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Oligoglyceric Acid Synthesis by Autocondensation of Glyceroyl Thioester

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Summary. The autocondensation of the glyceroyl thioester *S*-glyceroyl-ethanethiol yielded oligoglyceric acid. The rates of autocondensation and hydrolysis of the thioester increased from pH 6.5 to pH 7.5 in 2,6-lutidine and imidazole buffers. Autocondensation and hydrolysis were much more rapid in imidazole buffers than in 2,6-lutidine buffers of the same pH. The efficiency of ester bond synthesis was about 20% for 40 mM *S*-glyceroyl-ethanethiol in 2,6-lutidine and imidazole buffers near neutral pH. The size and yield of the oligoglyceric acid products increased when the concentration of the thioester was increased. The relationship of these results to prebiotic polymer synthesis is discussed.

Key words: Glyceroyl thioester — Polymerization — Polyester — Oligoglyceric acid — Thioester — Prebiotic chemistry — Molecular evolution

Introduction

In an effort to understand how useful chemical energy was produced in the prebiotic environment for the origin of life, we have studied chemical reactions that resemble the initial energy-yielding reaction of glycolysis. Since this glycolytic reaction involves the oxidation of glyceraldehyde-3-phosphate to give an "energy-rich" glyceroyl thioester that is used to drive the synthesis of ATP, we have studied the nonenzymatic formation of thioesters from glyceraldehyde and a thiol, and have examined thioester-driven phosphoanhydride synthesis. We showed that glyceraldehyde and a thiol could be converted to lactoyl thioester under anaerobic conditions and glyceroyl thioester in the presence of oxygen (Weber 1984a,b). We also obtained evidence of alanyl thioester synthesis in similar anaerobic reactions in the presence of ammonium ion (Weber 1985). Our studies of thioester-driven phosphoanhydride synthesis demonstrated that thioesters can act as an energy source for the synthesis of pyrophosphate, tripolyphosphate, and phosphorylimidazole (Weber 1981, 1982).

Glyceraldehyde's role in prebiotic chemistry may not have been limited to its being an energy source for phosphoanhydride synthesis. Our earlier studies suggest that glyceraldehyde could act as a source of both energy and monomers for the synthesis of prebiotic macromolecules, since lactoyl, glyceroyl, and alanyl thioesters derived from glyceraldehyde are in fact "activated" monomers that have the energy needed for polymerization to polyesters or polyamides. Amino acid thioesters previously have been shown to condense to give peptides (Weber and Orgel 1979). We now report the autocondensation of the hydroxy acid thioester, Glc-SEt, that yields oligoglyceric acid.

The synthesis of glyceraldehyde on the primitive Earth most likely occurred by the oligomerization of formaldehyde (Gabel and Ponnamperuma 1967; Reid and Orgel 1967; Mizuno and Weiss 1974). Formaldehyde has been synthesized under a variety of prebiotic conditions (Garrison et al. 1951; Miller 1957; Getoff et al. 1960; Hubbard et al. 1971; Bar-Nun and Hartman 1978; Miller and Schlesinger 1984), and models of the Earth's primitive atmosphere show the photochemical synthesis of form-

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Abbreviations: Glc, glyceric acid; $(Glc)_n$, glyceric acid oligomers, where n = chain length; Glc-SEt, *S*-glyceroyl-ethanethiol; $(Glc)_2$-SEt, *S*-glyceroylglyceroyl-ethanethiol; $(Glc)_3$-SEt, *S*-glyceroylglyceroylglyceroyl-ethanethiol; Glc-hydrox, glyceric acid hydroxamate; Glc-Im, *N*-glyceroyl-imidazole; Im, imidazole; DMF, *N, N*-dimethylformamide

Table 1. Chromatographic and electrophoretic mobilities[a]

Substance	Relative mobility		
	System 1	System 2	System 3
Glc	1.00	1.00	1.00
$(Glc)_2$	0.74	0.84	0.77
$(Glc)_3$	0.50	0.65	0.62
$(Glc)_4$	0.32	0.47	0.52
$(Glc)_5$	0.21	0.33	0.46
$(Glc)_{>5}$	0–0.21	0–0.33	0–0.40
Glc-SEt	2.12	—	—
$(Glc)_2$-SEt	1.93	—	—
$(Glc)_3$-SEt	1.57	—	—
Glc-hydrox	0.55	—	—

[a] Mobilities are given relative to glyceric acid

aldehyde in the early atmosphere and its transport to the Earth's surface by rain-out (Pinto et al. 1980; Canuto et al. 1983; Kasting and Pollack 1984).

Experimental

Materials. L-Glyceric acid (hemicalcium salt), Dowex 50W-X4 resin, and 5,5'-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma Chemical Co.; L-[^{14}C(U)]serine from New England Nuclear; 4-dimethylaminopyridine, ethanethiol, 1,3-dicyclohexylcarbodiimide, and sodium nitrite from Aldrich Chemical Co.; hydroxylamine hydrochloride, 2,6-lutidine(2,6-dimethylpyridine), and methyl red from Matheson, Coleman and Bell; and Centrex microfiltration units (nylon filter, 0.2-μm pore size) from Schleicher and Schuell.

Glyceric acid hydroxamate was prepared by the method of Thompson (1951).

Chromatography and Electrophoresis. Paper chromatography was carried out by descending elution on Whatman 3MM paper in System 1 with *n*-butanol–formic acid–water (8:1:1, v/v/v) and in System 2 with *tert*-butyl alcohol–formic acid–water (7:1.5:1.5, v/v/v). High-voltage paper electrophoresis on Whatman 3MM paper used a buffer of 0.03 M potassium phosphate (pH 7.1) in System 3. Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. The products containing [^{14}C]glyceric acid were located by running the electrophoretograms through a Baird RSC-363 radiochromatographic scanner. The areas of the paper that contained the radioactive products were cut out, placed in vials that contained 20 ml of scintillator made with Liquifluor (New England Nuclear), and counted in a Beckman scintillation counter. Radioactive products were identified by co-chromatography with commercially available standards, whenever possible. Organic acids were detected by spraying with methyl red. Thioesters were seen as dark spots under ultraviolet light.

Preparation of L-[^{14}C]-glyceric acid. A modified version of the method of Lok et al. (1976) was used to synthesize L-[^{14}C]glyceric acid from L-[^{14}C]serine. L-Serine (10.3 mg, 0.098 mmol) and 12 μl of concentrated HCl were added to a solution of L-[^{14}C(U)]serine (250 μCi, 1.7 μmol) in 0.60 ml of water. The solution was cooled to 2°C and then sodium nitrite (7.0 mg, 0.1 mmol) was added in 1.0-mg portions every 30 min. The reaction solution was allowed to stand 24 h in a cold room at 9°C. Two more 1.0-mg additions of sodium nitrite were made and the reaction solution was allowed to stand at 9°C for 24 h and at ambient temperature for an additional 24 h. Purification of L-[^{14}C]glyceric acid was achieved by paper chromatography on Whatman 3MM paper (R_f = 0.57; developing solvent: *tert*-butyl alcohol–formic acid–water, 7:1.5:1.5, v/v/v). [^{14}C]Glyceric acid was eluted from the paper with water, which was removed in vacuo. The residue was redissolved in 1.2 ml of water and the solution was filtered through a Centrex microfiltration unit (nylon filter, 0.2-μm pore size, Schleicher and Schuell). The water was removed in vacuo and the residue was dried in a desiccator over P_2O_5 and NaOH pellets for 24 h. The residue was dissolved in 300 μl of DMF. The yield of L-[^{14}C]glyceric acid was 34% based on radioactivity. The identity of L-[^{14}C]glyceric acid is supported by its co-chromatography and co-electrophoresis with commercially obtained L-glyceric acid in Systems 1, 2, and 3.

Preparation of S-Glyceroyl Ethanethiol. The method of Neises and Steglich (1978) was used to synthesize Glc-SEt. Glyceric acid (hemicalcium salt) was first converted to the free acid by passing 1 g of the hemicalcium salt dissolved in about 20 ml of H_2O through a column containing 40 ml of Dowex 50W-X4 resin that had been prewashed with 20 ml of 2 M HCl followed by 225 ml of water. The eluent was dried in vacuo and then in a desiccator over P_2O_5 in vacuo for 24 h. The residue was stored at −80°C until used in order to prevent spontaneous oligomerization, which occurs at ambient temperature. The free acid form of glyceric acid (84.8 mg, 0.8 mmol) was dissolved in 340 μl of DMF. Next, 50 μl of a DMF solution of L-[^{14}C]glyceric acid (14 μCi, 2.5 mCi/mmol), 4-dimethylaminopyridine (4.0 mg, 0.032 mmol), and ethanethiol (118 μl, 99 mg, 16 mmol) was added to this solution. The reaction vial was sealed and cooled to 2°C. 1,3-Dicyclohexylcarbodiimide (107 mg, 5.2 mmol) was added and reacted while stirring for 5 min at 2°C and then 1 h at ambient temperature. Forty microliters of glacial acetic acid and 500 μl of DMF were added, the reaction solution was filtered through a Centrex microfiltration unit, and the precipitate was washed with an additional 750 μl of DMF. The DMF of the filtrate was removed in vacuo, the residue was taken up in 700 μl of methanol, and a small amount of precipitate was removed by centrifugation. Purification of Glc-SEt was achieved by preparative thin-layer chromatography of this crude preparation on a Silica Gel GF Plate (1500-μm layer) from Analtech, Inc. (R_f = 0.66; developing solvent: chloroform–methanol–acetic acid, 85:15:5, v/v/v). The adsorbent layer that contained Glc-SEt, which was visible under ultraviolet light, was scraped off and the thioester was eluted with methanol in a Centrex microfiltration unit by centrifugation. The methanol was removed in vacuo and the residue was dissolved in 750 μl of water and filtered through a Centrex microfiltration unit. The water was removed in vacuo. The residue was taken up in a final 500 μl of water, filtered through a Centrex microfiltration unit, and stored at −80°C until used in reactions. The yield was 0.121 mmol, 15% based on the amount of glyceric acid used in the preparation.

Glc-SEt was characterized by hydrolysis (pH 12, 30 min) to glyceric acid and a thiol, and by reaction with neutral hydroxylamine to give glyceric acid hydroxamate (Stadtman 1957). The absorption spectrum (200–300 nm) resembled that of other thioesters (Stadtman 1957 and references therein). At the wavelength of maximum absorbance (240 nm), the molar extinction coefficient was found to be 4700 by relating the absorbance at 240 nm of Glc-SEt to the concentration of thiol released by its ammonolysis (Lynen 1951). Thiol concentration was measured with 5,5'-dithiobis-(2-nitrobenzoic acid) (Zahler and Cleland 1968).

Autocondensation of Glc-SEt. In a typical solution reaction, 40 μl of buffer, 20 μl of water, and 20 μl of 160 mM L-[^{14}C]Glc-SEt were added to a reaction tube. At various times, 15-μl aliquots were removed for electrophoresis in System 3, which separated

glyceric acid oligomers from each other and from Glc-SEt, which remained near the origin. The region of the electrophoretogram that contained Glc-SEt was cut out and sewed onto another strip of Whatman 3MM paper for chromatography in System 2. The radioactive reaction products on the electrophoretogram and chromatogram were located and measured as described earlier.

The dry-state reaction was carried out by first drying in vacuo 10-μl aliquots of 160 mM L-[^{14}C]Glc-SEt in 160 mM imidazole-HCl (pH 7.0) contained in small test tubes. Each residue was then heated at 40°C under a nitrogen stream. At specified times, reaction tubes were removed from the heating block, 10 μl of 2.5 M formic acid was added to each tube, and the resulting solutions were subjected to chromatography in System 1. The radioactive products were located and measured as described earlier.

Characterization of Reaction Products. The identity of the oligoglyceric acids is supported by their stability in weak acid and their rapid alkaline hydrolysis in 0.2 M NaOH, that is complete within 2 min. The chain lengths of $(Glc)_2$, $(Glc)_3$, and $(Glc)_4$ are confirmed by their reaction with alkaline hydroxylamine (Hestrin 1949; Jencks et al. 1960) to give their expected Glc-hydrox:Glc ratios. The structure of $(Glc)_2$-SEt is supported by its absorption spectrum (200–300 nm), which resembles those of other thioesters (Stadtman 1957 and references therein), and by its containing about twice as many Glc residues per thioester linkage as Glc-SEt. This determination assumes that the extinction coefficients for $(Glc)_2$-SEt and Glc-SEt are the same. The identity of $(Glc)_3$-SEt is supported by the presence of radioactive Glc residues in its structure and by its absorbance of ultraviolet light on chromatograms, a behavior that is characteristic of thioesters.

Results

Table 2 shows that the autocondensation of 40 mM Glc-SEt in 2,6-lutidine and imidazole buffers yields $(Glc)_2$ and $(Glc)_3$. In these buffers, the rate of of Glc-SEt decomposition increases as the pH increases from 6.5 to 7.5. Moreover, the rate of Glc-SEt decomposition is much more rapid in the imidazole buffers compared to the 2,6-lutidine buffers of the same pH. For example, at pH 7.0, the decomposition half-life of Glc-SEt is about 16 days in the 2,6-lutidine buffer and about 1.1 days in the imidazole buffer. In 2,6-lutidine, the efficiency of ester bond synthesis is about 14% at pH 6.5 and increases to about 22% at pH 7.0. From pH 7.0 to 7.5, the efficiency remains constant. In imidazole buffers, the efficiency is more difficult to estimate because $(Glc)_2$ and $(Glc)_3$ undergo hydrolysis during the reaction. Nevertheless, when this hydrolysis is taken into account, the efficiency of ester bond synthesis in imidazole buffers appears to be about the same as in 2,6-lutidine buffers.

The effect of 25 mM $MgCl_2$, 25 mM $CaCl_2$, and 25 mM $ZnCl_2$ on the autocondensation of 40 mM Glc-SEt was also studied at ambient temperature and pH 7.0. Only $ZnCl_2$ was found to have a significant effect on the reaction when compared to a control reaction in which these salts were replaced by NaCl of the same ionic strength. Our measurements show that $ZnCl_2$ increases the rate of Glc-SEt decomposition and $(Glc)_2$ synthesis in the 2,6-lutidine buffer, and slightly decreases the rate of $(Glc)_2$ synthesis in the imidazole buffer. Precipitated solids, which are thought to be insoluble Zn^{2+} salts, are present in reaction with $ZnCl_2$ in the 2,6-lutidine buffer, but not in the imidazole buffer.

Table 3 shows the autocondensation of Glc-SEt at 400 mM and in the dry state. Condensation of 400 mM Glc-SEt yields oligomers as large as the pentamer $(Glc)_5$. Under dry conditions, oligomers larger than $(Glc)_5$ are produced from Glc-SEt. These results, together with our studies at 40 mM Glc-SEt, show that increasing the concentration of Glc-SEt results in the synthesis of larger oligomers. This re-

Table 2. Formation of oligoglyceric acid by autocondensation of 40 mM Glc-SEt in 2,6-lutidine and imidazole buffers at ambient temperature

Buffer	pH	Time (days)	Percentage of total cpm			
			Glc-SEt	Glc	$(Glc)_2$	$(Glc)_3$
0.4 M 2,6-lutidine-HCl	6.5	0	98.0	0.6	0.5	0.9
		1	96.8	2.0	0.6	0.6
		5	92.4	5.6	1.1	0.9
		10	85.4	10.8	2.8	1.0
		20	71.8	21.1	5.8	1.3
	6.8	0	98.3	0.4	0.5	0.8
		1	95.3	3.1	0.8	0.8
		5	85.9	9.6	3.2	1.3
		10	72.7	18.7	7.0	1.6
		20	52.9	33.7	11.5	1.9
	7.0	0	98.7	0.3	0.5	0.5
		2	86.0	7.8	4.7	1.4
		5	74.9	13.4	9.2	2.4
		10	62.4	21.9	13.3	2.6
		20	45.4	36.3	16.1	2.2
	7.2	0	99.0	0.2	0.4	0.4
		2	82.2	9.5	6.3	1.9
		5	68.9	16.7	11.6	2.9
		10	54.7	27.3	15.3	2.6
		20	38.0	43.2	16.8	2.1
	7.5	0	98.9	0.2	0.4	0.5
		2	75.6	12.2	9.7	2.7
		5	57.1	24.5	15.3	3.0
		10	42.5	38.5	16.9	2.3
		20	26.0	57.6	15.2	1.3
0.4 M imidazole-HCl	6.5	0	99.0	0.3	0.4	0.3
		2	64.8	27.3	6.1	1.7
		5	36.4	49.3	11.8	2.5
		10	14.2	70.0	13.9	2.0
		20	1.9	85.5	11.9	0.8
	7.0	0	98.1	0.3	0.7	0.8
		2	32.7	49.0	14.4	3.8
		5	4.7	75.2	17.4	2.7
		10	0.4	85.1	13.6	0.9
		20	0.2	92.3	7.3	0.2
	7.5	0	94.4	0.5	2.6	2.4
		2	12.9	63.8	19.2	4.1
		5	0.9	81.4	16.2	1.4
		10	0.6	89.6	9.5	0.4
		20	0.9	95.9	3.0	0.1

Table 3. Formation of oligoglyceric acid by autocondensation of Glc-SEt at high concentration and in the dry state

Reaction conditions	Time (days)	Percentage of total cpm								
		Glc-SEt	$(Glc)_2$-SEt	$(Glc)_3$-SEt	Glc	$(Glc)_2$	$(Glc)_3$	$(Glc)_4$	$(Glc)_5$	$(Glc)_{>5}$
0.4 M Glc-SEt at ambient temp.										
In 0.8 M imidazole-HCl (pH 7.0)	1	33.0	2.5	—[a]	28.7	18.4	10.8	4.3	2.3	—
	5	2.6	0.2	—	57.9	30.5	7.4	1.1	0.3	—
In 0.8 M 2,6-lutidine-HCl (pH 7.0)	5	62.1	5.5	—	11.7	11.2	5.9	2.2	1.3	—
	10	51.9	1.7	—	17.0	18.1	7.9	2.3	1.1	—
0.16 M Glc-SEt in 0.16 M imidazole-HCl (pH 7.0) that was dried in vacuo and heated at 40°C	0.08	61.1	13.8	2.8	10.4	5.3	3.1	1.0	1.9	0.6
	0.17	36.4	14.0	4.6	19.4	12.3	7.2	3.4	1.8	0.8
	1.0	8.9	6.0	2.8	21.8	24.0	15.0	8.9	6.7	5.8

[a] Dash indicates that this product was not detected in any radioscans of this reaction; therefore, it was not measured by scintillation counting

sult is attributed to the increase in the ratio of the autocondensation rate to the hydrolysis rate of Glc-SEt that occurs when the concentration of Glc-SEt is increased. In our studies, we did not find any evidence for the synthesis of the cyclic diester of glyceric acid. However, this result does not rule out the possibility that the diester is synthesized and rapidly hydrolyzed, or that it is not seen on radioscans because it was not separated during chromatography from other radioactive products, such as $(Glc)_2$-SEt.

Discussion

The autocondensation of 40 mM Glc-SEt to give oligoglyceric acid occurs with an efficiency of about 20%. This efficiency of ester bond synthesis is greater than that observed for peptide bond synthesis with 2(3′)-aminoacyl esters of nucleotides (Weber and Orgel 1978, 1980, 1981), but less than that with aminoacyl thioesters (Weber and Orgel 1979). The scheme in Fig. 1 summarizes the reactions that are thought to occur during Glc-SEt autocondensation. As shown, Glc-SEt can undergo (1) autocondensation to yield $(Glc)_2$-SEt; (2) hydrolysis to Glc, which can react with Glc-SEt to give $(Glc)_2$; or (3) reaction with imidazole that produces Glc-Im, an intermediate that can hydrolyze to Glc (not shown) or react with Glc-SEt to give $(Glc)_2$-SEt. Further reaction of $(Glc)_2$-SEt with Glc-SEt (or Glc-Im) yields $(Glc)_3$-SEt. Repetition of this acylation reaction converts $(Glc)_n$-SEt to $(Glc)_{n+1}$-SEt; hydrolysis of these thioesters produces their corresponding oligoglyceric acids. Chain growth is also shown to take place by reaction of Glc-SEt (or Glc-Im) with $(Glc)_n$ to give $(Glc)_{n+1}$. Glc-Im is proposed as a possible intermediate in reactions carried out in imidazole buffers, because rates of autocondensation and hydrolysis of Glc-SEt are dramatically increased by the presence of imidazole, and imidazole has been shown to react with thioesters to give reactive *N*-acylimidazoles (Stadtman 1954), which are known to react with hydroxyl groups to give esters (Weber and Fox 1973; Profy and Usher 1984). Imidazole could also act in these reactions as a general acid–base catalyst.

Prebiotic Significance

Glc-SEt autocondensation is considered a model of condensation reactions that may have produced polyglyceric acid on the prebiotic Earth. This autocondensation reaction, together with (1) the synthesis of glyceroyl thioester by oxidation of glyceraldehyde in the presence of a thiol (Weber 1984a,b) and (2) the synthesis of glyceraldehyde from formaldehyde (Gabel and Ponnamperuma 1967; Reid and Orgel 1967; Mizuno and Weiss 1974), constitutes a model pathway for the prebiotic synthesis of polyglyceric acid from formaldehyde, a one-carbon substrate. If we assume that the oxidant for glyceroyl thioester synthesis is a second molecule of triose (glyceraldehyde or dihydroxyacetone), the standard free energy ($\Delta G^{0\prime}$)[1] of the overall pathway that converts six molecules of formaldehyde into a residue of polyglyceric acid and one molecule of glycerol is calculated to be a favorable −32.4 kcal/mol (convention III; Jencks 1976). Furthermore, the loss of glycerol to the environment would make the energetics of this reaction even more favorable.

This pathway from formaldehyde to polyglyceric acid has several characteristics that make it an attractive model for an early type of polymer synthesis that was involved in the origin of life. First, the synthesis of polyglyceric acid from formaldehyde is

[1] This $\Delta G^{0\prime}$ was calculated from the $\Delta G^{0\prime}$ values of Thauer et al. (1977) and an estimated $\Delta G^{0\prime}$ of hydrolysis of a β-ester linkage of polyglyceric acid of −7.6 kcal/mol (Weber 1986)

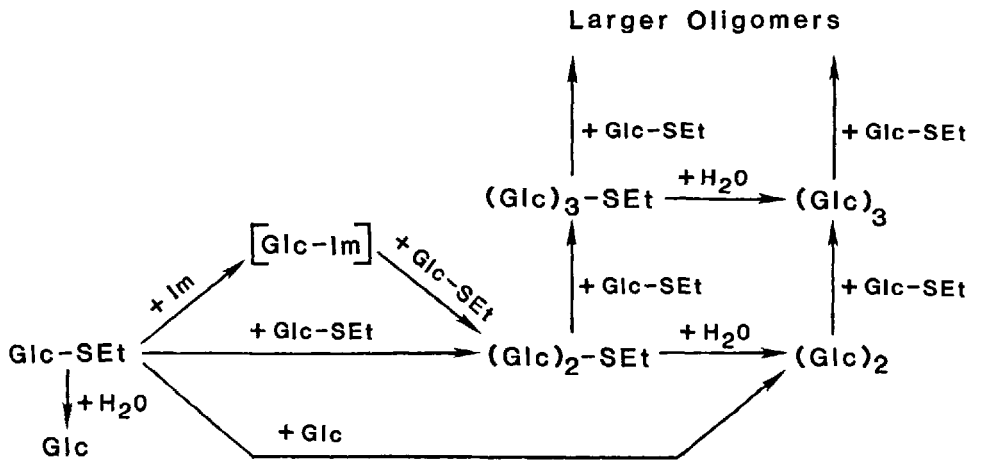

Fig. 1. Scheme of reactions thought to occur during Glc-SEt autocondensation

relatively simple. The monomer is produced in an activated form, a glyceroyl thioester, which possesses the energy required for prebiotic polyester synthesis. This eliminates the need for a condensing agent and its bimolecular reaction with monomer. Also, hydrolysis of some activated monomer liberates acid (glyceric acid, pKa = 3.55), which could protect polyglyceric from alkaline hydrolysis (Euranto 1969; Braud et al. 1985). Second, the model unites the origin of metabolism and the origin of polymer synthesis into a single process. This unification takes place because the oxidation of glyceraldehyde to glyceroyl thioester can function as the initial energy-yielding reaction of early glycolysis, and as the source of activated monomer for early polymer synthesis. Finally, the pathway provides a good starting point for the development of a phosphoanhydride-based energy metabolism, because it produces glyceroyl thioester, which has enough energy to drive phosphoanhydride synthesis (Weber 1981, 1982), and forms glyceric acid as a hydrolytic by-product whose acidity could facilitate the solubilization of phosphate that is tied up in apatite (Schwartz 1971). Also, the further development of a glycolytic metabolism that efficiently produced phosphoanhydrides may initially have used polyglyceric acid as a catalyst that was capable of rudimentary replication of the sequence of D- and L-residues in its structure (Weber 1986).

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