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Note

Isotachophoretic control analysis of the enzymatic synthesis of 7-aminodesacetoxycephalosporanic acid

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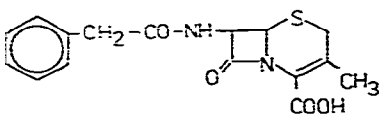
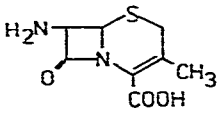
The increasing resistance to antibiotics of various pathogenic microorganisms has necessitated development of new antibiotics. Amongst these, semisynthetic cephalosporins have some advantages compared to other antibiotics: (a) they act against a wide spectrum of microorganisms; (b) various types of cephalosporins may be prepared by chemical modification of 7-aminocephalosporanic acid[1].

Another starting compound for the synthesis of various types of cephalosporins is 7-aminodesacetoxycephalosporanic acid (7-ADCA). It is prepared by enzymatic cleavage of desacetoxycephalosporin G (DCG), phenylacetic acid (PhAch) being lost. Analytical control of this delicate step is necessary.



DCG



7-ADCA

This paper demonstrates that isotachophoresis is a suitable method for the control of enzymatic cleavage of DCG.

EXPERIMENTAL

DCG was prepared from the natural penicillin G[2–5] and isolated as its crystalline potassium salt. 7-ADCA was prepared as described below, and purified by recrystallization. All other chemicals were supplied by Lachema (Brno, Czechoslovakia).

For the enzymatic conversion, a 10% solution of DCG was incubated with cells possessing penicillinacylase activity[6] at 37°C. The pH value of the reaction mixture was kept at 7.6, by means of 2 *M* NaOH or 4 *M* NH_3.

The analyses were carried out in an apparatus for cascade isotachophoresis[7] equipped with a potential gradient detection system[8]. A high concentration leading

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electrolyte (HCLE), 0.2 *M* 2,6-dimethylpyridine + 0.1 *M* HCl, pH 6.85, and a low concentration leading electrolyte (LCLE), 0.02 *M* 2,6-dimethylpyridine + 0.01 *M* HCl, pH 6.85, were employed. 0.3% Hydroxypropyl-cellulose was added to both leading electrolytes to improve separation by the enhancement of viscosity and reduction of electroendoosmosis[9]. 0.1 *M* Sodium 5,5'-diethylbarbiturate was used as the terminating electrolyte. The analysis first proceeded on the level of the HCLE at 550 μA for 12 min and then continued at 300 μA with the detection of zones on the level of the LCLE.

RESULTS AND DISCUSSION

Preliminary experiments have shown poor separation of DCG and 7-ADCA in acidic media. At pH 6 all components were separated, but no pure and stable terminating substance with adequate effective mobility was found. Thus 2,6-dimethylpyridine as counter-ion and a pH of 6.85 were used. The obtained separation can be seen in Fig. 1. Bicarbonate interference due to absorption of atmospheric CO_2 was kept negligible by using fresh terminating electrolyte for each analysis.

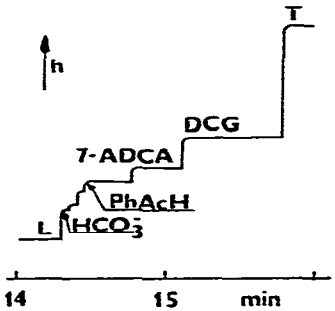
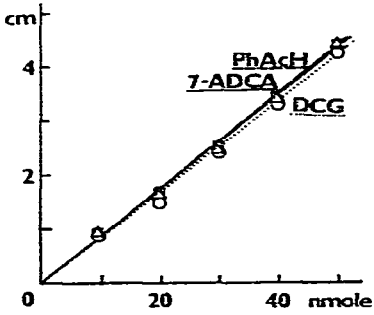


Fig. 1. Isotachophoregram of the reaction mixture 5 min after initiation of the reaction. Sample injected: 0.6 μl. h = Detector signal. Leading (L) and terminating (T) anions were chloride and 5,5'-diethylbarbiturate, respectively. For separation conditions see Experimental.



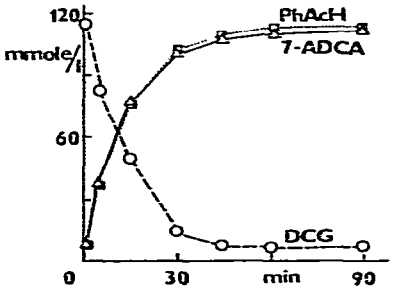


Fig. 2. Dependence of step length on the analyzed amount. Sample injected: 1–5 μl of a standard mixture containing 10 mmole/l each of phenylacetic acid, 7-ADCA and DCG, respectively. Chart speed: 8 cm/min. For separation conditions see Experimental.

Fig. 3. Course of the enzymatic cleavage of DCG, as measured by isotachophoresis.

For quantification purposes, calibration graphs (step length *vs.* amount analyzed) were constructed in the range 10–50 nmole (Fig. 2). The calibration was linear, with correlation coefficients of 0.997, 0.997 and 0.998 and standard deviations of the regression line of 1.37, 1.31 and 0.98 nmole for DCG, 7-ADCA and phenylacetic acid, respectively.

The procedure described was successfully applied for monitoring of the enzymatic conversion of DCG into 7-ADCA and enabled control of its course (Fig. 3).

The short analysis time for both the parent compound (DCG) and reaction products (7-ADCA and phenylacetic acid), together with the simple quantification, small sample size and lack of pretreatment, demonstrate that isotachophoresis is a convenient analytical method in the control of 7-ADCA production.

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