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Note

Thin-layer chromatography of oxidised and reduced lipoate and lipoamide and their persulfides

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Procedures have been described for the separation by thin-layer chromatography (TLC) of α-lipoate from other polar aliphatic disulfides[1] and from the products of its photolysis[2]. However, no methods have been published for the separation of the oxidised and reduced forms of lipoic acid and lipoamide or their persulfides. These latter compounds have been reported to be intermediate products in the scission of thiosulfate catalysed by the enzyme rhodanese from beef liver[3].

The object of our studies was to determine whether persulfides of dihydrolipoate and dihydrolipoamide were intermediate products of the thiosulfate-splitting reaction catalyzed by rhodanese isolated from *Thiobacillus* A2. In order to do this, it was necessary to develop a chromatographic system using a solvent which was neither very acidic, which would cause the hydrolysis of the sulfide moiety, nor very alkaline, which would cause the oxidation of dihydrolipoate, dihydrolipoamide, and their persulfides to α-lipoate and lipoamide.

MATERIALS AND METHODS

Dihydrolipoate, α-lipoic acid and lipoamide were purchased from Sigma (London, Great Britain). Dihydrolipoamide was prepared from lipoamide by the method of Reed *et al.*[4]. The persulfides of dihydrolipoate and dihydrolipoamide were prepared by allowing these compounds in aqueous solution to react individually with sulfide, each at a concentration of 0.01 *M*, for 3 h at room temperature[3].

Rhodanese was purified from *Thiobacillus* A2 grown on 0.5% (w/v) thiosulfate[5] by a procedure similar to that previously described[6]. The enzyme was assayed by a modification of the method of Volini and Westley[7] using either dihydrolipoate or dihydrolipoamide as sulfide acceptor.

TLC was performed using silica gel 60 F_{254} precoated aluminium sheets (Merck, Darmstadt, G.F.R.) (layer thickness 0.2 mm). The solvents, made from analytical grade reagents, were as follows: (1) *n*-propanol–35% NH_3 (7:3); (2) *n*-butanol–35% NH_3 (7:3); (3) *n*-propanol–acetic acid–35% NH_3 (7:2:3); (4) *n*-butanol–acetic

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acid–35% NH_3 (7:2:3); (5) *n*-propanol; (6) *n*-butanol; (7) *n*-propanol–acetic acid–35% NH_3 (7:3:2); (8) *n*-butanol–acetic acid–35% NH_3 (7:3:2).

Marker compounds were applied by means of a micropipette 1.0 cm from the lower edge of the chromatography sheet in the following amounts: 5 μl each of 0.1 *M* α-lipoate, 0.04 *M* lipoamide in 80% (v/v) ethanol, 0.1 *M* dihydrolipoate in 0.1 M NaOH, and 0.1 *M* dihydrolipoamide; 25 μl each of the preparations of dihydrolipoate persulfide and dihydrolipoamide persulfide. In addition, various mixtures of these compounds, and also 25–50 μl of the rhodanese assay systems after reaction times of 2 h were applied. In the reaction mixtures which had originally contained 0.01 *M* dihydrolipoate or dihydrolipoamide, 1.3×10^{-3} *M* dihydrolipoate persulfide and/or α-lipoate and 2.0×10^{-3} *M* dihydrolipoamide persulfide and/or oxidised lipoamide, respectively, were formed. These concentrations were calculated using a molar extinction coefficient of 150 for α-lipoate and lipoamide[8]. As the maximum absorbance for all these compounds is at 230–235 nm, it was not possible to distinguish one from the others by spectrophotometry.

After development of the chromatography sheets in one of the aforementioned solvents for a distance of approx. 10 cm (3–5 h), the sheets were air dried and the compounds were detected either with a Camag Universal UV lamp, which provided radiation at 254 nm, or by exposure to iodine vapour. Both α-lipoate and lipoamide were very easily detected by UV light; dihydrolipoate and dihydrolipoamide absorbed the light less strongly, whereas the persulfides were not present in concentrations great enough to be detected by this method. After 5-min exposure to iodine vapour, both the α-lipoate and lipoamide spots turned brown, whereas the spots of all the other compounds remained lighter than the background, similar to those reported by Brown and Edwards[9]. After longer exposure, all compounds could be readily detected as brown spots.

RESULTS AND DISCUSSION

Solvents 1–4 could successfully separate α-lipoate from lipoamide, but were sufficiently alkaline to cause oxidation of all other compounds tested. The R_F values for α-lipoate and lipoamide, respectively, are as follows: solvent 1, 0.73 and 0.91; solvent 2, 0.34 and 0.80; solvent 3, 0.78 and 0.84; solvent 4, 0.19 and 0.57. In the presence of all other solvents tested, little or no alteration of the test compounds occurred. As shown in Table I, α-lipoate, dihydrolipoate and dihydrolipoate persulfide were not successfully separated by solvents 5 and 6, but the latter compound was well separated from the former two by solvents 7 and 8. Likewise, solvent 5 could not separate lipoamide, dihydrolipoamide, and dihydrolipoamide persulfide, but dihydrolipoamide was separated from lipoamide and dihydrolipoamide persulfide by solvent 6. Solvents 7 and 8 were successful in distinguishing dihydrolipoamide persulfide from lipoamide and dihydrolipoamide, although these latter two compounds were not separated from each other.

When an aliquot of the rhodanese assay system after a reaction time of 2 h was subjected to chromatography using solvent 7 and exposed to iodine vapour, brown spots could be easily detected with R_F values of 0.92 and 0.59 when dihydrolipoate was used as substrate, and R_F values of 0.89 and 0.59 when dihydrolipoamide was used as substrate. Similarly, using solvent 8, R_F values of 0.91 and 0.41 were

TABLE I

TLC OF OXIDISED AND REDUCED LIPOATE AND LIPOAMIDE AND THEIR PERSULFIDES

Compound	R_F			
	Solvent 5	*Solvent 6*	*Solvent 7*	*Solvent 8*
α-Lipoate	0.77	0.45	0.90	0.93
Dihydrolipoate	0.77	0.48	0.92	0.93
Dihydrolipoate persulfide	0.80	0.45	0.60	0.43
Lipoamide	0.90	0.54	0.88	0.81
Dihydrolipoamide	0.95	0.62	0.86	0.81
Dihydrolipoamide persulfide	0.95	0.52	0.60	0.43

observed when dihydrolipoate was used as substrate, and R_F values of 0.80 and 0.43 when dihydrolipoamide was used. In all cases when exposed to iodine vapour, the spots of lower R_F values remained lighter than the background for over 5 min, turning darker upon continued exposure.

The results of this study show that the persulfides of dihydrolipoate and dihydrolipoamide can successfully be separated from their parent compounds. Using this fact, we present evidence to indicate that rhodanese of *Thiobacillus* A2 could indeed use dihydrolipoate and dihydrolipoamide as acceptor of the sulfide moiety from thiosulfate as has been suggested previously[6] and is thus similar to the mammalian enzyme in that the persulfides of these compounds are formed as intermediate products[7].

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