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NOTE

Synthesis of ^{14}C-labelled methylglyoxal and S-D-lactoylglutathione

James D. Clelland and Paul J. Thornalley[1]

Department of Chemistry and Biological Chemistry
University of Essex
Wivenhoe Park, Colchester CO4 3SQ, U.K.

SUMMARY

[2-^{14}C]Methylglyoxal and S-D-[2-^{14}C]lactoylglutathione were synthesised from [2-^{14}C]acetone. Acetone was oxidised to methylglyoxal by selenium dioxide and was purified by distillation (yield: 23% based on acetone). Methylglyoxal was converted to S-D-lactoylglutathione by reaction with reduced glutathione catalysed by glyoxalase I (EC 4.4.1.5). S-D-Lactoylglutathione was purified by anion exchange chromatography on Dowex 1 (yield: 82%, based on methylglyoxal).

KEY WORDS carbon-14, methylglyoxal, S-D-lactoylglutathione, glyoxalase, glutathione.

INTRODUCTION

Methylglyoxal CH_3COCHO is formed in biological systems from dihydroxyacetone phosphate in a reaction catalysed by methylglyoxal synthase (EC 4.1.99.11) (1).

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[1] For correspondence

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$$^{2-}O_3POCH_2COCH_2OH \xrightarrow[\text{Synthase}]{\text{Methylglyoxal}} CH_3COCHO + HPO_4^{2-}$$

It is metabolised in all cells by the glyoxalase system which catalyses the conversion of methylglyoxal to D-lactate, via the intermediate S-D-lactoylglutathione. Glyoxalase I (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from the hemithioacetal formed non-enzymatically from methylglyoxal and reduced glutathione (2).

$$CH_3COCHO + GSH \rightleftharpoons CH_3COCH(OH)\text{-}SG \xrightarrow{\text{Glyoxalase I}} CH_3CH(OH)CO\text{-}SG$$

S-D-Lactoylglutathione is hydrolysed to reduced glutathione and D-lactate in biological systems by glyoxalase II (EC 3.1.2.6) (3)

$$CH_3CH(OH)CO\text{-}SG + H_2O \xrightarrow{\text{Glyoxalase II}} CH_3CH(OH)CO_2H + GSH$$

The glyoxalase system is present in all cells. The biological function of the glyoxalase system is unknown but is the subject of a current review (4).

Recent research has shown that the activities of glyoxalase enzymes are modified in the cell growth cycle, differentiation, during cellular secretion of cytoplasmic granules, and in disease processes (tumour growth and diabetes mellitus) (5,6,7,8). The significance of these changes in glyoxalase activity and concomitant regulation of the cellular concentrations of methylglyoxal and S-D-lactoylglutathione are currently under investigation. In order to study the fate of methylglyoxal and S-D-lactoylglutathione in cell systems such as their metabolism and binding to biological macromolecules, radiolabelled derivatives were required for tracer experiments.

^{14}C-Labelled methylglyoxal has been synthesised by microbial metabolism of [U-^{14}C]glycerol in a mutant of E. coli (9). Since these and other microbial cultures are not readily available in many laboratories, an alternative chemical procedure was developed to synthesise methylglyoxal from acetone, and an extended procedure to produce S-D-lactoylglutathione from methylglyoxal.

In this report we describe the synthesis and purification of [2-^{14}C]methylglyoxal and S-D-[2-^{14}C]lactoylglutathione from [2^{14}C]-acetone. Although the yield of the initial conversion of acetone to methylglyoxal was 23%, this is a convenient and relatively economic synthetic route to radiolabelled glyoxalase substrates.

MATERIALS AND METHODS

Materials

[2-^{14}C]Acetone, 1 mCi, was purchased from Amersham International plc (Amersham, Buckinghamshire, U.K.) with a specific activity of 56 mCi/mmol. Selenium dioxide, reduced glutathione, diamide (1,1'-azobis(N,N-dimethylformamide)), glyoxalase I (grade XI, lyophilised powder from yeast), glyoxalase II (lyophilised powder from bovine liver) and 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB) were obtained from Sigma Chem. Co. (Poole, Dorset, U.K.). All other reagents were of standard analytical grade.

Methods

Synthesis of [2-^{14}C]methylglyoxal from [2-^{14}C]acetone

[2-^{14}C]Methylglyoxal was synthesised by oxidation of [2-^{14}C]-acetone with selenium dioxide (10). Optimum reaction conditions were: [2-^{14}C]acetone (1 mmol, 1 mCi) incubated with 1 mmol of selenium dioxide in 0.35 ml of distilled water in an evacuated sealed vial at 60°C for 2.5 hours. Methylglyoxal was isolated by distillation. The purity was analysed by thin layer chromatography of the 2,4-dinitrophenylhydrazones on silica gel.

The concentration of methylglyoxal in the aqueous product solution obtained was assayed by conversion to S-D-lactoylglutathione, catalysed by glyoxalase I in the presence of reduced glutathione, and following the decrease in absorbance at 240 nm during hydrolysis after addition of glyoxalase II; $\Delta\epsilon_{240}$= -3.1 $mM^{-1}cm^{-1}$ (11).

Synthesis of S-D-[2-^{14}C]lactoylglutathione

[2-^{14}C]Methylglyoxal (11 mM) was incubated with reduced glutathione (11 mM) in 10 mM sodium phosphate, pH 6.6, with 25 units of glyoxalase I at 37°C for 60 minutes. Diamide (1 M in methanol) was added dropwise to the reaction mixture until a yellow colour persisted, indicating the oxidation of residual reduced glutathione. The product mixture (10 ml) was adjusted to pH 2.36 with 90% formic acid solution and loaded onto a column (2.6 x 40 cm) of Dowex I, formate form, equilibrated with 122 mM formic acid. The column was eluted with 122 mM formic acid until peaks of reduced and oxidised diamide had been eluted (detected by monitoring absorbance of the eluate at 254 nm), and with a linear gradient of 122-610 mM formic acid thereafter. Fractions were collected and analysed for radioactivity (by scintillation counting) and S-D-lactoylglutathione content (by measuring the decrease in absorbance at 240 nm on the addition of glyoxalase II; $\Delta\epsilon_{240}$ = -3.1 $mM^{-1}cm^{-1}$ (11)). Fractions containing S-D-lactoylglutathione were combined and lyophilised. The dry, white powder remaining was reconstituted in distilled water and stored in small aliquots at -20°C. (The compounds eluted from the column, as indicated by absorbance of 254 nm, were characterised by performing an identical synthesis with cold acetone. Fractions corresponding to an absorbance peak were pooled, lyophilised and the residual compound identified by proton n.m.r.).

Purity of the radiolabelled S-D-lactoylglutathione was analysed by thin layer chromatography on silica gel, analysis for

contaminating reduced glutathione by incubation with DTNB; $\Delta\epsilon_{412}$ = 13.6 $mM^{-1}cm^{-1}$ for thiol groups (12).

RESULTS

Synthesis of [2-^{14}C]methylglyoxal

Analysis of the purity of the aqueous product mixture by thin layer chromatography gave only one spot, corresponding to methyglyoxal (Table 1). The proton n.m.r. spectrum of methylglyoxal gave aldehydic proton peaks at 5.24 and 4.33 ppm and methyl proton peaks at 2.25 and 1.31 ppm for the monohydrate and dihydrated forms of methylglyoxal. The yield of methylglyoxal produced from acetone was 23%. Methylglyoxal was stored in aqueous solution at -20°C without significant decomposition over several months.

TABLE 1 Thin layer chromatographic analysis of [2-^{14}C]methylglyoxal on silica gel

Hydrazone derivative of:	R_f
Methylglyoxal	0.80
Acetone	0.95
Pyruvic acid	0.11
[2-^{14}C]methylglyoxal	0.80

Developing solvent: Chloroform/ethanol 25:2

Synthesis of S-D-[2-^{14}C]lactoylglutathione

The crude product was purified by anion exchange chromatography on Dowex I, formate form. The elution profile from the column is presented in Fig. 1. Surprisingly, some radioactivity was eluted with peaks previously identified as oxidised and reduced diamide.

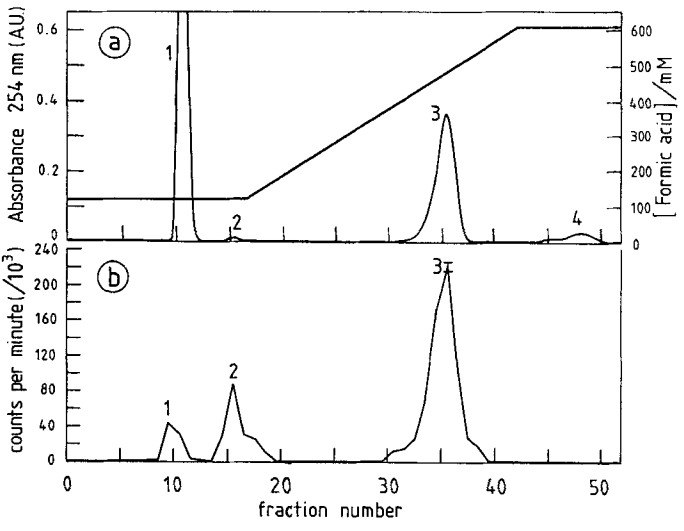

Fig. 1 **Purification of S-D-[2-^{14}C]lactoylglutathione by ion exchange chromatography on Dowex 1, formate form.**

a. Absorbance of eluate at 254 nm with overlay of the formic acid concentration of the eluant and gradient profile. Peak 1 - oxidised diamide, peak 2 - reduced diamide, peak 3 - S-D-lactoylglutathione and peak 4 - oxidised glutathione. b. Radioactivity content of collected fractions, as judged by scintillation counting. Fraction size 15 ml.

The S-D-lactoylglutathione was well resolved from other components. After isolation of the radiolabelled product by lyophilisation of the aqueous formic acid eluate, analysis of the product by thin layer chromatography gave only one spot (Table 2) and no reactivity with DTNB. Analysis of the S-D-lactoylglutathione product by glyoxalase II gave a yield of 82% (based on methylglyoxal).

TABLE 2 Thin layer chromatographic analysis of S-D-[2-^{14}C]lactoylglutathione on silica gel

Compound	R_f			
Solvent system	**a**	**b**	**c**	**d**
Reduced glutathione	0.228	0.124	0.172	0.242
Oxidised glutathione	0.207	0.082	0.032	0.423
S-D-Lactoyl-glutathione	0.450	0.247	0.210	0.272
S-D-[2-^{14}C]lactoyl-glutathione	0.450	0.247	0.210	0.272

Solvent system: a. butan-1-ol:acetic acid:water, 10:5:5; pentan-1-ol:acetic acid:water, b. 10:5:5, c. 10:5:1, d. 12:10:1

Detection: ninhydrin (0.2%) in ethanol

The pure S-D-lactoylglutathione, free acid, was stored in aqueous, unbuffered solution (~ 3 mM). S-D-Lactoylglutathione decomposes by non-enzymatic hydrolysis to reduced glutathione and lactic acid at pH > 7.4. Storage in unbuffered, acid conditions permits reliable preservation without the need to remove buffer salts before use in biological experiments.

DISCUSSION

A previous independent report of a similar synthesis of [^{14}C]methylglyoxal from [^{14}C]acetone under non-optimised conditions quoted a yield of only 16% (14). It was possible to obtain much higher yields (~ 70%) for the oxidation of acetone by selenium dioxide but when an excess of acetone was used (not practicable for

radiosynthesis) (6). The optimised (23%) yield obtained here may be due to the failure to degrade organoselenium intermediates (15).

The mechanism of the oxidation of acetone to methylglyoxal by selenium dioxide has not been fully elucidated. The effective oxidant is selenous acid, H_2SeO_3.The attack of selenous acid by the O- or C- of the enol form of acetone (I) has been proposed (Fig. 2). The ketoselenic acid formed (II) in both cases undergoes a Pummerer rearrangement to form the product methylglyoxal (III). A kinetic study of the oxidation of α-oxo methyl groups by selenous acid has shown the reaction to be first order with respect to each component: ketone, selenium dioxide and acid (16). However, methylglyoxal may undergo further reaction with selenous acid to oxidise the remaining methyl group. This reaction may also contribute to the low yield of methylglyoxal obtained in the synthesis, which is consistent with our observation that the formation of methylglyoxal from acetone was maximum after 2.5 h of reaction time and decreased rapidly thereafter.

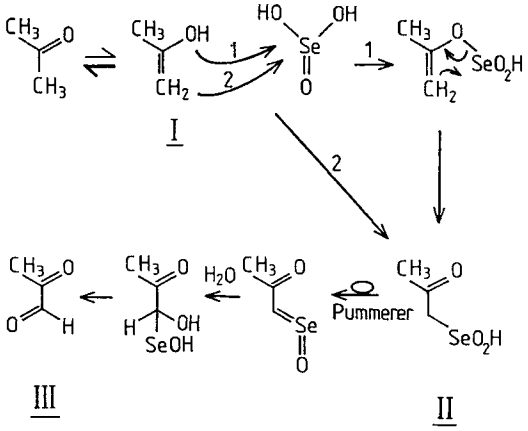

Fig. 2 **Mechanism proposed for the oxidation of acetone to methylglyoxal by selenous acid.** Taken from Ref. 15.

S-D-[2-^{14}C]lactoylglutathione was synthesised in good yield from [2-^{14}C]methylglyoxal and reduced glutathione in a reaction catalysed by glyoxalase I. We have recently optimised methods for the large-scale synthesis of S-D-lactoylglutathione and other S-acylglutathiones by this and similar methods (17). In the purification method employed, diamide was used to oxidise contaminating reduced glutathione to oxidised glutathione since S-D-lactoylglutathione can be readily resolved from oxidised glutathione but not from reduced glutathione by chromatography on Dowex I (13,17,18). From the scintillation counting data, some radioactivity was associated with the reduced and oxidised diamide peaks. This may be due radiolabelled methylglyoxal binding to the reduced and oxidised diamide.

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REFERENCES

1. Cooper R.A. - Ann. Rev. Microbiol. 38: 49 (1984)
2. Sellin S.,Aronsson A.-C., Eriksson L.E.G., Larsen K., Tibbelin G. and Mannervik B. - Functions of Glutathione, Raven Press, New York, pp. 187-197 (1983)
3. Uotila L. - Glutathione. Chemical, Biochemical and Medical Aspects (Dolphin D., Poulson R. and Avramovic O., eds.), Part A, pp. 767-804 (1989)
4. Thornalley P.J. - Biochem. J. 269: 1 (1990)
5. Hooper N.I., Tisdale M.J. and Thornalley P.J. - Cell. Mol. Biol. 34: 399 (1988)
6. Hooper N.I., Tisdale M.J. and Thornalley P.J. - Leuk. Res. 11: 1141 (1987)

7. Thornalley P.J. and Bellavite P. - Biochim. Biophys. Acta 931: 120 (1987)
8. Thornalley P.J. and Atkins T.W. - Med. Sci. 17: 777 (1989)
9. Krymkiewicz N. - FEBS Lett. 19: 51 (1973)
10. Matsumoto I. - Musashino Chem. Res. Inst. Ltd., Japan Kokai Tokkyokoho 81,40632
11. Thornalley P.J. - Biochem. J. 254: 751 (1988)
12. Ellman G.L. - Arch. Biochem. Biophys. 82: 70 (1959)
13. Ball, J.C. and Van der Jagt, D.L. - Biochemistry 20: 899 (1981)
14. Hall S.S., Doweyko A.M. and Jordan F. - J. Amer. Chem. Soc. 100: 5934-5939
15. Paulmier C. - Selenium Reagents and Intermediates in Organic Synthesis, Pergamon Press, Oxford, UK, p. 360
16. Singh H and Anand S.N. - J. Ind. Chem. Soc. 51: 363 (1979)
17. Clelland J.D. and Thornalley P.J. - submitted for publication
18. Uotila L. - Biochemistry 12: 3938 (1973)