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Note

High-performance liquid chromatography of some coenzyme M (2-mercaptoethanesulphonic acid) derivatives by ion pairing on reversed-phase columns

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The recently discovered coenzyme M (2-mercaptoethanesulphonic acid), HS–CoM[1], has been found in all methanogens tested, but not in other organisms[2]. Methylated coenzyme M (CH_3–S–CoM) has been shown to be the first product to accumulate in substrate quantities during carbon dioxide reduction by cell extracts of *Methanobacterium bryantii*[1]. From more recent results presented by Thiel[3] and by Hermans *et al.*[4], it appears that other derivatives of coenzyme M, *e.g.* hydroxymethyl-coenzyme M (CH_2OH–S–CoM), formyl-coenzyme M (CHO–S–CoM) and acetyl-CoM (CH_3–CO–S–CoM), may possibly play a role in the metabolism of these bacteria. In our endeavour to investigate this possibility, we sought a fast and sensitive method which entails minimal sample manipulation to make an unambiguous quantitative determination of these compounds. The polarity and thermal lability of these compounds eliminated gas chromatography, while the work of Hermans *et al.*[4] ruled out isotachophoresis on complex whole-cell extracts. High-performance liquid chromatography (HPLC) seemed eminently suitable for this application. In this paper we report the chromatographic behaviour of some coenzyme M derivatives, *e.g.*, HS–CoM, CH_3–CO–S–CoM and $(S–CoM)_2$, on several columns using various elution conditions.

MATERIALS

The equipment used was a Spectra-Physics SP 8000 B liquid chromatograph equipped with a Model SP8400 (UV–VIS) variable-wavelength detector and a Model SP 8010 autosampler.

Methanol (Merck, Johannesburg, South Africa) and water from a Millipore Milli-Q system were used throughout this work and were always prefiltered (0.45 μm pore size) through PTFE discs. PIC-A® reagent (tetrabutylammonium hydrogen sulphate) was obtained from Waters Assoc., Johannesburg, South Africa. Whenever buffers or counter-ions were used, these were dissolved in each solvent separately and then the solutions filtered. The composition of the mobile phase was controlled by a low-pressure ternary proportioning valve according to programmed instructions.

Separations were performed with various packing materials in commercial stainless-steel columns (250 × 4.6 mm I.D.) equipped with precolumns. All experi-

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ments were conducted at 40°C, after preliminary experiments in the range of 30–70°C had shown that optimal separation was obtained at this temperature. Coenzyme M was purchased (Merck), while the derivatives were prepared in our laboratory. Their authenticity was checked by ^{1}H nuclear magnetic resonance spectroscopy. Their synthesis will be described elsewhere. Standards of each were prepared by dissolving 10 mg in 10 ml of water, and a mixture containing all four at 2.5 mg/10 ml was used for injections (20-μl sample loop). The instrument was programmed to inject consecutively water, the mixture containing the four compounds, and the four compounds separately at 100, 80, 60, 40, 20 and 0% methanol phase, the remainder being water phase. Cells of *M. bryantii* were cultivated in a 10-litre New Brunswick fermenter as described by Theil[3] on a defined medium, under an atmosphere of hydrogen–carbon dioxide (80:20).

The cells were harvested by centrifugation and the cell protein determined by the heated biuret Folin method[5]. A cell suspension was prepared containing 20 mg of cell protein in 20 ml of 0.01 *M* bicarbonate buffer (pH 7.0). This suspension was sonified for 30 min at the maximum output of a Branson sonifier (the temperature rising to 80°C, destroying all enzymes) and spiked with 10 mg of each of the HS–CoM, CH_3–S–CoM and CH_3–CO–S–CoM, then centrifuged at 20,000 *g* for 20 min and filtered through a 0.45-μm pore size filter disc.

RESULTS AND DISCUSSION

The chromatographic behaviour of coenzyme M and its derivatives is governed primarily by the sulphonic acid group, which has a pK_a of approximately 2 (ref. 6). Because silica-based columns are stable only in the pH 2–8 range, the coenzyme M derivatives have to be chromatographed in their ionized state. The following columns were used: Zorbax C_{18}, Zorbax C_8, Zorbax C_1, Zorbax-NH_2, Ultrasphere ODS and μBondapak C_{18}. All the columns gave no retention of the CoM derivatives when using methanol–water mobile phases. The Zorbax-NH_2 column gave very long retention times for the CoM derivatives when the methanol and water phases contained 10 m*M* citric acid (pH 2.3), capacity factor ($k' > 20$ with 100% methanol. The best results were obtained with the μBondapak C_{18} column and a methanol–water mobile phase containing 0.05 *M* tetrabutylammonium hydrogen sulphate as counter-ion. The run-time for each chromatogram was 30 min, thus enabling us to follow the derivatives until their k' value became *ca.* 20. Preliminary experiments indicated that the best wavelength for detecting all four compounds was 220 nm. The dependence of the k' values of the standards on the percent methanol in the mobile phase when using 0.005 *M* tetrabutylammonium hydrogen sulphate as counter-ion is illustrated in Fig. 1. The oxidized form of the coenzyme M contains two sulphonic acid groups ($^{-}O_3S$– and is retained much longer than the other derivatives; however, since its participation in anabolism is most unlikely, its detection is not essential. Thus the separation of the other three standards was concentrated upon and is shown in Fig. 2. Fig. 3 illustrates the separation of the coenzyme derivatives (0.50 mg in 1 ml) in a sonified whole-cell extract (1 mg/ml total cell protein). The linearity of the detector was confirmed in the 2–40 μg (0.1–2 mg/ml, 20-μl injection loop) range.

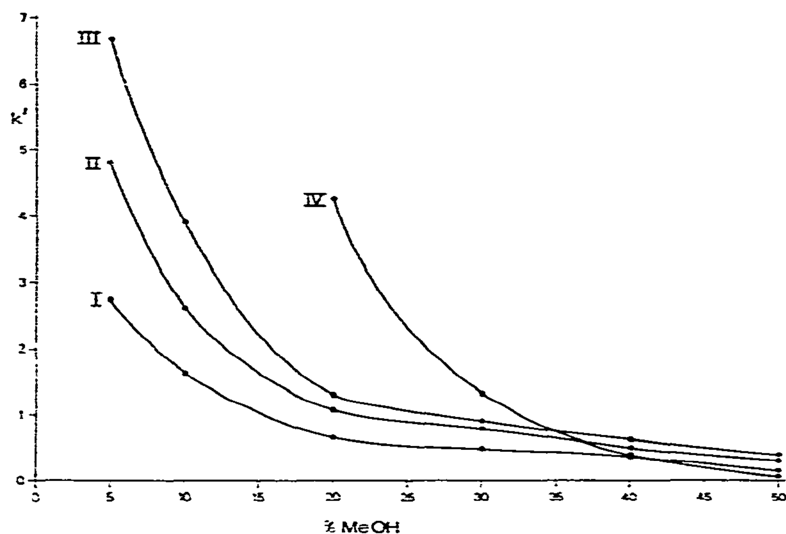

Fig. 1. Chromatographic behaviour of the four standards on a μBondapak C_{18} column (Waters Assoc.) with methanol (MeOH)–water mixtures, each solvent containing 0.005 *M* tetrabutylammonium hydrogen sulphate. Flow-rate, 2 ml/min. Pressure, *ca.* 1500 p.s.i. Temperature, 40°C. I = H–S–CoM; II = CH_3–S–CoM; III = CH_3CO–S–CoM; IV = $(S–CoM)_2$.

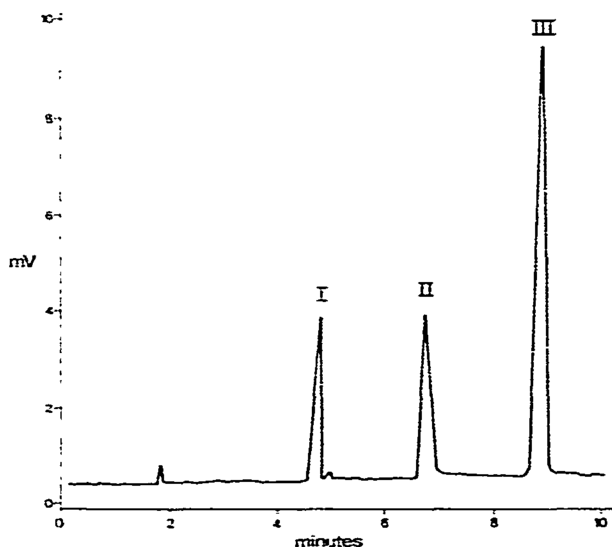

Fig. 2. Reversed-phase ion-paired separation H–S–CoM (I), CH_3–S–CoM (II) and CH_3–CO–S–CoM (III), each compound at 0.25 mg/ml in water. The isocratic mobile phase consisted of 5% 0.005 *M* methanolic tetrabutylammonium hydrogen sulphate and 95% 0.005 *M* aqueous tetrabutylammonium hydrogen sulphate. Flow-rate, 1 ml/min. Temperature, 40°C.

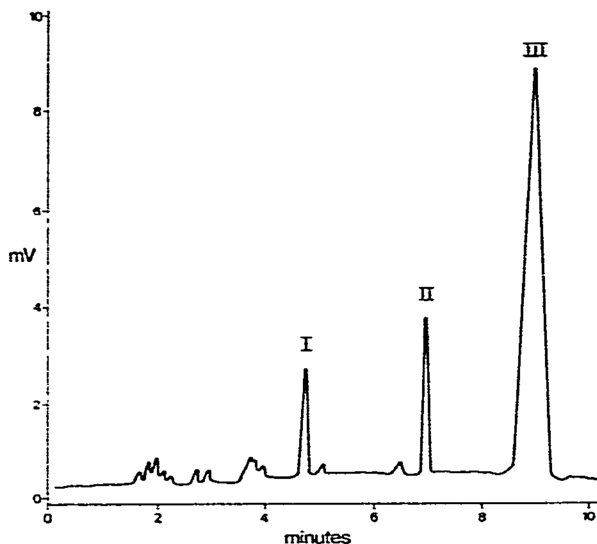

Fig. 3. Chromatogram of the three standards shown in Fig. 2, in the presence of 1 mg/ml total cell protein of *M. bryantii*, sonified and centrifuged whole-cell extract. Chromatographic conditions as in Fig. 2.

CONCLUSION

The derivatives of coenzyme M can be resolved by ion-pairing techniques on reversed-phase columns. This method can be used to analyse these derivatives in centrifuged and filtered sonified whole cell extracts. The method requires minimal sample clean-up, making it very suitable for assaying many samples in a short period of time.

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