J Mol Evol (1983) 19:237–243

Journal of
Molecular Evolution

Thiol-Catalyzed Formation of Lactate and Glycerate from Glyceraldehyde

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Summary. The formation of lactate from glyceraldehyde is catalyzed by the thiol, N-acetylcysteine, at ambient temperature in aqueous sodium phosphate (pH 7.0). The rate of lactate formation is more rapid at higher concentrations of sodium phosphate and is essentially the same in the presence and absence of oxygen. The formation of lactate is efficient, but proceeds slowly with an 8.8% yield of lactate after 16 days from 10 mM glyceraldehyde in the presence of 12.5 mM N-acetylcysteine and 500 mM sodium phosphate (pH 7.0). The formation of glycerate from glyceraldehyde, that occurs in the presence of oxygen and to a small extent when oxygen has been removed, is also catalyzed by the thiol, N-acetylcysteine, under the same conditions. The dramatic increase in the rate of glycerate formation that is brought about by the thiol, N-acetylcysteine, is accompanied by an equally dramatic decrease in the rates of production of glycolate and formate. Presumably, the thiol-dependent formation of lactate and glycerate occurs via their respective thioesters. The significance of these reactions to molecular evolution is discussed.

Key words: Glyceraldehyde – Intramolecular – Rearrangement – Lactic Acid – Oxidation – Glyceric acid – Prebiotic

Introduction

Lactoyl thioesters have been shown to form readily from pyruvaldehyde and a thiol in aqueous sodium phosphate (Weber 1982a; Hall et al. 1978). Since Riddle and Lorenz (1968) and Fedoronko and Konigstein (1969) had reported that phosphate catalyzed the formation of pyruvaldehyde from glyceraldehyde in aqueous solution, it was reasonable to expect the formation of lactoyl thioester from glyceraldehyde in the presence of a thiol and phosphate. We now report the formation of lactate from glyceraldehyde that is catalyzed by the thiol, N-acetylcysteine, in aqueous sodium phosphate (pH 6.5–7.0). Lactate formation presumably occurs via the "energy-rich" thioester intermediate, N-acetyl-S-lactoylcysteine. The thiol, N-acetylcysteine, was also found to catalyze the formation of glycerate from glyceraldehyde in oxidation reactions carried out with oxygen.

N-acetylcysteine was selected as the thiol in our reactions because it resembles a cysteine residue in a prebiotic peptide and cysteine (cystine) has been synthesized under prebiotic conditions (Sagan and Khare 1971; Hong and Becker 1979). The prebiotic formation of glyceraldehyde is thought to have occurred by the oligomerization of formaldehyde (Gabel and Ponnamperuma 1967; Reid and Orgel 1967; Mizuno and Weiss 1974). Formaldehyde has been produced under presumed prebiotic conditions (Garrison et al. 1951; Miller 1957; Getoff et al. 1960; Hubbard et al. 1971; Bar-Nun and Hartman 1978).

Lactoyl and acetyl thioesters (Weber 1981a, 1982a) may have provided the energy needed for the synthesis of phosphoanhydrides, which acted as phosphorylating and condensing agents in the prebiotic environment. Acetyl thioester has been shown to act as a condensing agent for the synthesis of phosphoanhydrides, like pyrophosphate, tripolyphosphate and phosphorylimidazole (Weber 1981b). Acetyl thioester has also been shown to provide the energy for the synthesis of pyrophosphate on hydroxyapatite, an abundant phosphate mineral (Weber 1982b). Earlier studies of the prebiotic formation of phosphoanhydrides have employed thermal

Abbreviations: Ac-Cys, N-acetylcysteine; Ac-Cys (Lac), N-acetyl-S-lactoylcysteine

methods or chemical condensing agents, other than thioesters (Weber 1982b and references therein).

Experimental

Materials. N-acetyl-L-cysteine, 2,2'-dithiobis-(5-nitropyridine), D-glyceraldehyde, pyruvic acid (sodium salt), DL-glyceric acid (hemi-calcium salt), pyruvaldehyde (methylglyoxal, 40% aqueous solution), β-D(-)fructose, L(-)sorbose, dihydroxyacetone, and hydrazine sulfate (0.56 M solution (pH 8.6)) were obtained from Sigma Chemical Co.; anhydrous oxalic acid from Aldrich Chemical Co.; 2,4-dinitrophenylhydrazine and methyl red from Matheson, Coleman and Bell; lactic acid from J.T. Baker Chemical Co.; glycolic acid from Fisher Scientific Co.; formic acid from Eastman Kodak Co.; lead tetraacetate from Mallinckrodt; D-[UL-^{14}C]fructose, DL-[1-^{14}C]lactic acid (sodium salt) and [1-^{14}C]glycerol from Amersham; [^{14}C]formic acid (sodium salt) from ICN chemical and radioisotope division; [2-^{14}C]pyruvic acid from New England Nuclear.

The D-glyceraldehyde was purified by thin-layer chromatography by the procedure described later for D-[^{14}C]glyceraldehyde; the concentration of D-glyceraldehyde was measured by the method of Beck (1957). N-Acetyl-S-lactoylcysteine was synthesized from pyruvaldehyde and N-acetylcysteine by the method described in our earlier publication (Weber 1982a). Dendroketose was prepared as described by Gutsche et al. (1967). N,N'-Diacetylcystine was available from our earlier synthesis (Weber 1981a).

Chromatography and Electrophoresis. Paper chromatography was carried out by descending elution on Whatman 3 MM paper in System I with the upper phase from ethyl acetate, pyridine, water (2:1:2 v/v), in System II with the upper phase from n-butanol, formic acid, water (4:1:5 v/v), and in System III with n-propanol, concd. ammonium hydroxide (7:3 v/v). High-voltage paper electrophoresis in System IV used Whatman 3 MM paper with a 0.03 M potassium phosphate buffer (pH 7.1). Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. The products formed from D-[^{14}C]glyceraldehyde were located by running the chromatograms and 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 from New England Nuclear, and counted in a Beckman Scintillation Counter. Reaction products were identified by co-chromatography with commercially available radioactive and non-radioactive standards, whenever possible. Thiols were visualized with the 2,2'-dithiobis-(nitropyridine) spray (Grassetti and Murray 1969). Disulfides and thioesters were seen as dark spots under ultraviolet light. Organic acids were detected by spraying with methyl red in a borate buffer (Lederer and Lederer 1957). Pyruvaldehyde, glyceraldehyde, dihydroxyacetone and sugars were visualized by spraying with p-anisidine (Putnam 1957).

Preparation of D-[^{14}C]glyceraldehyde. D-[UL-^{14}C]Glyceraldehyde was prepared by a scaled-down version of the method described by Perlin (1962). D-Fructose (2.3 mg, 12.8 μmoles) was added to 0.25 ml of a 20% ethanol solution of D-[UL-^{14}C] fructose (250 μCi, 225 mCi/mmole) and this solution dried in a desiccator in vacuo over phosphorus pentoxide and sodium hydroxide. The residue was dissolved in 2.5 μl of water and 125 μl of glacial acetic acid. Lead tetraacetate (82% purity, 17 mg, 31.4 μmoles) was added and reacted with vigorous stirring for 10 min at 16– 18°C and then for 60 min at ambient temperature. A solution (approx. 70 μl) of 5 g of anhydrous oxalic acid in 5 ml of glacial acetic acid was added to the reaction mixture until a negative starch-iodide test was obtained. The starch-iodide test was carried out by adding 1 μl of the supernatant of the reaction mixture to 5 μl of a solution of 1% starch and 1% KI. A negative test was indicated by the absence of a yellow color and no precipitation.

Next, the supernatant of the reaction mixture was removed after centrifugation and the precipitate was washed with twice its volume of glacial acetic acid. The supernatant from this wash was isolated by centrifugation and it was combined with the original supernatant and dried at 35°C in vacuo. Twenty microliters of toluene was added and removed in vacuo at 35°C in order to remove acetic acid. This procedure was carried out four times. The residue was taken up in 25 μl of 0.1 M sulfuric acid and reacted 48 h at ambient temperature in order to hydrolyze the esters of glycolic acid and formic acid. The volume of the preparation was adjusted to 0.4 ml with water and Bio-Rad AG-1X8 (200–400 mesh, bicarbonate form) resin was added until the pH was 6.5 in order to remove acids in the preparation. The resin was isolated by centrifugation and washed once with 0.2 ml water. The combined supernatants were treated for 2.5 min with 1 μl of Bio-Rad Chelex 100 resin (sodium form), which was removed by centrifugation. The preparation was concentrated to 0.3 ml in vacuo and applied to a Silica Gel GF preparative thin-layer plate (1500 micron layer) purchased from Analtech Inc. The plate was developed in n-propanol : water (7:0.5 v/v), which separated glyceraldehyde (R_f = 0.81) from dihydroxyacetone (R_f = 0.74) and fructose (R_f = 0.61). The edges of the plate were cut off and the glyceraldehyde detected with the anisidine spray (Putnam 1957). The region of the silica gel that contained the glyceraldehyde was scraped off the glass plate and the glyceraldehyde eluted with methanol. The eluent was dried in vacuo, taken up in 1.0 ml of water, and passed through a 13 mm Millipore FG filter (0.2 μm pore size) in a Swinnex filter apparatus. The preparation was stored at –80°C. Analysis of the preparation by electrophoresis and chromatography showed that the preparation contained impurities with electrophoretic mobilities

Table 1. Chromatographic and electrophoretic mobilities (Rm)[a]

	System I (Rm)	System II (Rm)	System III (Rm)	System IV (Rm)
Lactic acid	1.00	1.00	1.00	1.00
Glyceric acid	0.71	0.56	0.64	1.04
Glycolic acid	0.74	0.82	0.77	1.21
Formic acid	0.87	–	0.99	1.63
Pyruvic acid	1.35	0.96	–	1.22
Glyceraldehyde	1.33	–	–	0.05
Dihydroxyacetone	2.47	–	–	0.04
Fructose	1.68	–	–	0.05
Sorbose	1.68	–	–	0.05
Dendroketose	2.01	–	–	0.07
Glycerol	2.18	–	–	0.05
Glycolaldehyde	1.43	–	–	0.05
Ac-Cys	1.38	–	–	0.89
Ac-Cys(Lac)	1.53	0.99	–	0.70
N,N'-diacetyl-cystine	0.68	–	–	1.05
Pyruvaldehyde	3.05	–	–	0.05
Unknown-A	–	–	–	0.77
Unknown-B	1.99	–	–	–
Unknown-C	–	–	–	0.52

a Chromatographic and electrophoretic mobilities are given relative to lactic acid

in System IV of formic acid (0.3%), glycolic acid (1.6%), unknown-C (0.7%) and with chromatographic mobilities in System I of fructose (2.0%), dihydroxyacetone (2.2%), unknown-B (1.4%). The yields of products from reactions of glyceraldehyde were corrected for these impurities, except for dihydroxyacetone which is known to isomerize to glyceraldehyde.

Reactions with D-[^{14}C]Glyceraldehyde. Reaction solutions were prepared under a nitrogen blanket from solutions of substrates that had been flushed for 20 min with nitrogen in order to remove oxygen. In a typical reaction, 90 µl of 1.0 M sodium phosphate (pH 7.0), 18 µl of 0.50 M N-acetylcysteine, 36 µl of water, 18 µl of 0.10 M D-glyceraldehyde and 18 µl of D-[^{14}C]glyceraldehyde were added with gastight Hamilton syringes to a test tube that had been flushed with nitrogen. The resulting solution was flushed 5 min with nitrogen and then degassed in a desiccator for 5 min at 2 mm Hg pressure. Nitrogen was admitted to the desiccator and the reaction solutions sterilized by filtration through autoclaved Millipore FG filters (13 mm, 0.2 µm pore size) in Swinnex filter holders. Twenty microliter portions of these reaction solutions and 5 µl of toluene were transferred with sterilized Drummond microcap disposable pipettes into sterilized reaction tubes, which were subsequently glass sealed at 1 mm Hg pressure. Some reactions were carried out without sterile precautions. Reactions with oxygen were performed by bubbling oxygen through the reaction solutions for 10 min and sealing the timed reaction tubes under atmospheric pressure. All reactions were carried out at ambient temperature in the dark. After a given time of reaction, the reaction tubes were opened and their content stored at -80°C until 5 µl aliquots were analyzed by chromatography and electrophoresis, as described earlier. The analysis of N-acetyl-S-lactoylcysteine was performed by chromatography of a 10 µl aliquot of a mixture of 5 µl of the reaction solution, 5 µl of authentic N-acetyl-S-lactoylcysteine (Weber 1982a) and 3 µl of 5 M acetic acid on Whatman DE-81 paper with the upper phase from n-butanol, 3 M acetic acid (2:1 v/v) for 24 h. The ultraviolet-absorbing spot, which corresponded to N-acetyl-S-lactoylcysteine (mobility relative to lactate (R_m) = 0.61), was cut out and attached to a sheet of Whatman 3 MM paper. System II was used to re-chromatograph the N-acetyl-S-lactoylcysteine, which was measured by scintillation counting, as described earlier.

Results

The reactions of glyceraldehyde that are depicted in Fig. 1 and Fig. 2 were carried out in 500 mM sodium phosphate (pH 7.0) at ambient temperature. Fig. 1(a1) shows the formation of organic acids from glyceraldehyde in the presence of the thiol, N-acetylcysteine, and absence of oxygen. Lactate forms at a rate of about 0.4% per day throughout the reaction period. Early in the reaction glycerate formation stops in less than 2 days (probably less than 1 day as indicated by a similar reaction with 10 mM thiol). Although the trace of oxidant responsible for glycerate formation has not been identified, molecular oxygen is considered a reasonable possibility, since glycerate formation is increased in the presence of oxygen, as discussed later. The yields of glycolate and formate are not considered significant, since they may have been produced by hydrolysis of traces of their esters that contaminate the preparation. As shown in Fig. 1(a2) the formation of organic acids is accom-

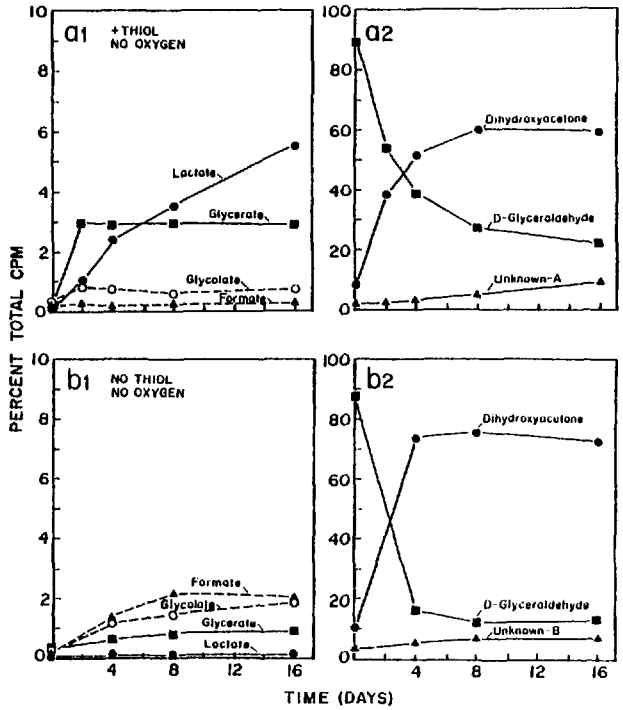


Fig. 1. Formation of products from 10 mM [^{14}C]glyceraldehyde in the absence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature, (a1, a2) with 50 mM N-acetylcysteine; (b1, b2) without N-acetylcysteine

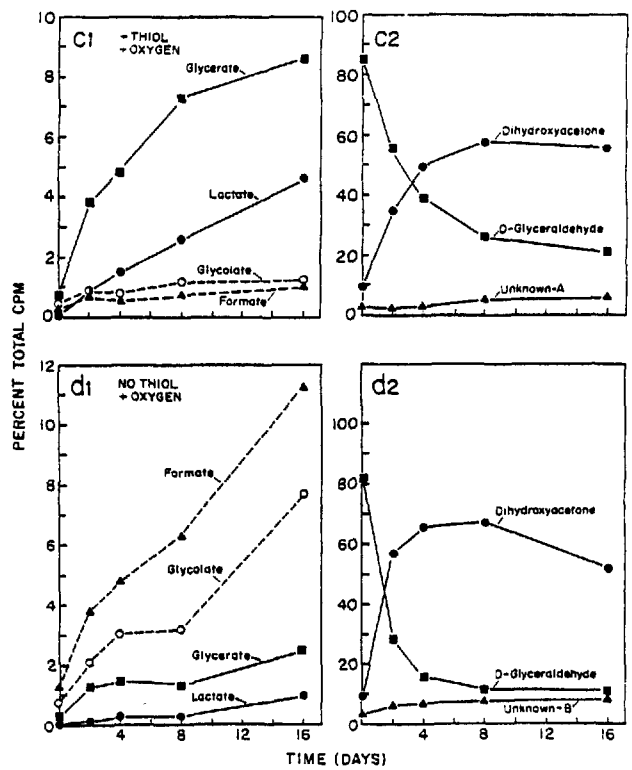


Fig. 2. Formation of products from 10 mM [^{14}C]glyceraldehyde in the presence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature, (c1, c2) with 50 mM N-acetylcysteine; (d1, d2) without N-acetylcysteine

panied by the isomerization of glyceraldehyde to dihydroxyacetone and the formation of an unidentified product, unknown-A.

Fig. 1(b1) depicts the formation of organic acids from glyceraldehyde in the absence of thiol. As seen in Fig. 1(b1), lactate formation does not occur to a significant extent without thiol. The rate of formation of glycerate is also greatly reduced and is less than the rate of formation of glycolate and formate. A comparison of Fig. 1(a2) with Fig. 1(b2) shows that the isomerization of glyceraldehyde to dihydroxyacetone is apparently more rapid in the absence of thiol than in its presence. The formation of unknown-B is also shown in Fig. 1(b2).

Fig. 2(c1) depicts the formation of organic acids from glyceraldehyde in the presence of oxygen and the thiol, N-acetylcysteine. A comparison of Fig. 2(c1) with Fig. 1(a1) shows that the presence of oxygen results in a large increase in the production of glycerate, but only a small increase in the formation of glycolate and formate. Lactate formation is not significantly changed by oxygen. Fig. 2(c2) shows that the isomerization of glyceraldehyde to dihydroxyacetone and the formation of unknown-A are not significantly affected by oxygen; however, the yields of glyceraldehyde and dihydroxyacetone are reduced due to their oxidation to organic acids.

Fig. 2(d1) shows the formation of organic acids in the presence of oxygen without thiol. Glycolate and formate are the dominant products and glycerate production is much slower than the same reaction in the presence of thiol (Fig. 2(c1)). A small amount of lactate also forms under these conditions. Fig. 2(d2) shows the accompanying isomerization of glyceraldehyde to dihydroxyacetone and the formation of unknown-B.

Table 2 lists the results from experiments that were identical to those shown in Figs. 1(a1)(a2) and (b1)(b2), except that they were carried out under sterile conditions. These control reactions were performed in order to verify that the products from glyceraldehyde are formed by chemical reactions and not bacterial activity. Table 2 shows that the yields of products obtained in the presence of thiol under sterile conditions are very close to those obtained without sterile precautions (Fig. 1(a1), (a2). A comparison of the yields obtained in the absence of thiol under sterile conditions with those obtained without sterile precautions (Fig. 1(b1), (b2)) shows a small but probably significant reduction in the rate of formation of glycolate, formate and glycerate in the sterile reactions. This small reduction in the rate of oxidation of glyceraldehyde may be due to the removal of very small amounts of insoluble iron salts by filtration during the sterilization procedure. Iron salts like sodium ferro(ferri) pyrophosphate-phosphate mixtures have been shown to catalyze the oxidation of sugars, including glyceraldehyde (Spoehr 1924; Spoehr and Smith 1926; Spoehr and Milner 1934). Although this comparison of the product yields under sterile and non-sterile conditions indicates that sterilization is not required, all subsequent experiments have been carried out under sterile conditions as a precaution.

Table 3 shows the effect of phosphate concentration and thiol concentration on the formation of products from glyceraldehyde. As seen in Table 3, reducing the concentration of N-acetylcysteine increases the rate of formation of lactate and causes a decrease in the yield of unknown-A and in the final ratio of glyceraldehyde to dihydroxyacetone. A reduction in the phosphate concentration decreases the rate of lactate formation and slows the isomerization of glyceraldehyde to dihydroxyacetone. An examination of the pH dependency of the reaction of 10 mM glyceraldehyde in 500 mM sodium phosphate with 25 mM N-acetylcysteine showed that only the yields of lactate and unknown-A are effected by pH. At pH 6.5, 7.0 and 7.5 the yields of lactate are, respectively, 7.34%, 6.67% and 9.98%; the yields of unknown-A are respectively 3.81%, 2.97% and 5.90%.

Since lactate synthesis from glyceraldehyde was thought to proceed via the thioester intermediate, N-

Table 2. Formation of products from 10 mM [^{14}C]glyceraldehyde in 500 mM sodium (pH 7.0) in the presence and absence of 50 mM N-acetylcysteine at ambient temperature under sterile conditions in the absence of oxygen

		Percent of total CPM							
	Time (days)	Lactate	Glycerate	Glycolate	Formate	Glyceraldehyde	Dihydroxy-acetone	Unknown-A	Unknown-B
+Ac-Cys	0	0.00	0.12	0.41	0.02	90.31	5.53	2.24	–
	4	1.09	2.58	0.51	0.13	43.11	47.61	2.76	–
	8	2.20	2.73	0.72	0.23	27.95	60.52	5.27	–
	16	4.83	2.71	0.83	0.23	23.04	61.13	8.51	–
	32	8.34	2.55	0.72	0.23	20.79	53.45	14.58	–
-Ac-Cys	0	0.00	0.01	0.41	0.23	86.42	9.63	0.06	1.23
	4	0.01	0.31	0.72	1.07	18.33	70.45	0.56	5.32
	8	0.04	0.49	1.14	1.38	14.13	73.62	1.09	6.25
	16	0.18	0.77	1.77	1.59	13.93	73.01	1.92	6.86
	32	0.37	1.21	2.60	1.80	13.52	70.76	3.60	7.47

Table 3. Formation of products from [^{14}C]glyceraldehyde at several concentrations of sodium phosphate and N-acetylcysteine at ambient temperature under sterile conditions in the absence of oxygen

[Phosphate]	[Ac-Cys]	Time (days)	Percent of total CPM						
			Lactate	Glycerate	Glycolate	Formate	Glyceraldehyde	Dihydroxy-acetone	Unknown-A
500 mM	50 mM	0	0.02	0.10	0.62	0.13	92.46	5.53	2.55
		8	3.28	2.69	0.93	0.33	28.77	58.57	6.11
		16	5.52	2.44	0.83	0.33	24.11	58.43	8.83
	25 mM	0	0.03	0.29	0.62	0.02	89.70	7.78	2.34
		8	3.47	3.14	1.04	0.44	18.23	70.76	2.24
		16	6.67	2.54	0.93	0.44	17.82	67.68	2.97
	12.5 mM	0	0.01	0.31	0.51	0.13	88.27	8.91	1.72
		8	4.33	3.11	0.62	0.44	15.77	73.11	1.30
		16	8.80	2.92	1.04	0.44	14.85	70.86	1.72
250 mM	50 mM	0	0.02	0.30	0.51	0.13	93.08	4.61	2.45
		8	1.91	2.91	0.93	0.44	34.20	53.96	5.06
		16	3.39	3.00	1.04	0.33	23.24	61.64	7.89
125 mM	50 mM	0	0.00	0.01	0.41	0.13	94.00	3.89	1.72
		8	1.24	2.42	0.72	0.33	42.49	48.02	3.70
		16	2.49	2.33	0.83	0.33	28.57	59.18	6.32

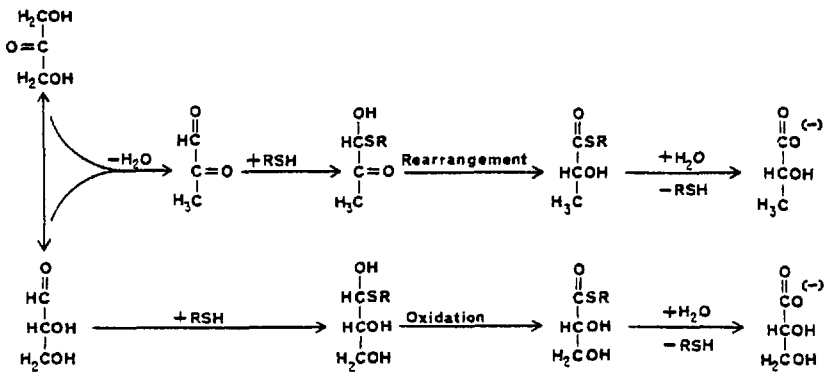
acetyl-S-lactoylcysteine, the formation of this lactoyl thioester was measured in a reaction mixture of 10 mM glyceraldehyde, 10 mM N-acetylcysteine and 500 mM sodium phosphate (pH 7.0). The yields of N-acetyl-S-lactoylcysteine and [lactate] at zero time, 1 day and 2 days of reaction are, respectively, 0.03% [0.02%], 0.28% [0.59%] and 0.23% [1.51%]. In the absence of the thiol, N-acetylcysteine, the yields of N-acetyl-S-lactoylcysteine and lactate are negligible (< 0.04%).

Discussion

We have shown that the thiol, N-acetylcysteine, catalyzes the formation of lactate and glycerate from glyceraldehyde. As shown in the scheme on the next page, the formation of lactate is thought to begin by the phosphate-catalyzed dehydration of glyceraldehyde and/or dihydroxyacetone to give pyruvaldehyde (Riddle and Lorenz 1968; Fedoronko and Konigstein 1969). The pyruvaldehyde rapidly forms a hemithioacetal with the thiol, N-acetylcysteine (RSH), that subsequently undergoes a phosphate-catalyzed rearrangement to give the thioester, N-acetyl-S-lactoylcysteine (Hall et al. 1978; Weber 1982b). This thioester ultimately hydrolyzes to yield lactate. The proposed pathway from glyceraldehyde to lactate is supported by (1) the detection of postulated intermediate, N-acetyl-S-lactoylcysteine, in the reaction mixture, and (2) the thiol- and phosphate-dependence of lactate formation that would be expected from the above-mentioned studies of the partial reactions that comprise the pathway. The formation of lactoyl thioester probably can occur at concentrations considerably below the 0.01 M glyceraldehyde that we studied, since lactoyl thioester formation from glyceraldehyde is essentially an irreversible process with a free energy change ($\Delta G_o'$) of roughly -17 kcal/mole. This $\Delta G_o'$ is calculated from (1) the estimated $\Delta G_o'$ of lactoyl thioester hydrolysis (-8/kcal/mole) that is assumed to be similar to the value reported for acetyl thioester hydrolysis (Jencks 1976) and (2) the $\Delta G_o'$ of formation of lactate from glyceraldehyde (-25.8 kcal/mole, Decker et al. (1970)).

It is likely that the dehydration of glyceraldehyde to give pyruvaldehyde is the slowest reaction in the pathway, since both the formation of lactoyl thioester from pyruvaldehyde (Weber 1982b) and its hydrolysis ($t_{1/2}$ = 6 days) in 0.5 M sodium-phosphate (pH 7.0) are considerably more rapid than lactate formation. Also, the isomerization of glyceraldehyde to dihydroxyacetone that reaches equilibrium at a 5 to 1 ratio of dihydroxyacetone to glyceraldehyde is apparently more rapid than the dehydration of glyceraldehyde to pyruvaldehyde that is estimated from the rate of lactate formation. Fedoronko and Konigstein (1969) also reported that phosphate-catalyzed isomerization of glyceraldehyde is more rapid than its dehydration to pyruvaldehyde and they gave a value of 5 to 1 for the ratio of dihydroxyacetone to glyceraldehyde at equilibrium. The reported equilibrium ratio of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate is approximately 22 (Reynolds et al. 1971 and references therein), a significantly higher value than we observe for the non-phosphorylated substances.

The reaction scheme also shows a possible pathway for glycerate synthesis from glyceraldehyde. Glyceraldehyde is shown to form a hemithioacetal with N-acetylcysteine (RSH) that undergoes oxidation to yield N-acetyl-S-glyceroylcysteine. The presumed glyceroyl thioester intermediate then hydrolyzes to give



glycerate. At this time we are attempting to demonstrate the formation of the glyceroyl thioester intermediate. We have not found any earlier report of the thiol-catalyzed formation of glycerate from glyceraldehyde in the presence of oxygen. The discussion of the prebiotic relevance of thiol-catalyzed formation of glycerate from glyceraldehyde is deferred until we have shown the formation of the presumed glyceroyl thioester intermediate.

The oxidation of glyceraldehyde in the absence of thiol to yield mostly formate and glycolate resembles the alkaline degradation of aldoses by oxygen that gives formate and aldonic acids having one less carbon (Warshowsky and Sandstrom 1952; Green 1980). However, in our reactions the molar yield of formate is much greater than that of glycolate. This observation indicates that there may be a second two-carbon product that we have not isolated from the reaction mixture. This fragment may be glycolaldehyde, which is not resolved from glyceraldehyde by our electrophoretic and chromatographic methods.

The formation of "energy-rich" lactoyl thioesters from glyceraldehyde is considered a plausible prebiotic reaction, since it operates in the presence of water with simple substrates and catalysts at low concentrations. The interconversion of glyceraldehyde (dihydroxyacetone) and larger sugars by aldolization and dealdolization may have allowed the readily reversible storage of glyceraldehyde in the form of more stable, larger sugars on the prebiotic Earth (Konigstein and Fedoronko 1975; Gutsche et al. 1967; Degani and Halmann 1968; Mizuno and Weiss 1974). Lactoyl thioester formation from glyceraldehyde resembles fermentation (glycolysis) in that useful free energy in both cases is derived from the rearrangement of glyceraldehyde to lactic acid. The prebiotic formation of lactoyl thioesters would have provided a source of energy in a chemical form that may have been used directly or indirectly, via phosphoanhydrides (Weber 1981b, 1982b), to drive the synthesis of biopolymers on the primitive Earth.

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

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Received November 15, 1982/Revised February 1, 1983