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Formation of the Thioester, N,S-Diacetylcysteine, from Acetaldehyde and N,N'Diacetylcystine in Aqueous Solution with Ultraviolet Light

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Summary. The thioester, N,S-diacetylcysteine, is formed during the illumination of phosphate buffered (pH 7.0) aqueous solutions of acetaldehyde and N,N'-diacetylcystine with ultraviolet light. The yield of N,S-diacetylcysteine relative to N-acetylcysteine and unidentified products progressively increases as ultraviolet light below 239 nm, 253 nm and 281 nm is cut off with optical filters. When ultraviolet light below 320 nm is removed with an optical filter, there is no detectable reaction. Illumination of 0.025 M N,N'-diacetylcystine with 0.5 M and 1.0 M acetaldehyde with filtered ultraviolet light gives, respectively, 20% and 80% yields of N,S-diacetylcysteine. In the reaction with 1.0 M acetaldehyde, N-acetylcysteine forms early in the reaction and later decreases with its conversion to N,S-diacetylcysteine. The prebiotic significance of these reactions is discussed.

Key words: Thioester – Disulfide – Acetaldehyde – Ultraviolet light – Molecular evolution

Introduction

Thioesters are formed as the first "energy-rich" intermediates in several substrate-level oxidative phosphorylations (Slater 1966). Relevant biochemical reactions include the oxidation of (1) glyceraldehyde-3-phosphate, (2) α-keto acids and (3) acetaldehyde (Goldman and Vagelos 1964; Harris and Waters 1976; Bridger 1974). These reactions proceed by an initial oxidation of an aldehyde (bound to a thiol or thiamine) that yields a thioester. Phosphorolysis of the thioester gives an acyl phosphate, which is used to phosphorylate nucleoside diphosphates.

The use of thioester intermediates in these energy-yielding reactions and their indispensable role in metabolism (Fluharty 1974) can be attributed to their special chemical properties. These properties are (1) a large negative free energy of hydrolysis (ΔG = -7.5 kcal/mole, Jencks 1976), (2) a good acylating ability and (3) tail activation, which involves carbanion stabilization on the α-carbon of the acyl group (Bruice and Benkovic 1966).

The participation of thioesters in substrate-level oxidative phosphorylation, a type of energy-transduction that is considered ancient (Broda 1975), suggests that thioesters were involved in energy-metabolism at an early stage in molecular evolution (Hartman 1975; Buvet 1978). In this paper we describe a model of prebiotic thioester formation in which thioesters are formed by illumination of an aqueous solution of acetaldehyde and a disulfide with ultraviolet light. Previously, Takagi (1976) had shown the photochemical reductive acylation of disulfides in aldehyde solvents. N,N'-diacetylcystine was selected as the disulfide because it structurally resembles a cystine residue in a polypeptide. Cystine (cysteine) and acetaldehyde have been synthesized under plausible prebiotic conditions (Sagan and Khare 1971; Hong and Becker 1979; Miller 1957).

Experimental

Materials

Ac-Cys, dithioerythritol, 2,2'-dithiobis-(5-nitropyridine), L-cystine, and L-cysteine (hydrochloride) were purchased from Sigma Chemical Co., acetaldehyde from Aldrich Chemical Co., acetic anhydride from Mallinckrodt and L-cystine[^{14}C] from New England Nuclear.

Abbreviations: Ac–Cys, N-acetylcysteine; Ac–Cys(Ac), N,S-diacetylcysteine; Ac–Cys, N,N'-diacetylcystine.
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Ac–Cys

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Chromatography and Electrophoresis

High voltage electrophoresis in System I used Whatman 3MM with a 0.03 M potassium phosphate buffer (pH 7.1). Paper chromatography was carried out by descending elution in System II with n-butanol:3 M acetic acid (2:1 v/v) for 30 h on Whatman DE-81 paper and System III with n-butanol:5 M acetic acid (2:1 v/v) for 18 h on Whatman 3MM paper. Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. Thiols were visualized with the 2,2'-dithiobis-(5-nitropyridine) spray (Grassetti and Murray 1969). Disulfides and thioesters were visible as dark spots under ultraviolet light.

The products formed from radioactive. N,N'-diacetylcystine-[^{14}C] were located and estimated by running the chromatograms and electrophoretograms through a Baird Atomic RSC-363 radiochromatographic scanner with integrator. The reaction products were identified by co-electrophoresis and co-chromatography with commercially available standards, wherever possible.

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

	System I R_m	System II R_m	System III R_m
Ac–Cys	1.00	0.39	1.00
Ac–Cys | Ac–Cys	1.22	0.07	0.81
Ac–Cys (Ac)	0.86	1.00	1.04
Unknowns-1	0.64–0.81	[0.50] [0.20] [0.07] [b]	
Unknown-2	0.98	1.27	

[a]Chromatographic and electrophoretic mobilities in System II are given relative to Ac-Cys(Ac) and in Systems I and III are given relative to Ac-Cys

[b]R_m values for substances eluted from System I electrophoretogram (R_m = 0.64–0.81) and rechromatographed in System II

Synthesis of N,S-Diacetylcysteine

N,S-Diacetylcysteine was synthesized from cysteine and acetic anhydride by the method of Smith and Gorin (1961). This chromatographically homogeneous substance had the typical absorbance spectrum of a thioester (ϵ max at 233 nm, Stadtman 1957). Reaction of the thioester with glycine at pH 8.0 yielded acetylglycine and in a 0.125 M sodium phosphate buffer (pH 7.0) its hydrolytic half-life was 35 days, which would be expected for a thioester under these conditions (Lorand and Stenberg 1976). Rapid hydrolysis of the substance at pH 13 is also consistent with the presence of a thioester linkage (Bruice and Benkovic 1966).

Synthesis of N,N'-Diacetylcystine

The method used to prepare N,N'-diacetylcystine was similar to that used by Hollander and duVigneaud, 1931. l-Cystine (0.05 mole, 12.0 g) was suspended in 50 ml of water and dissolved by adding 8 M potassium hydroxide until the solution was pH 12. At 0°C to 3°C, acetic anhydride (0.15 mole, 15.3 g) was added in small portions as the pH of the solution was maintained between 10 and 10.5 with 8 M potassium hydroxide. After the addition of acetic anhydride, the solution was allowed to stand 1 h at room temperature at pH 10 and then adjusted to pH 3 with concentrated hydrochloric acid. The solution was concentrated in vacuo, and the viscous residue extracted 3 times with 100 ml portions of an acetone-water mixture (93:7 (v/v). The acetone extract was concentrated in vacuo and dried in a desiccator over phosphorus pentoxide and sodium hydroxide. The residue was dissolved in ethanol. The precipitate that formed was removed by centrifugation and the remaining solution applied to Silica Gel-G (Woelm) preparative thin-layer plates (1500 micron layer) purchased from Analtech Inc. The plates were developed with chloroform:methanol:acetic acid (80:15:10 v/v). The thin-layer silica gel coating, together with adherent N,N'-diacetylcystine (R_f = 0.40) was scraped off the glass plate and the disulfide eluted with methanol. The eluent was concentrated in vacuo and dried in a desiccator over phosphorus pentoxide and sodium hydroxide. The residue was dissolved in ethanol and disulfide precipitated by adding the ethanol solution to ethyl ether. The yield of N,N'-diacetylcystine was 22% based on cystine. Analysis by chromatography and electrophoresis showed the preparation to be a homogeneous substance that was reduced by dithioerythritol to give N-acetylcysteine (Zahler and Cleland 1968). Elemental analysis – calculated for $C_{10}H_{16}N_2S_2O_6$ [0.8 H_2O]: C, 35.46, H, 5.20; N, 8.27; O, 32.15; S, 18.91. Found: C, 35.94; H, 5.24; N, 8.17; O, 32.99; S, 18.29.

Radioactive N,N-diacetylcystine[^{14}C] was prepared, as described above, by acetylation of l-cystine with acetic anhydride. The purification of N,N'diacetylcystine[^{14}C] was accomplished by chromatography on Whatman 3MM paper with n-butanol:5 N acetic acid (2:1 v/v). The chromatographic and electrophoretic mobility of this substance was identical to non-radioactive. N,N'-diacetylcystine. Reduction of N,N-diacetylcystine[^{14}C] with dithioerythritol yielded N-acetylcysteine[^{14}C] (Zahler and Cleland 1968).

Photochemical Reactions

Reaction solutions were prepared under a nitrogen blanket from solutions of the substrates that had been flushed with nitrogen for 30 min in order to remove oxygen. In a nitrogen-swept dry box, 40 μl aliquots of each reaction solution were transferred to small quartz reaction tubes (inner dia. 3.2 mm, outer dia. 4.2 mm) and the tubes sealed. Each quartz tube was illuminated with ultraviolet light for a specified length of time in a turntable photochemical reactor purchased from Ace Glass Inc. The reaction tubes were positioned 7.5 cm from the light source, a 200 watt Hanovia medium-pressure mercury lamp. The light source was turned off for 5 min during the removal of reaction tubes, which were subsequently stored at -70°C until analyzed. During the photochemical reaction, the temperature of the reaction tubes was maintained at 23°C by the movement of the reaction tubes through a water reservoir surrounding the turntable photochemical reactor.

Some photochemical reactions were carried out with optical filters that cut off short-wavelength ultraviolet light below a specified wavelength. In these reactions the small quartz reaction tubes were positioned in the center of a larger quartz tube (outer dia. 13 mm, inner dia. 11 mm) that contained a solution filter. This arrangement provided a solution filter with a path length of 3.4 mm. These solution filters include: (1) 5 N acetic acid (50% T, 247 nm; 10% T, 242 nm; 1% T, 239 nm), (2) carbon tetrachloride (50% T, 263 nm; 10% T, 257 nm; 1% T, 252 nm), (3) toluene (50% T, 285 nm; 10% T, 283 nm; 1% T, 281 nm), and (4) acetone (50% T, 329 nm; 10% T, 324 nm; 1% T, 320 nm).

Results

Fig. 1 a-d depicts the product yields obtained from 0.025 M N,N'-diacetylcystine in the presence of 0.5 M acetaldehyde during illumination with ultraviolet light. As shown in Fig. 1 a, illumination with unfiltered ultraviolet light yields more Ac-Cys than Ac-Cys(Ac) and several unknown products. Fig. 1 b shows that when ultraviolet light below 239 nm is removed with a optical filter, the yield of Ac-Cys(Ac) increases and the yields Ac-Cys and the unknown substances decrease. As seen in Fig. 1 c and Fig. 1 d, the yield of Ac-Cys(Ac) progressively improves relative to other products when ultraviolet light below, respectively, 252 nm and 281 nm, is removed by optical filters. When ultraviolet light below 320 nm is removed with an optical filter, there is no detectable reaction. Since the Hanovia lamp emits light principally at 297 nm, 303 nm and 313 nm in the range 280–320 nm, the photochemistry observed in Fig. 1 d and Fig. 2, which follows, can be attributed to ultraviolet light between 295 nm and 320 nm. Fig. 1 a-d also show that the yield of Ac-Cys(Ac) does not increase indefinitely but rather reaches a plateau near the end of the illumination.

Figure 2 depicts the yields of products obtained from 0.025 M N,N'-diacetylcystine in the presence of 1.0 M

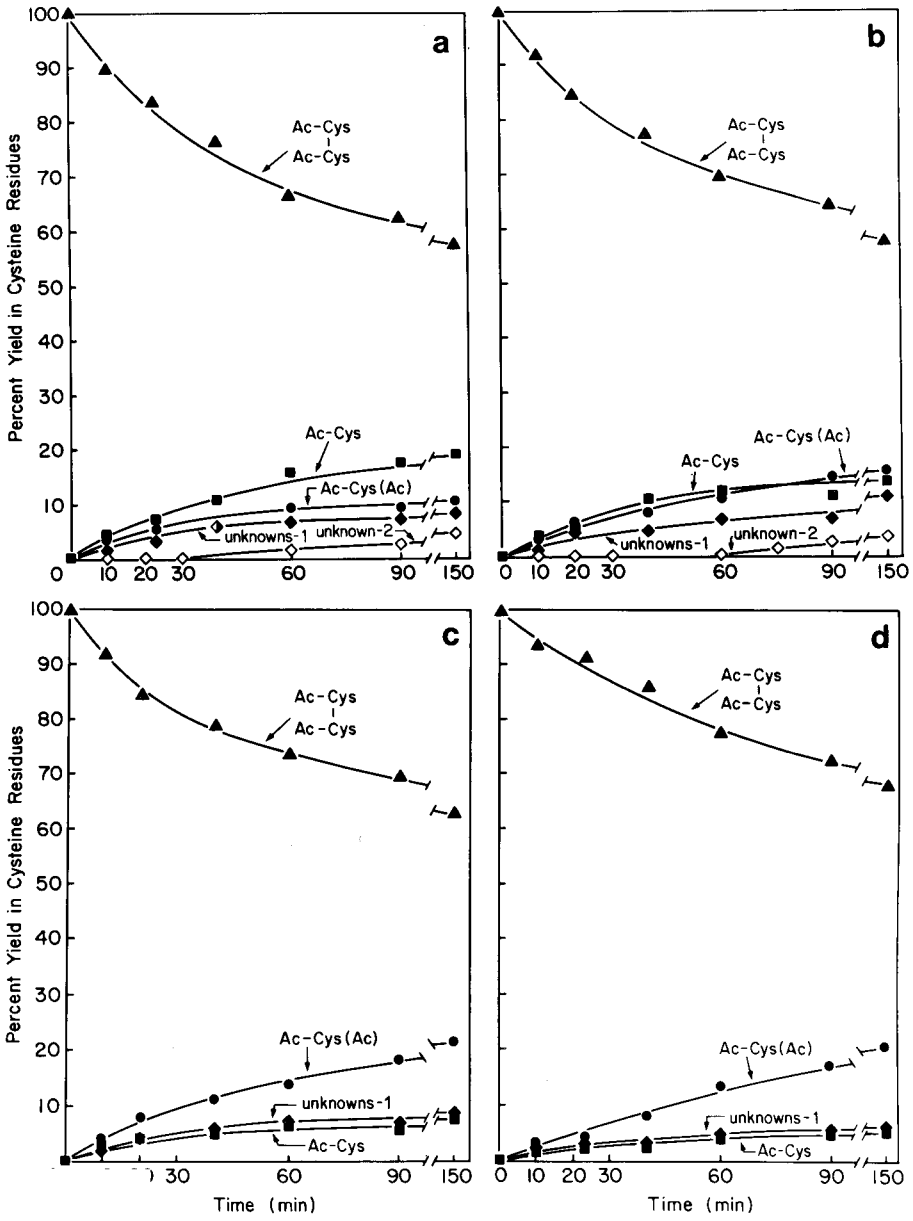


Fig. 1 a-d. Time course of formation of products from the illumination of 0.025 M N,N'-diacetylcystine and 0.5 M acetaldehyde in a 0.125 M sodium phosphate buffer (pH 7.0) with ultraviolet light. The ultraviolet light was (**a**) not filtered, (**b**) filtered through a 239 nm cut-off filter, (c) filtered through a 252 nm cut-off filter and (**d**) filtered through a 281 nm cut-off filter. The optical filters cut-off over 99% of the ultraviolet light below the specified wavelength

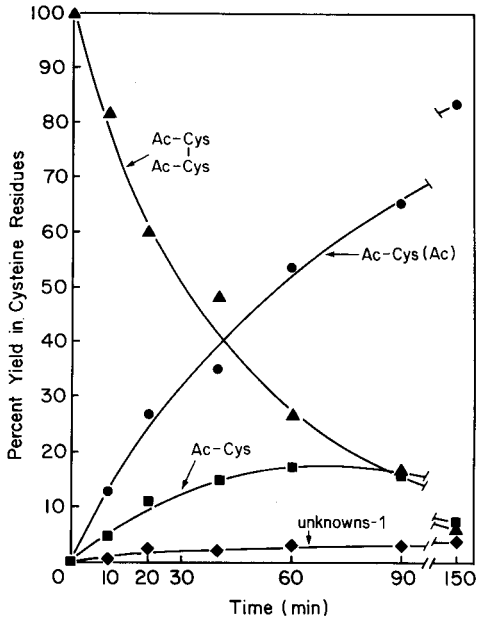


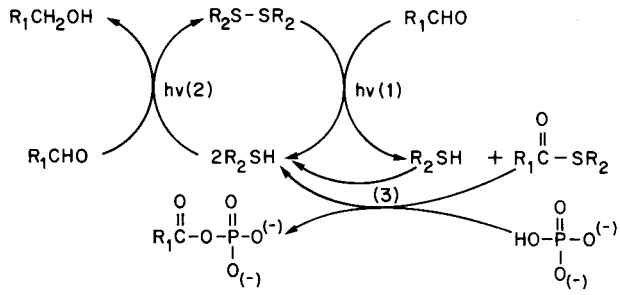
Fig. 2. Time course of formation of products from the illumination of 0.025 M N,N'-diacetylcystine and 1.0 M acetaldehyde in a 0.125 M sodium phosphate buffer (pH 7.0) with ultraviolet light. The ultraviolet light was optically filtered to remove over 99% of the light below 281 nm

acetaldehyde during illumination with filtered ultraviolet light. After 150 min of illumination the yield of Ac-Cys (Ac) is over 80%. The 80% yield of Ac-Cys(Ac) with 1.0 M acetaldehyde is four times the yield obtained with 0.5 M actealdehyde under the same conditions (see Fig. 1d). Ac-Cys is seen to accumulate during the first 60 min of the reaction and then decrease during the last 90 min of reaction. There is only a small amount of formation of unknown products. Phosphate is not required for the photochemical production of thioester, since illumination of 1.0 M acetaldehyde and 25 mM N,N'-diacetylcystine in an unbuffered solution at pH 2.5 with unfiltered light yields over 60% Ac-Cys(Ac).

Discussion

We have shown the photochemical synthesis of a thioester from an aqueous solution of a disulfide and acetaldehyde at neutral pH. This reaction produces the highest yield of thioester relative to other products with ultraviolet light between 295 nm and 320 nm. Although the mechanism of thioester formation has not been investigated, it seems likely that free radicals are involved in this reaction. Both aldehydes and disulfides yield free radical products when illuminated with ultraviolet light (Pitts and Wan 1966; Knight 1974; Calvert and Pitts 1966). It is also likely that the aldehyde accepts the radiant energy in the primary photoprocess, since only the aldehyde has an appreciable absorbance of ultraviolet light ($\lambda > 330$ nm) in similar reactions reported by Takagi (1976).

Takagi's earlier work on the ultraviolet illumination ($\lambda > 330$ nm) of organic disulfides in aldehyde solvents gave an equimolar yield of thioester and thiol, a result consistent with the stoichiometry of reaction hv (1), shown in the figure below. In our aqueous system with filtered ultraviolet light ($\lambda > 280$ nm), the yield of thioester is always greater than the yield of thiol. Also, with 1.0 M acetaldehyde the yield of thiol decreases late in the reaction. These results suggest that the thiol is being re-oxidized to the disulfide, which is used to form more thioester. Re-oxidation of the thiol is thought to be ultimately coupled to the reduction of acetaldehyde to ethanol, as shown in reaction hv (2). As shown in reaction (3) transfer of the acyl group of the thioester



to phosphate would produce acylphosphate anhydride, which could be used to drive the formation of pyrophosphates (Disabato and Jencks 1961). In the overall reaction scheme, the thiol (disulfide) acts catalytically to produce the useful free energy of hydrolysis of thioesters with the concomittant disproportionation of two moles of aldehyde to one mole of carboxylic acid and one mole of alcohol. This process resembles in some respects fermentation (glycolysis) in which the carboxylic acid and alcohol products are found in one molecule, lactic acid. Fermentation has generally been considered the most primitive form of energy metabolism, because it operates in the absence of oxygen by substrate-level phosphorylations, which do not require compartmentation (Broda 1975; Buvet 1978; Egami 1974).

We believe reactions between photo-excited aldehyde and disulfides that yielded thioesters occurred in the aqueous environment of the primitive Earth. Although related reactions between aldehydes and volatile disulfides could have produced thioesters in the atmosphere, it seems unlikely that these thioesters contributed to the energy needs of developing life on the Earth's surface. In the reducing environment of the primitive Earth, sulfur was present in considerable amount as H_2S in the atmosphere and HS^- in the hydrosphere (Ochiai 1978; Hart 1979). The absorption of ultraviolet light by H_2S (ϵ_{max} at 190 nm = 1.6×10^3) and HS^- (ϵ_{max} at 230 nm = 7.5×10^3, Jocelyn 1972) would screen

out damaging ultraviolet light below roughly 270 nm. This ultraviolet screen would protect disulfide groups in polypeptides that are susceptible to damage by ultraviolet light (Asquith and Shah 1971; Risi et al. 1967), while photo-excited aldehydes react to form thioesters.

If a ultraviolet screen was not present, the polypeptides could be shielded from ultraviolet light by the shadow of an opaque object or by water roughly 10 meters deep (Berkner and Marshall 1965). These protected polypeptides could scavenge thioesters that were produced photochemically from aldehydes and simple disulfides (thiols) in the surrounding aqueous environment (Raulin 1978). A simple scavenging mechanism would involve diffusion and subsequent acyl transfer from the thioester to imidazole or thiol groups of protected polypeptides (Bruice 1966; Stirling 1958; Stadtman 1952).

In our system, the yield of Ac-Cys(Ac) reaches a plateau that depends upon the acetaldehyde concentration. The maximum accumulation of Ac-Cys(Ac) with 1.0 M and 0.5 M acetaldehyde are estimated to be 85% and 20%, respectively. These values indicate that the maximum accumulation of Ac-Cys(Ac) at 0.1 M acetaldehyde would be 1–2% (1 mM). Although this concentration of thioester appears low, it could be maintained indefinitely in an aqueous enivronment as long as acetaldehyde and possibly other aldehydes, like glyceraldehyde and methylglyoxal, are available for disproportionation. The thioester, possibly via pyrophosphates (Oro and Stephen-Sherwood 1976; Hulshof and Ponnamperuma 1976), could maintain the synthesis of biopolymers over a long period of time to give prebiotically significant amounts of the biopolymers.

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References

Asquith RS, Shah AV (1971) Biochim Biophys Acta 244:547–556
Berkner LV, Marshall LC (1965) J Atmos Sci 22:225–261
Bridger WA (1974) Succinyl-CoA Synthetase. In: Boyer PD (ed) The Enzymes, vol X. Academic Press, New York, p 581
Broda E (1975) The Evolution of Bioenergetic Processes. Pergamon Press, Oxford, p 43
Bruice TC, Benkovic SJ (1966) Bioorganic Mechanisms, vol 1. Benjamin, New York, p 259
Buvet R (1978) Compared Energetics of Primordial and Biological Metabolisms. In: Noda H (ed) Origin of Life. Japan Scientific Societies Press, Tokyo, p 411
Calvert JG, Pitts JN Jr (1966) Photochemistry. Wiley, New York, p 368
DiSabato G, Jencks WP (1961) J Am Chem Soc 83:4400–4405
Egami F (1974) Orig Life 5:405–413
Fluharty AL (1974) Biochemistry of the Thiol Group. In: Patai S (ed) The Chemistry of the Thiol Group. Wiley, London, p 589
Goldman P, Vagelos PR (1964) Acyl-Transfer Reactions (CoA-Structure, Function). In: Florkin M, Stotz EH (eds) Comprehensive Biochemistry, vol 15. Elsevier, Amsterdam, p 71
Grassetti DR, Murray JF Jr (1969) J Chromatogr 41:121–123
Harris JI, Waters M (1976) Glyceraldehyde-3-Phosphate Dehydrogenase. In: Boyer PD (ed) The Enzymes, 3rd edn, vol XIII. Academic Press, New York, p 1
Hart MH (1979) Origins of Life 9:261–266
Hartman H (1975) J Mol Evol 4:359–370
Hollander L, DuVigneaud V (1931) J Biol Chem 94:243–252
Hong JH, Becker RS (1979) J Mol Evol 13:15–26
Hulshof J, Ponnamperuma C (1976) Origins of Life 7:197–224
Jencks WP (1976) Free Energies of Hydrolysis and Decarboxylation. In: Fasman GD (ed) Handbook of Biochemistry and Molecular Biology, 3rd edn. Physical and Chemical Data, vol I. CRC Press, Cleveland, p 296
Jocelyn PC (1972) Biochemistry of the SH Group. Academic Press, New York, p 50
Knight AR (1974) Photochemistry of Thiols. In: Patai S (ed) The Chemistry of the Thiol Group. Wiley, New York, p 455
Lorand L, Stenberg P (1976) Endo-γ-Glutamine: ε-Lysine Transferases Enzymes Which Cross-link Proteins. In: Fasman GD (ed) Handbook of Biochemistry and Molecular Biology, 3rd edn. Proteins, vol II. CRC Press, Cleveland, p 669
Miller SL (1957) Ann NY Acad Sci 69:260–275
Ochiai EI (1978) Origins of Life 9:81–91
Oro J, Stephen-Sherwood E (1976) Orig Life 7:37–47
Pitts JN Jr, Wan JKS (1966) Photochemistry of Ketones and Aldehydes. In: Patai S (ed) The Chemistry of the Carbonyl Group. Wiley, New York, p 823
Raulin F (1978) Incorporation of Sulfur in Chemical Evolution. In: Noda H (ed) Origin of Life. Japan Scientific Societies Press, Tokyo, p 177
Risi S, Dose K, Rathinasamy TK, Augenstein L (1967) Photochem Photobiol 6:423–436
Sagan C, Khare BN (1971) Science 173:417–420
Slater EC (1966) Oxidative Phosphorylation. In: Florkin M, Stotz EH (eds) Comprehensive Biochemistry, vol 14. Elsevier, Amsterdam, p 327
Smith HA, Gorin G (1961) J Org Chem 26:820–823
Stadtman ER (1952) J Biol Chem 196:535–546
Stadtman ER (1957) Preparation and Assay of Acyl Coenzyme A and Other Thioesters; Use of Hydroxylamine. In: Colowick SP, Kaplan NO (eds) Methods in Enzymology, vol III. Academic Press, New York, p 931
Stirling CJM (1958) J Chem Soc 4524–4530
Takagi M, Goto S, Matsuda T (1976) J Chem Soc Chem Commun 92–93
Zahler WL, Cleland WW (1968) J Biol Chem 243:716–719

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