[Chem. Pharm. Bull. 36(3)1218—1222(1988)]

Sodium Deoxycholate–Polyacrylamide Gel Electrophoresis of Lipopolysaccharides at Low Temperature

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(Received August 5, 1987)

Sodium deoxycholate (DOC)–polyacrylamide gel electrophoresis (PAGE) has previously been used to examine in detail the chemical heterogeneity of lipopolysaccharide (LPS). It is now shown that DOC–PAGE carried out at 4 °C can give reproducible banding patterns even when a large amount of LPS (20 mg) is applied to the slab gel (4 × 150 × 300 mm). By DOC–PAGE, we found that LPS obtained from *E. coli* UKT-B showed 14 silver-staining bands on gels but lacked the bands corresponding to a series of high-molecular-weight LPS usually observed in the S form LPS.

These findings suggest that the DOC–PAGE at 4 °C should be useful in fractionating LPS intactly to study LPS preparations with unknown structures.

Keywords——lipopolysaccharide; polyacrylamide gel electrophoresis; sodium deoxycholate–polyacrylamide gel electrophoresis; SDS–PAGE; DOC–PAGE

Introduction

Lipopolysaccharide (LPS) obtained from gram-negative bacteria has been thought to be chemically heterogenous mainly because of the difference in the number of O-antigenic side chain units per molecule of LPS, and a number of attempts have been made to demonstrate the heterogeneity by the use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)[1-5] and some other techniques.[6-8] In the case of SDS–PAGE, anomolous behavior was reported in the electrophoretic migration of LPS.[9-11]

We introduced sodium deoxycholate (DOC) instead of SDS into PAGE because of the strong dissociating capacity of DOC, which effectively disperses aggregates of LPS into subunits[12,13] and allows more accurate estimation of the chemical heterogeneity of LPS.[14-16] But, when a large amount of LPS is fractionated by PAGE at room temperature, the gel becomes hot due to the generation of heat during electrophoresis. This is undesirable, because some components of LPS such as fatty acid esters might be partly decomposed in alkaline solution at high temperature during the electrophoresis.

In contrast to SDS, DOC has a rather mild denaturating capacity for biological substances and can be utilized in PAGE under low temperature conditions because of its high solubility even at 4°C.[17]

The present communication shows that LPS can be solubilized (or dispersed) in DOC and analyzed by PAGE in the presence of the same detergent at 4 °C, and that by this method a characteristic and reproducible banding pattern of LPS is obtained from *E. coli* UKT-B.

Experimental

Chemicals——All chemicals used in the present experiments were of reagent or analytical grade and were purchased from commercial sources. Deionized and distilled water was used in all solutions.

Lipopolysaccharides (LPS)——LPS obtained from *E. coli* UKT-B (S form) was isolated according to the 45% hot phenol–water method[18] and purified by ultracentrifugation. LPS from *E. coli* UKT-B is a candidate Japanese reference endotoxin for the *Limulus* test.[19] Purified LPS from *Salmonella abortus equi* was supplied by Dr. C. Galanos (Freiburg, F. R. Germany).

Sodium Deoxycholate–Polyacrylamide Gel Electrophoresis (DOC–PAGE)——DOC–PAGE was carried out with a modified buffer system of Laemmli,[20] where the separating gels were formed with 14% polyacrylamide (PA) and 0.5% DOC instead of SDS and the stacking gel with 4% PA and 0.5% DOC. LPS samples were mixed 1 : 1 with sample buffer solution containing 1.0% DOC, 0.1 M Tris–glycine buffer, pH 6.8, and 20% glycerol. Where necessary, the samples were sonicated for one minute to dissolve them completely.

Fractionation of LPS——For the separation of LPS according to molecular size, LPS was fractionated by DOC–PAGE and recovered from the gels as follows. LPS from *E. coli* UKT-B (20 mg) was electrophoresed in 14% polyacrylamide gel (PAG) in the presence of DOC at 4 °C; the temperature of electrode buffer solutions in both the upper and lower chambers was accurately regulated within ±0.5 °C by the use of a thermoregulator (Sharp Thermoelectric, model TE-106). In this system, the gel is immersed in the lower chamber. Three regions (2.6 to 4.6, 6.6 to 7.6 and 7.6 to 8.6 cm from the top of the separating gel) of the slab gel (4 × 150 × 300 mm) were excised and the LPS included in each of these regions was eluted with 0.05 M phosphate buffer, pH 7.2. The elutes were concentrated by lyophilization. To remove contaminating mono- and polyacrylamide from the concentrated LPS solution, LPS samples were further fractionated by Sephadex G-100 (Pharmacia Fine Chemical) column (30 cm × 25 mm) chromatography at room temperature in 0.05 M phosphate buffer, pH 7.2 and LPS fractions were recovered. To remove buffer components, LPS fractions were extensively dialyzed against distilled water at 4 °C. The dialyzed fractions were lyophilized and used for investigating the reproducibility of LPS migration profiles in the DOC–PAGE.

Silver Staining——LPS bands in gels were detected by the silver staining method of Tsai and Frasch[21] with a modification of the fixation procedure as follows. After electrophoresis, PAG (1 × 100 × 140 mm) was immediately fixed by placing the gel into a 40% ethyl alcohol– 5% acetic acid solution (200 ml) in a clean glass dish which was slowly shaken at over 20 °C (this is indispensable; otherwise DOC cannot be completely eluted from gel, and subsequent

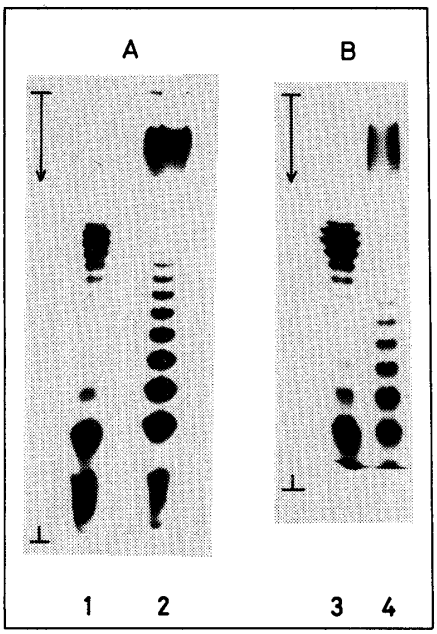

Fig. 1. Electrophoretic Migration Profiles of LPS in 0.5% DOC–14% PAG at 4 °C (A) or 20 °C (B)

Electrophoreses were carried out at 4 °C (lanes 1 and 2) or 20 °C (lanes 3 and 4) with a constant current of 18 mA until the tracking dye entered the separating gel and at 25 mA until the tracking dye migrated about 8 cm into the gel. Seven micrograms of *E. coli* UKT-B LPS (lanes 1 and 3) or *S. abortus equi* LPS (lanes 2 and 4) were applied to the gels.

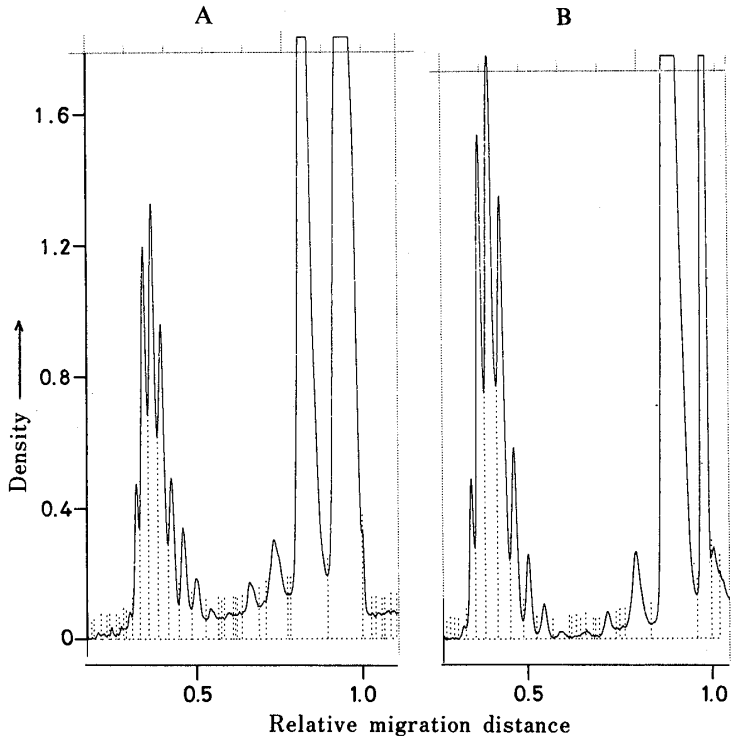

Fig. 2. Scanning Densitometric Analysis of LPS Electrophoresed in DOC–PAG at 4 °C (A) or 20 °C (B)

E. coli UKT-B LPS (5 mg) was applied to a large slab gel (1 × 150 × 300 mm) containing 14% PA and 0.5% DOC. Densities of LPS bands were measured at 450 nm after silver staining. Electrophoreses were carried out with a constant current of 20 mA until the tracking dye entered the separating gel and at 40 mA until the migrational front migrated 8.6 cm into the gel.

staining is disturbed) overnight or until the milk-white color derived from DOC in the gel disappeared. Subsequent procedures were carried out according to the method of Tsai and Frasch.[21] Densitometric scanning of the stained LPS was carried out with a Shimadzu chromatoscanner, model CS-930.

Results

E. coli UKT-B LPS was subjected to DOC–PAGE at 20°C and visualized by silver staining. The results obtained are shown in Fig. 1-B with a migration profile of *S. abortus equi* LPS as a typical control of S form LPS. Compared to *Salmonella* LPS, *E. coli* UKT-B LPS showed a characteristic banding pattern. It lacked upper bands corresponding to a series of high-molecular-weight LPS in the migration profile of *Salmonella* LPS. Similar electrophoreses were carried out at 4 °C. As Fig. 1-A shows clearly, banding patterns of LPS at 4°C were similar to those obtained at 20 °C except that each band, especially the front band observed at 4°C was broader than the corresponding one at 20 °C.

To investigate the influence of temperature on the electrophoretic migration profiles of LPS in DOC–PAGE, LPS was electrophoresed using a large slab gel (1 × 150 × 300 mm) at 4 or 20 °C, visualized by silver staining and directly analyzed by densitometric scanning. Densitometric migration profiles obtained at both temperatures are shown in Fig. 2-A and B, which reveal that LPS from *E. coli* UKT-B is composed of 14 bands. From these results, relative migration distances of bands at 4 °C were plotted against those obtained at 20 °C. As Fig. 3 shows, a good correlation was found between them.

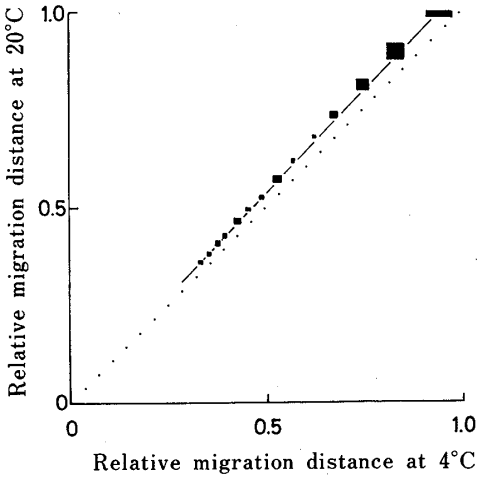

Fig. 3. Relation between Relative Migration Distances of LPS in DOC–PAGE at 4 °C and Those at 20 °C

Relative migration distances were calculated from the densitometric scanning profiles shown in Fig. 2. $y = 1.055x + 0.014$. $\gamma^2 = 0.9985$.

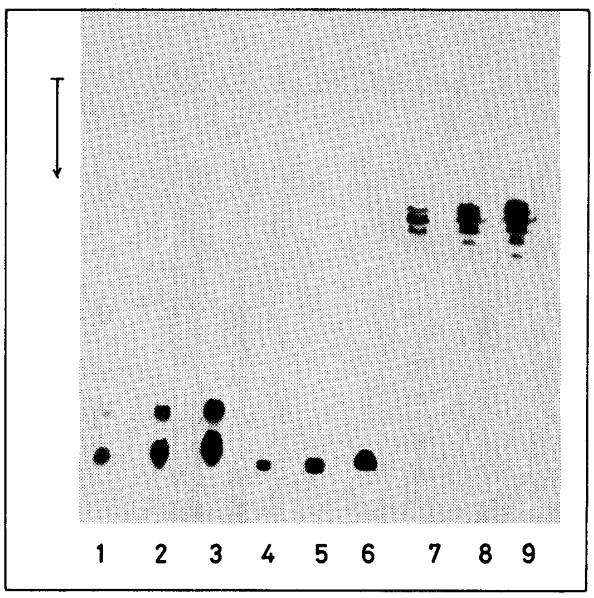

Fig. 4. Electrophoretic Reproducibility of Banding Pattern of LPS Recovered from Gel in the DOC–PAGE

E. coli UKT-B LPS (20 mg) was applied to a slab gel (4 × 150 × 300 mm) containing 14% PA and 0.5% DOC. Electrophoresis was carried out with a constant current at 80 mA until the tracking dye entered the separating gel and at 140 mA until the front migrated 8.6 cm into the gel. After electrophoresis, the gel was divided into three regions as described in Experimental and the LPS recovered from each region were re-electrophoresed in 14% PA with 0.5% DOC. Electrophoretic conditions were the same as in Fig. 1.
Region A, lanes 7—9: 3, 6 and 9 μg, respectively.
Region B, lanes 1—3: 1, 2 and 4 μg, respectively.
Region C, lanes 4—6: 1, 2 and 4 μg, respectively.

To confirm the reproducibility of relative migration distance, or banding pattern of LPS in DOC–PAGE, LPS was fractionated as described in Experimental. As shown in Fig. 4, when each of these fractions was solubilized and rerun on the gel, the materials from each region produced distinct banding patterns and each band migrated to its corresponding original position. In fractions 7,8 and 9, no trace of a band could be detected in the migrational front region, where a trace band is usually observed when LPS fractionated by PAGE at room temperature is rerun.

Discussion

For examination of the chemical heterogeneity of LPS, in the previous report we presented an improved procedure of PAGE in the presence of DOC and demonstrated clearly the size heterogeneity due to the differences in the number of O-antigenic side chain units per molecule using a series of *Salmonella* chemotype LPS[14-16] as shown in Fig. 5. Such a heterogeneity of LPS has not yet been demonstrated in SDS–PAGE. In this report, we show that a large amount of LPS can be solubilized in DOC and analyzed by DOC–PAGE at 4 °C. This method was used to demonstrate that *E. coli* UKT-B LPS consists of 14 bands but lacks many bands corresponding to a series of high-molecular-weight LPS with 15—32 repeating units of oligosaccharide per molecule in comparison with *Salmonella* S form LPS. Its characteristic banding pattern clearly shows that *E. coli* UKT-B LPS has a very much lower content of polysaccharide (hydrophilic part) per unit weight than *Salmonella* LPS (S form). This finding is consistent with the fact that *E. coli* UKT-B LPS is soluble in $CHCl_3$–CH_3OH mixed solvent (2 : 1, v/v).

SDS–PAGE has recently become a powerful method of separating protein molecules according to size.[22] It has also been applied to the study of LPS, but the resolution was rather poor.[23] In SDS–PAGE, the banding patterns of LPS fractionated on gels could not always be reproduced. Considering that no band due to the dissociation or formation of LPS aggregates or due to the partial decomposition of LPS during electrophoresis was observed in DOC–PAGE, as shown in Fig. 4, our method seems to give good resolution and effective fractionation of LPS, as well as reproducible banding patterns.

LPS and other biological substances are not always stable under conditions normally used in SDS–PAGE. LPS is reported to be temperature-labile under acidic or alkaline

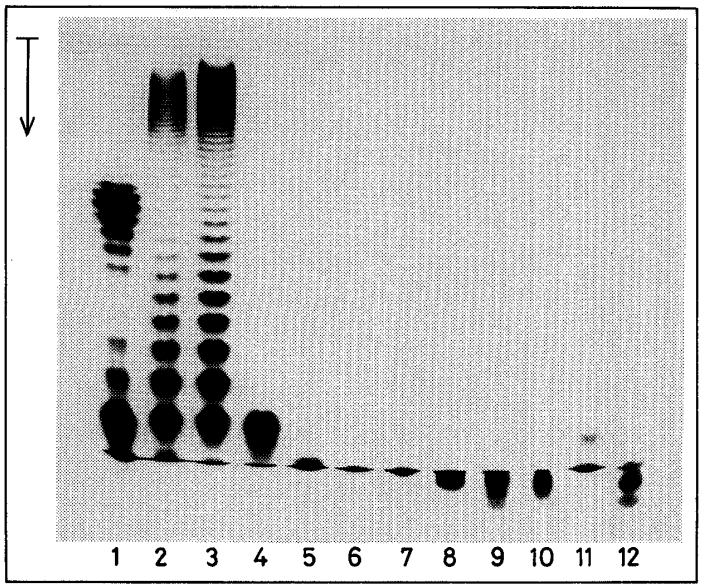

Fig. 5. Migration Patterns of LPS in the 14% PAG in the Presence of 0.5% DOC

Lane 1, *E. coli* UKT-B (S form); lanes 2 and 3, *S. abortus equi* (S form); lane 4, *S. typhimurium* (SR form); lanes 5—10, *S. minnesota* Ra, Rb, Rc, Rd_1, Rd_2 and Re, respectively; lanes 11 and 12, *E. coli* Ra and Re, respectively (from ref. 16).

conditions.[24-26] If the electrophoretic procedure could be applied for the molecular characterization of LPS at low temperature, the difficulty might be overcome, but SDS precipitates during electrophoresis below 15 °C.[27,28] This study was initiated primarily to develop an improved PAGE system for LPS. The advantages of DOC as a solubilizer for LPS and subsequent PAGE include good dissociation of LPS aggregates into subunits and no precipitation of DOC at 4°C.[17] In addition, in DOC–PAGE, boiling of the samples in DOC is not necessary, and this may also help to prevent loss of the intact structure of LPS.

In conclusion, DOC–PAGE at 4 °C is superior to SDS–PAGE for the molecular characterization of LPS. Additional improvement in the present method, including examination of the optimum pH of running buffer solution, is in progress.

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