section 2.3. We found that when D-glyceric acid (D-Glc) in the reaction mixture was increased from 0% to 12.5% the yield of insoluble polymer decreased rapidly. The yields of insoluble polymer were 57% with 0% D-Glc; 34% with 2.5% D-Glc; 16% with 5% D-Glc; 7% with 7.5% D-Glc; 4% with 10% D-Glc; and 2.5% with 12.5% D-Glc.

We also examined the solubility of the acid form of poly-L-glyceric acid in several solvents. Polymers prepared by heating at 80°C for 1h, 5h, and 24h had solubilities respectively of 95%, 87% and 22% in dimethylsulfoxide; 85%, 19% and 5% in N,N'-dimethylformamide; 35%, 18% and 5% in water, formic acid and trifluoroacetic acid. All polymers were essentially insoluble in acetic acid, dichloroacetic

acid and trifluoroethanol. The relatively high solubility of poly-L-glyceric acid in dimethylsulfoxide is probably a result of the disruption of polymer-polymer hydrogen bonds by the formation of hydrogen bonds between the polymer and dimethylsulfoxide solvent (Martin *et al.*, 1967).

Figure 4 depicts the hydrolysis of water soluble poly-DL-glyceric acid at various pH values. As shown, poly-DL-glyceric acid became more unstable towards hydrolysis as the pH was raised from pH 4 to pH 7. This behavior of poly-DL-glyceric acid is consistent with the general dependence of ester hydrolysis on hydroxide ion concentration (Euranto, 1969). The slopes of the logarithmic plots in Figure 4 also show that the rate of polymer hydrolysis decreased during the reaction. If we assume pseudo first order kinetics for hydrolysis, then at the initial reaction rate was about seven times faster than the final rate. In addition, electrophoretic analysis of the products of the pH 7 reaction at 0.2 day gave a $(Glc)_2$ mole percent yield of 7.9% that was significantly greater than the yields of Glc-3.5%, $(Glc)_4$-3.7% and $(Glc)_3$-5.0%. As mentioned later these observations suggest the involvement of an intramolecular pathway in hydrolysis of poly-DL-glyceric acid.

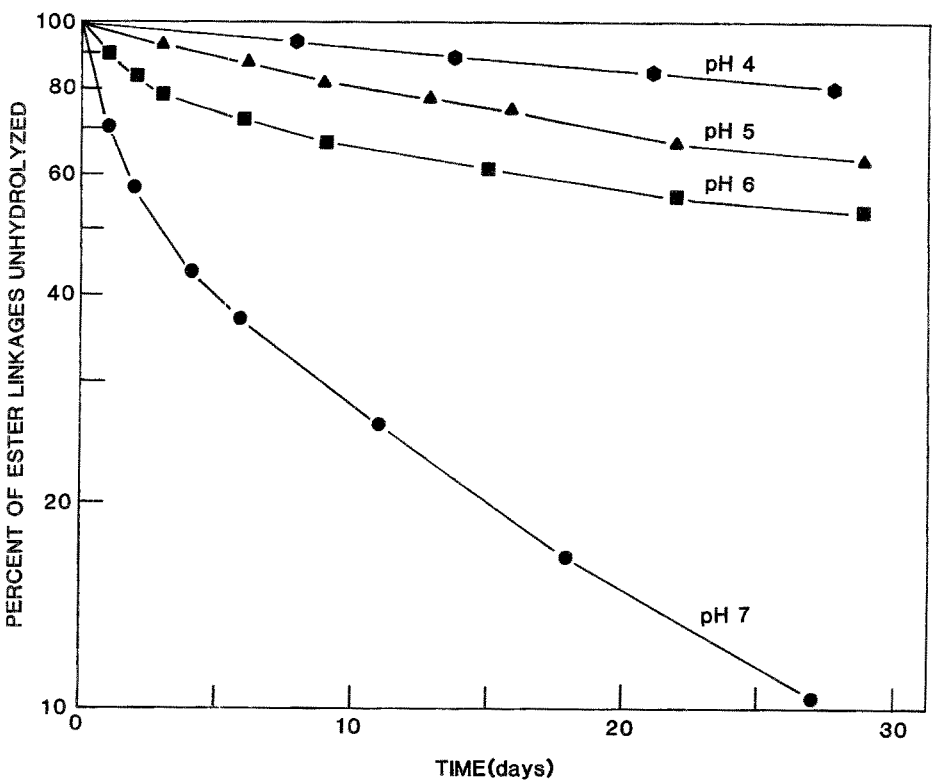

Fig. 4. Hydrolysis of 40 mM poly-DL-glyceric acid as a function of pH. The ordinate scale is logarithmic. Buffers used were 80 mM sodium phosphate (pH 7.0), 80 mM sodium succinate (pH 6.0), 80 mM sodium acetate (pH 5.0), and 40 mM sodium glycerate (pH 4.0).

4. Discussion

We found that the thermal condensation of glyceric acid at 80°C provides a simple, virutally racemization-free method for the synthesis of polyglyceric acid. This method allows us to prepare poyglyceric acid of different sizes and enantiomeric compositions for chemical studies of this polymer which may have functioned as an autocatalyst in the origin of life (Weber, 1987a; Weber, 1987b).

Our studies show that L-glyceric acid was converted much more rapidly to an insoluble polymer than racemic DL-glyceric acid. Our results indicate that this difference between the L- and DL-condensations is not due to L-glyceric acid polymerizing more rapidly than DL-glyceric acid, but rather, it is caused by the DL-polymer being more soluble than the L-polymer. This idea is supported by measurements of the average chain length of the L- and DL-polymers which show that the L-polymer is more insoluble than DL-polymer twice its size. For example, the L-polymer in Figure 2(a) at 10 days with an average chain length of 8 residues is 70% insoluble whereas, the DL-polymer in Figure 2(b) at 10 days with a length of 14 residues is completely soluble. Likewise, the L-polymer in Figure 3(a) at 4h with a length of 13 residues is 80% insoluble, but the DL-polymer in Figure 3(b) at 4h with a length of 25 residues is only 5% insoluble. Furthermore, the substantial decrease in the yield of insoluble polymer that occurs when a small amount of D-glyceric acid is added to a condensation of L-glyceric acid also reinforces the idea that polyglyceric acid containing both D and L residues is considerably more soluble than the homochiral polymer. This solubility behavior is not unusual, since generally the atacticity of racemic polymers decreases their crystallizability and increases their solubility compared to stereoregular optically active polymers (Bamford *et al.*, 1956; Grenier and Prud'homme, 1983). For example, poly-DL-alanine and poly-DL-serine are soluble in water, but their optically active counterparts poly-L-alanine and poly-L-serine are insoluble (Astbury *et al.*, 1948; Bohak and Katchalsky, 1963; Tooney and Fasman, 1968). Also the solubility of poly-L-serine is increased dramatically when a few percent of its residues are isomerized to the D-form (Bohak and Katchalsky, 1963). However, it is important to point out that polyglyceric acid is structurally more complex than these polypeptides, since it can be branched and have ester linkages involving α- and β-hydroxyl groups of glyceric acid residues. In addition, the acyl groups of glyceric acid residues probably can migrate between the vicinal α- and β-hydroxyls. In fact, equilibrium between the α- and β-positions is probably attained rapidly for polymers in solution at pH 7, since the analogous rate of migration of the acyl group of glycerol monoacetate($t_{1/2}$ = 12 min, pH 7, 37°C) is rapid and exceeds the rate of hydrolysis by more than a factor of 6000 (Wolfenden *et al.*, 1964). This equilibrium between the α- and β-positions is currently being studied in the model compound – glyceroylglyceric acid methyl ester. Our earlier calculations indicate that the β-ester linkage is preferred at equilibrium (Weber, 1987b).

Polyglyceric acid also showed unusual hydrolytic behavior that is characterized by an initial hydrolysis rate which is about seven times faster than the final rate. This unusual behavior suggests the involvement of two hydrolytic pathways. The first is the typical

intermolecular attack of water or hydroxide ion on oligomers of all sizes. The second is faster and occurs by intramolecular attack (transesterification) of the free α-hydroxyl group of a glycerol residue on an adjacent glyceroyl ester that yields a polymer fragment containing the hydroxyl leaving group and a second fragment terminated with a six-membered cyclic diester of glyceric acid that subsequently undergoes rapid hydrolysis at its labile cis-ester bonds (Huisgen and Ott, 1959; Bruice and Benkovic, 1966). This speculation on two pathways is consistent with the dramatic decrease in hydrolytic rate observed during poly-DL-glyceric acid hydrolysis, since the contribution of the rapid intramolecular pathway is expected to decrease during hydrolysis as reactive oligomers $\geq(Glc)_3$ are converted to less reactive $(Glc)_2$ and branched $(Glc)_3$ that cannot be hydrolyzed by the intramolecular pathway. The larger yield of $(Glc)_2$ compared to Glc also suggests involvement of intramolecular hydrolysis, since the intramolecular pathway can yield $(Glc)_2$ at both the hydroxyl and carboxylate termini, but formation of Glc is restricted to the carboxylate terminus.

Prebiotic Significance

Our studies show that glyceric acid polymerizes readily under drying conditions. The ease of polymerization, which occurs even at ambient temperature, suggests that this pathway may have yielded polyglyceric acid on the primitive Earth. However, the polymerization would have been restricted to moderately acidic environments, since it requires the acid form of glyceric acid ($pK_a = 3.55$, Jencks and Regenstein, 1976). An attractive alternative to this dehydration polymerization is the proposed oxidative synthesis of polyglyceric acid from glyceraldehyde that could occur in the presence of water (Weber, 1987b). Although the prebiotic synthesis of glyceric acid has not been studied, the related hydroxy acids – glycolic acid and lactic acid – have been shown to be formed in spark discharge experiments (Miller, 1955, 1957). Since these α-hydroxy acids were probably synthesized by hydrolysis of the nitriles produced by the Strecker addition of hydrogen cyanide to formaldehyde (glycolic acid) and acetaldehyde (lactic acid) (Miller and Van Trump, 1981), it seems likely that glyceric acid could have been synthesized by a similar pathway from hydrogen cyanide and glycolaldehyde. It is interesting to note that the relative yields of α-hydroxy acids and α-amino acids in the Strecker synthesis (pH 8, 25°C) is dependent on the ammonia concentration with the yield of α-hydroxy acid exceeding α-amino acids when the concentration of ammonia drops below 0.014 M for lactic acid-alanine, 0.043 M for glycolic acid-glycine, and 0.001 M for fumaric acid-aspartic acid (Miller and Van Trump, 1981).

The general insolubility of homochiral polymers, like poly-L-glyceric acid, compared to racemic polymers, like poly-DL-glyceric acid, suggests that the regions of homochirality in a racemic polymer could aggregate forming homochiral domains. On the primitive Earth this aggregation may have separated polymer strands with more homochiral regions from those with less. Furthermore, if the formation of soluble or insoluble aggregates had a selective advantage, such as increasing polymer stability or synthesis, then racemic polymers that have extensive regions of homochirality would

have accumulated over time on the primitive Earth. This process which selects racemic polymer with homochiral regions may have been a prerequisite for eventual selection of homochiral biopolymers. Although the selection of homochiral sequences has not been experimentally studied with racemic polymers, it has been shown that homochiral regions of poly-(D/L = 0.25)-Lys-Leu) can be enriched by selective hydrolysis, since the aggregation of the homochiral regions into islands of optically pure β-pleated sheets makes these regions more stable towards acid hydrolysis (Brack, 1987; Brack and Spach, 1979, 1980; Spach and Brack, 1979). Racemic poly-DL-(Lys-Leu) was also shown to contain about 4% of β-sheet which probably formed by aggregation of homochiral regions; however, this polymer was not used in selection experiments (Brack and Spach, 1979, 1980).

Our studies of the hydrolysis of polyglyceric acid indicate that the survival of polyglyceric acid in the prebiotic environment would be favored by weakly acidic conditions (pH < 6). Although the processes that control the ocean's pH are not well understood, it seems likely that if the primitive atmosphere was high in CO_2 as discussed by Kasting and Pollack (1984) and Walker (1985), the primitive ocean would have had a pH below 6. Furthermore, hydrolytic degradation of polyesters like polyglyceric acid is self-stabilizing, since it produces carboxylic acid groups that reduce the rate of hydrolysis by lowering the pH in the vicinity of the polymer. In the prebiotic environment the degree of self-stabilization would have depended on the buffering capacity and mixing rate of the aqueous solution in contact with the polymer. In general, polymer hydrolysis would have been detrimental to the origin of life if it was faster than polymer synthesis; however, if hydrolysis was slower than polymer synthesis it may have contributed to the origin of life by providing a selective pressure that favors the survival of the most stable strands of polymer. The ability to synthesize polyglyceric acid with different enantiomeric compositions should facilitate experimentation on the possible effect of chirality on hydrolysis, and examination of other properties of polyglyceric acid that are related to its proposed role in the origin of life (Weber, 1987a).

Acknowledgements

This study was supported by NASA Grant No. NSG-7627. I thank Esther Varon for technical assistance in this study.

References

Astbury, W. T., Dalgliesh, C. E., Darmon, S. E., and Sutherland, G. B. B. M.: 1948, *Nature* 162, 596–600.

Baer, E., Grosheintz, J. M., and Fisher, H. O. L.: 1939, *J. Am. Chem. Soc.* **61**, 2607–2609.

Bamford, C. H., Elliott, A., and Hanby, W. E.: 1956, *Synthetic Polypeptides*, Academic Press, New York, p. 312–334.

Bohak, Z. and Katchalski, E.: 1963, *Biochemistry* **2**, 228–237.

Brack, A.: 1987, *Origins of Life* **17**, 367–379.

Brack, A. and Spach, G.: 1979, *J. Mol. Evol.* **13**, 35–46.

Brack, A. and Spach, G.: 1980, *J. Mol. Evol.* **15**, 231–238.

Bruice, T. C. and Benkovic, S. J.: 1966, *Bioorganic Mechanisms*, Vol. I, W. A. Benjamin, Inc., New York, pp. 16–22.
Euranto, E. K.: 1969, in S. Patai (ed.), *The Chemistry of Carboxylic Acids and Esters*, Interscience Publishers, New York, pp. 505–588.
Georgieva, M., Velinov, G., and Budevsky, O.: 1977, *Anal. Chim. Acta* **90**, 83–89.
Georgieva, M., Zikolov, P., and Budevsky, O.: 1980, *Anal. Chim. Acta*, **115**, 411–415.
Goddu, R. F. and Hume, D. N.: 1954, *Anal. Chem.* **26**, 1740–1746.
Grenier, D. and Prud'homme, R. E.: 1983, *Macromolecules* **16**, 302–310.
Hestrin, S.: 1949, *J. Biol. Chem.*, **180**, 249–261.
Horowitz, P. M. and Barnes, L. D.: 1983, *Anal. Biochem.*, **128**, 478–480.
Huisgen, R. and Ott, H.: 1959, *Tetrahedron* **6**, 253–267.
Jencks, W. P., Cordes, S., and Carriuolo, J.: 1960, *J. Biol. Chem.*, **235**, 3608–3614.
Jencks, W. P. and Regenstein, J.: 1976, in G. D. Fasman (ed.), *Handbook of Biochemistry and Molecular Biology*, 3rd Edn. Physical and Chemical Data, Vol. 1, CRC Press, Cleveland, pp. 305–351.
Kasting, J. F. and Pollack, J. B.: 1984, *J. Atmos. Chem.* **1**, 403–428.
Lederer, E. and Lederer, M.: 1957, *Chromatography*, Elsevier, New York, p. 184.
Lok, C. M., Ward, J. P., and van Dorp, D. A.: 1976, *Chem. Phys. Lipids* **16**, 115–122.
Martin, D., Weise, A., and Niclas, H. J.: 1967, *Angew. Chem. Internat. Edit.* **6**, 318–334.
Miller, S. L.: 1955, *J. Am. Chem. Soc.* **77**, 2351–2361.
Miller, S. L.: 1957, *Biochim. Biophys. Acta* **23**, 480–489.
Miller, S. L. and Van Trump, J. E.: 1981, in Y. Wolman (ed.), *Origin of Life*, D. Reidel Publ. Co., Dordrecht, pp. 135–141.
Nakanishi, K.: 1962, *Infrared Absorption Spectroscopy – Practical*, Holden-Day, Inc., San Francisco, pp. 17–42.
Sallach, H. J.: 1966, in W. A. Wood (ed.), *Methods in Enzymology*, Vol. IX, Academic Press, New York, pp. 221–228.
Spach, G. and Brack, A.: 1979, *J. Mol. Evol.* **13**, 47–56.
Tooney, N. M. and Fasman, G. D.: 1968, *Biopolymers* **6**, 81–96.
Wagner, G.: 1878, *Ber. Dtsch. Chem. Ges.*, vol. II, 679–680.
Walker, J. C. G.: 1985, *Origins of Life* **16**, 117–127.
Weber, A. L.: 1981, *J. Mol. Evol.* **18**, 24–29.
Weber, A. L.: 1982, *BioSystems* **15**, 183–189.
Weber, A. L.: 1984a, *J. Mol. Evol.* **20**, 157–166.
Weber, A. L.: 1984b, *Origins of Life* **15**, 17–27.
Weber, A. L.: 1985, *J. Mol. Evol.* **21**, 351–355.
Weber, A. L.: 1987a, *J. Mol. Evol.* **25**, 191–196.
Weber, A. L.: 1987b, *Origins of Life* **17**, 107–119.
Weber, A. L. and Orgel, L. E.: 1979, *J. Mol. Evol.* **13**, 193–202.
Windholz, M. and Budavari, S. (eds.): 1983, *The Merck Index*, 10th Edition, Merck and Co., Inc., Rahway, New Jersey, pp. 643–644.
Wolfenden, R., Rammler, D. H., and Lipmann, F.: 1964, *Biochemistry* **3**, 329–338.