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Note

Cleavage of Peptidoglycan Monomer under Cathodic Electrolysis Conditions

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The cleavage reaction of the peptidoglycan monomer **1** was investigated under cathodic electrolysis conditions. Using a platinum cathode in a divided electrolytic cell, at constant current density, the D-lactoylpentapeptide **2**, a potential active immunoadjuvant, was obtained. The influence of current densities and temperature of the catholyte on the yields of the product was also studied.

INTRODUCTION

It was described earlier that the peptidoglycan monomer from *Brevibacterium divaricatum*, characterized previously[1–3] as [2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D--glucopyranosyl)-2-deoxy-3-*O*-(D)-ethyl-1-carbonyl)-D-glucopyranose]-L-alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(D)-amide-(L)-D-alanyl-D-alanine] (**1**), under mild alkaline conditions undergoes splitting at the C-3 ether linkage[1,4,5] to give the D-lactoylpentapeptide: 2-hydroxypropionyl-L-alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(D)-amide-(L)-D-alanyl-D-alanine] (**2**) and the corresponding disaccharide chitobiose **3**.

In order to find a convenient procedure for the preparation of the interesting D-lactoylpentapeptide **2** and its derivatives as potential active immunoadjuvants, we investigated the reaction mentioned above, under the conditions of the preparative electrochemical method (cathodic electrolysis).

RESULTS AND DISCUSSION

Cathodic electrolyses of the peptidoglycan monomer were carried out in a divided electrolytic cell with a ceramic diaphragm using a platinum cathode and a lead anode at constant current densities of 1.0–2.0 A dm^{-2} and at different temperatures (room temperature and 5 °C). The reaction was followed by the TL chromatography method.[6] The catholyte was a peptidoglycan monomer solution in water with sodium sulphate and a solution of sodium sulphate with a lead anode, served as the anolyte. Electrolysis was performed under a nitrogen atmosphere.

After completion of the electrolysis, the catholyte was neutralized and partially evaporated under reduced pressure. The crude product, lactoyl pentapeptide **2**, was purified by several successive treatments, using both Sephadex and Silicagel columns. Then, **2** was characterized and identified as presented in the Experimental section.

Representative results of the cathodic electrolyses of **1**, depending on the current densities and temperatures of the catholyte, are presented in Table I.

TABLE I

Results of the cathodic reaction, 2% solution of the peptidoglycan monomer **1** *in water with 0.1% sodium sulphate, Pt cathode*

Experiment	Temperature °C	Current density A dm^{-2}	Yield of **2** %
1	25	1.3	59
2	5	1.3	65
3	5	1.0	64
4	5	2.0	49

It is obvious that the optimal reaction conditions were those in experiment 2. Isolation of the product was carried out after the known procedure.[1] The product of cathodic electrolyses **2** was obtained pure and the procedure was relatively simple.

In general, it can be concluded that the cleavage of the peptidoglycan monomer under the cathodic electrolysis conditions represents a convenient preparative procedure for obtaining D-lactoylpentapeptide **2** as a potentially active immunomodulator. It was found[7,8] that an enzymatic cleavage of the peptidoglycan monomer produced the biologically active pentapeptide: L-alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(D)-amide-(L)-D-alanyl-D-alanine].

The mechanism of the cleavage reaction was not the subject of this paper, but most probably, what is in question is the controlled hydrolysis of **1** in a convenient alkaline electrolytically produced medium.

EXPERIMENTAL

Peptidoglycan monomer was produced in Pliva, Zagreb. All chemicals used were of analytical grade (Merck, Aldrich). Potentiometric measurements (determination of the neutralization equivalent) were made on a 672 Titroprocessor, Metrohm, the ^{13}C-NMR spectrum was scanned on a Jeol FX-90Q (90 MHz) spectrometer. Preparative electrolyses were performed using a Fisher Electroanalyzer.

Cathodic electrolyses – a representative experiment: Typically, 1.0 g of the peptidoglycan monomer was dissolved in 50 cm^3 of water and, after addition of 50 mg of sodium sulphate, cathodically electrolyzed in an electrolytic cell with a ceramic diaphragm (alundum cup – Fischer) and a platinum cathode (7.54 cm^2). The reaction was carried out under a positive nitrogen pressure with magnetic stirring. The anolyte was a solution (10 cm^3) od 1% sodium sulphate in water with a lead foil anode. Cathodic electrolysis was followed by the TLC: Merck Silicagel $60F_{254}$, solvent system: *n*-propanol–water (70 : 30); chromatograms were detected as described

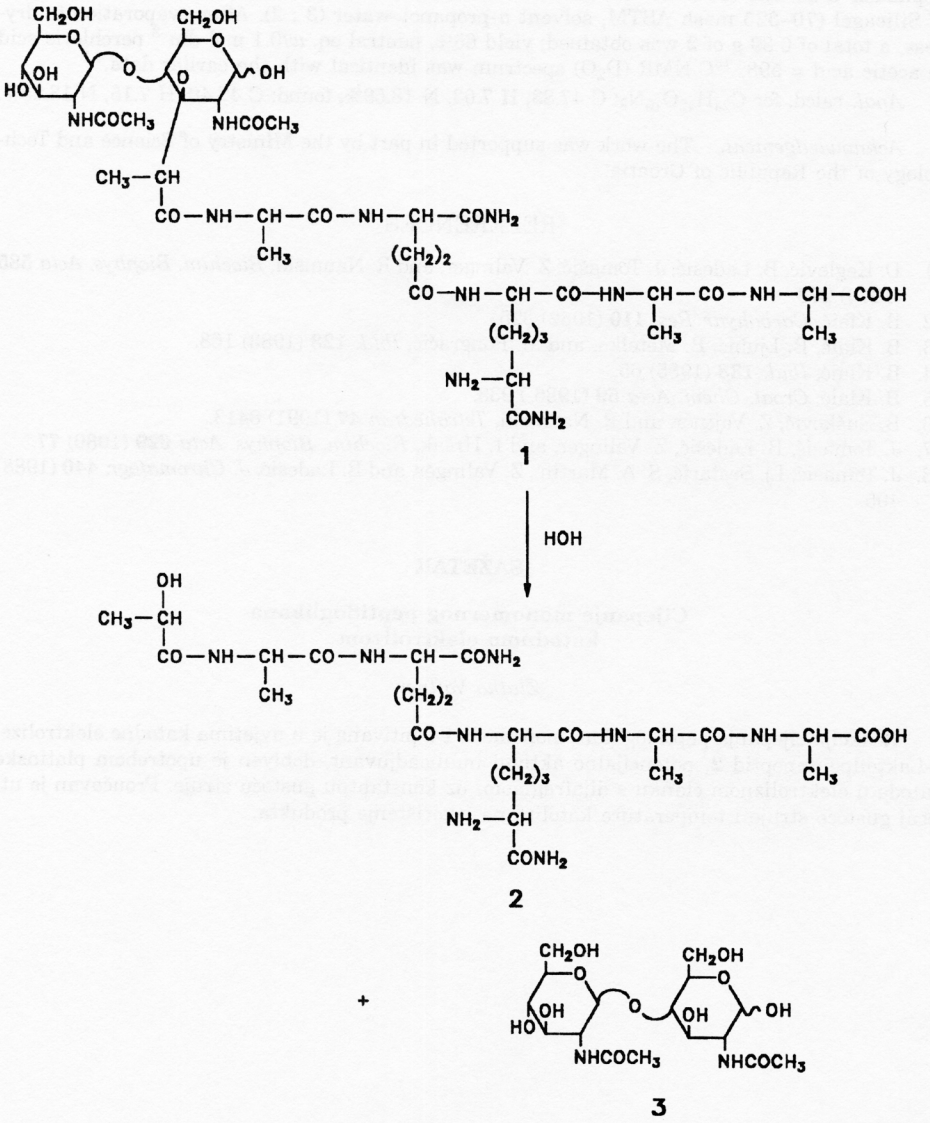

Figure 1. Cleavage of peptidoglycan monomer under the cathodic electrolysis conditions

earlier.[6] Using the constant current density of 1.3 A dm^{-2} at 5 °C, the reaction was completed after consumption of 3.8 F mol^{-1}.

Isolation of **2**: The pH value of the catholyte (*cca* 8.5) was first adjusted to 6.0 by 5% sulphuric acid and the solution was evaporated under reduced pressure to *cca* 5 ml. Aliquots corresponding to *cca* 100 mg of the peptidoglycan monomer were treated using a column of

Sephadex G-25 fine (30 x 1.5 cm, solvent water), and then by using (2 – 3 times) a column of Silicagel (70–325 mesh ASTM, solvent *n*-propanol–water (3 : 2). After evaporation to dryness, a total of 0.39 g of **2** was obtained; yield 65%, neutral eq. *w*/0.1 mol dm^{-3} perchloric acid in acetic acid = 598, ^{13}C-NMR (D_2O) spectrum was identical with the earlier data.[4,5]

Anal. calcd. for $C_{24}H_{42}O_{10}N_8$: C 47.83, H 7.03, N 18.59%; found: C 47.40, H 7.15, N 18.25%.

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SAŽETAK

Cijepanje monomernog peptidoglikana katodnom elektrolizom

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Reakcija cijepanja peptidoglikan monomera **1** ispitivana je u uvjetima katodne elektrolize. D-Laktoilpentapeptid **2**, potencijalno aktivni imunoadjuvant, dobiven je upotrebom platinske katode u elektroliznom članku s dijafragmom, uz konstantnu gustoću struje. Proučavan je utjecaj gustoće struje i temperature katolita na iskorištenje produkta.