SHORT COMMUNICATION

Physicochemical Characteristics of Listeria Specific Antigen 2

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Listeria specific antigen 2 (Ag2) was purified to within 97% of homogeneity, with a high yield, using both gel filtration and polyacrylamide gel electrophoresis. Ag2 is a glycoprotein. Its isoelectric point is about 4.2. As determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, its molecular weight is 16710 ± 450. Ag2 may aggregate easily since it was previously found in gel filtration in a peak corresponding to a molecular weight of 160000. No enzyme activity has been found in Ag2.

INTRODUCTION

Previous antigenic mapping of Listeria monocytogenes showed antigen 2 (Ag2) to be the main immunogen in both immunized rabbits and infected patients. This antigen was purified by an immunoadsorption procedure and was shown to be located on the surface of the bacteria (Delvallez et al., 1979). Its specificity, restricted to the genus Listeria, allowed the use of Ag2 for diagnostic purposes by enzyme-linked immunosorbent assay, which was able to detect anti-Ag2 IgG or IgM antibodies in human infection (Carlier et al., 1978).

The aims of this work were to study a procedure for purifying Ag2 that gave a higher yield than the immunoadsorption method, to measure the molecular weight, isoelectric point, amino acid and hexosamine composition of Ag2 and to investigate its possible enzyme activity.

METHODS

Soluble antigenic extracts of Listeria monocytogenes. A crude soluble extract of Listeria monocytogenes Serovar-4b (collection of Dr H. P. R. Seeliger) was obtained by X-press (LKB-Biotech) cell treatment and differential centrifugation; acrylamide agarose (ACA 44) gel filtration chromatography of the extract led to the preparation of a partially purified antigen 2 (PPAg2), as previously described (Delvallez et al., 1979).

A trichloroacetic acid (TCA) extract of L. monocytogenes was prepared by mixing crude soluble extract in 1 ml distilled water with 3 ml cold 10% (w/v) TCA and stirring for 15 min at 4 °C. After centrifugation at 5500 g for 30 min, the TCA-soluble material was recovered, dialysed against distilled water and lyophilized.

Specific anti-Ag2 sera. These were obtained from rabbits, as previously described (Delvallez et al., 1979). Polyacrylamide gel electrophoresis (PAGE). Analytical sodium dodecyl sulphate (SDS)-PAGE was
carried out according to Laemmli (1970), as modified by Delacourte et al. (1977), to determine the molecular weight of purified Ag2. Analytical PAGE without SDS in a 5 to 15% (w/v) polyacrylamide gradient and in 10% (w/v) polyacrylamide were performed to analyse PPAg2 and to localize the Ag2 for further preparative PAGE. Three strips of gel were stained with Coomassie blue, periodic acid-Schiff reagent (PAS) or Sudan black. An unstained strip of gel was cut in slices and the materials they contained were extracted (see below). Gels were scanned with a photometer integrator (Vernon, Paris, France).

Preparative PAGE used the same apparatus as for the analytical technique with 10% (w/v) acrylamide in thick plates (160 × 140 × 10 mm); 9 mg PPAg2 in 1 ml 62.5 mm-Tris/HCl buffer, 10% (w/v) glycerol, 0-001% bromophenol blue was layered across the plate. A potential of 80 V was applied until the bromophenol blue had migrated to the lower limit of the plate (overnight). A thin slice of gel was then cut off and stained to reveal the positions of the bands that had migrated; the remaining gel was then sliced to isolate each component.

Antigen extraction from gel slices. The slices were placed in 20 ml 0-15 M-NaCl solution and homogenized in a Virtiss grinder for 3 min. The gels were then agitated overnight at 4 °C and centrifuged at 5500g for 30 min. The supernatants, containing the extracted antigens, were recovered. This procedure was repeated three times with only 3 h of shaking. The three supernatants were mixed, dialysed against distilled water and lyophilized.

Isoelectrofocusing in thin-layer polyacrylamide gels. This was performed at 4 °C using an LKB Multiphor apparatus (LKB 2117). Ready-prepared polyacrylamide gel plates (LKB ampholine PAG plates) containing ampholytes in the pH range 3.5 to 9.5 were used. Samples (10 to 15 μl) of purified Ag2 solution (5-5 mg ml⁻¹) were applied to filter paper and placed on the gel surface at different positions between the electrodes. After 90 min electrophoresis (constant power, 50 mA and increasing voltage), the gel was stained with Coomassie blue.

Amino acid and hexosamine composition of Ag2. Amino acids and hexosamines were determined using a Beckman analyser, after hydrolysis of 200μg Ag2 in 5·6 M-HCl for 28 h at 100 °C.

Enzyme activities of Ag2. The following activities were investigated, according to Uriel (1971), on the Ag2 anti-Ag2 precipitation band obtained by immunoelectrophoresis as previously described (Delvallez et al., 1979) using crude soluble extract of Listeria monocytogenes and specific anti-Ag2 sera: malate dehydrogenase (EC 1.1.1.37), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), peroxidase (EC 1.11.1.7), carboxylic esterase (EC 3.1.1.1), lipase (EC 3.1.1.3), acetylholinesterase (EC 3.1.1.7), cholinesterase (EC 3.1.1.8), alkaline phosphatase (EC 3.1.3.1), acid phosphatase (EC 3.1.3.2), α-amylase (EC 3.2.1.1), β-glucosidase (EC 3.2.1.21), β-galactosidase (EC 3.2.1.23), leucine aminopeptidase (EC 3.4.11.1), carboxypeptidase A (EC 3.4.12.2), carboxypeptidase B (EC 3.4.12.3), trypsin (EC 3.4.21.4), chymotrypsin A (EC 3.4.21.1), aldolase (EC 4.1.2.13).

Immunodiffusion tests and immunoelectrophoretic analysis. These were performed as previously described (Delvallez et al., 1979).

RESULTS

Analysis of the ACA 44 gel filtration peak, containing Ag2. The purification procedure for Ag2 from Listeria monocytogenes employed three steps (Fig. 1). After analytical PAGE in 5 to 15% (w/v) polyacrylamide gradient gels without SDS, to avoid protein denaturation, Coomassie blue revealed three bands, while PAS and Sudan black showed nothing. The cathodic band (1') was faint and well separated from the other two main bands (2' and 3') which were close together (Fig. 2b). The parallel migration band on the unfixed and unstained remainder of the same plate was cut into small parts, care being taken to separate the three visible bands as indicated in Fig. 2. The components were extracted and tested using immunodiffusion tests and a specific anti-Ag2 serum. A precipitation line was obtained only with the 2' band, corresponding to the Ag2 previously described (Fig. 2c).

To obtain a better separation of these three components, PAGE was performed using polyacrylamide at a uniform concentration of only 10% (w/v) polyacrylamide. With Coomassie blue as dye, the cathodic band (1') migrated further than in the gradient gel and the other two main bands (2' and 3') were well separated (Fig. 2d).

Purification of Ag2 by preparative PAGE. The gel slice corresponding to the 2' band was cut out and the antigen was extracted with 0-15 M-NaCl. The yield of the Ag2 obtained is given in Fig. 1.

Purity and molecular weight determination of the isolated Ag2. Isolated Ag2 showed only one band on PAGE in 10% polyacrylamide (Fig. 2e). By SDS–PAGE the purified
**Bacteria**

**Step 1**
- X-press
- Pellet
- Crude soluble extract

Yield (mg) 60

**Step 2**
- ACA 44 gel filtration
- Unrelated peaks
- Peak of mol. wt 160000 (PPAg 2)

Yield (mg) 9

**Step 3**
- Polyacrylamide gel electrophoresis
- Unrelated antigens
- Ag2

Yield (mg) 1

Fig. 1. Purification procedure for Ag2.

Fig. 2. PAGE analysis of Ag2 (without SDS): (a) standard proteins (LMWC Pharmacia kit) and (b) PPAg2 in a 5 to 15% (w/v) polyacrylamide gradient gel; (c) immunodiffusion test between material extracted from the gel and a specific anti-Ag2 immune serum; (d) PPAg2 and (e) Ag2 isolated from preparative PAGE, in a 10% acrylamide concentration gel. Gels were stained with Coomassie blue.
Ag2 gave one main band, which migrated to a position that corresponded to a molecular weight of $16710 \pm 450$ s.d., and a faint band, quantitatively negligible (3% in scanning study), whose position corresponded to a molecular weight of 32350.

Isoelectric point of Ag2. Isoelectrofocusing of purified Ag2 showed a spot, stained by Coomassie blue, between pH 3.8 and 4.6.

Amino acid and hexosamine composition of Ag2. The main components of purified Ag2 were Asp, Glu, Gly, Ser, Ala, Leu, Lys, Thr, GalNAc (2%) and GlcNAc (5%). The TCA-soluble extract did not contain Ag2, as shown by immunodiffusion tests with anti-Ag2 immune serum.

Enzyme activity investigations on Ag2. None of the enzyme activities investigated was detected on the Ag2–anti-Ag2 immunoelectrophoretograms under the experimental conditions used.

**DISCUSSION**

A procedure using preparative PAGE to purify Ag2 from *Listeria monocytogenes* gave a better yield of antigen than the purification procedure previously described (Delvallez et al., 1979) which involved an immunoadsorption step. The yield of Ag2 would be sufficient for the production of this purified antigen for diagnostic purposes. The specificity of Ag2, restricted to the genus *Listeria*, without cross-reactions with other bacterial species, allowed its use in the serological diagnosis of human listeriosis by enzyme-linked immunosorbent assay (Carlier et al., 1978).

SDS-PAGE showed the molecular weight of the main band of isolated Ag2 to be $16710 \pm 450$. Ag2 was previously found in the peak of molecular weight 160000 in ACA 44 gel filtration chromatography (Delvallez et al., 1979). This discrepancy could be explained by aggregation or association of some subunits.

Isoelectrofocusing showed the mean isoelectric point of Ag2 to be about 4.2, which could explain the anodic electrophoretic migration of Ag2 in agarose gel as previously observed in immunoelectrophoresis (Delvallez et al., 1979).

The absence of Ag2 in the TCA extract of *L. monocytogenes* and the absence of bands in the polyacrylamide gels stained by the PAS reaction could be due to the low content of sugars.

None of the studied enzyme activities were detected in Ag2–anti-Ag2 precipitates after immunoelectrophoretic analysis, but since some antibodies are known to neutralize enzyme activity (Cinader, 1967), the possibility that Ag2 has some activity could not be excluded.

The purified *Listeria* genus-specific Ag2 glycoprotein can be easily produced and used as tool for the diagnosis of human listeriosis and for more fundamental investigations involving its role in the immune response to listeria infections.

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**REFERENCES**


