Anionic and Cationic Components of the K99 Surface Antigen from *Escherichia coli* B41

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(Received 13 February 1978)

INTRODUCTION

The K99 antigen, a plasmid-determined surface antigen found on most strains of *Escherichia coli* enteropathogenic for calves and lambs (Ørskov *et al.*, 1975), appears to be a colonization factor responsible for attachment of these organisms to the intestinal epithelium (Smith & Linggood, 1972). The antigen has a fimbrial-like structure and can be isolated by blending or gently shaking heated suspensions of the organism and precipitating the K99 with acetic acid (Burrows, Sellwood & Gibbons, 1976; Morris, Stevens & Sojka, 1977) or ammonium sulphate (Isaacson, 1977). K99 isolated by acetate precipitation from *E. coli* B41, the established K99 reference strain, was anionic with a pI of 4.2 and haemagglutinated guinea pig and sheep erythrocytes (r.b.c.) (Morris, Stevens & Sojka, 1978). Similarly, Burrows *et al.* (1976) reported that K99 from a $\kappa 12$ (K99⁺) recombinant precipitated from extracts at pH 4.5 on the addition of acetic acid and agglutinated sheep r.b.c. (but they did not report whether agglutination occurred with guinea pig r.b.c.). In contrast, K99 isolated from a $\kappa 12$ (K99⁺) recombinant by ammonium sulphate precipitation was cationic with a pI of 10 and failed to haemagglutinate r.b.c. from either guinea pigs (Isaacson, 1977) or sheep (Morris *et al.*, 1978).

The present communication reports the findings of an investigation of ammonium sulphate precipitated K99 isolated from *E. coli* B41 undertaken in an attempt to explain these anomalies.

METHODS

Preparation of K99 antigen. K99 antigen was extracted from *Escherichia coli* B41 as described previously (Morris *et al.*, 1977) and the antigen was precipitated from the supernatant by the addition of ammonium sulphate (Isaacson, 1977).

Antisera. OK antisera to the following strains of *E. coli* were prepared as described by Sojka (1965): B41 (O101:K99), B42 (O9:K35,K99), B44 (O9:K30,K99), B79 (O101:K32,K99), B85 (O9:K99), B111 (O101:K99), RVC118 (O9:K30?,K99), RVC1616 (O101:K30?,K99), Abbotstown (O149:K91,K88ac), G7 (O8:K87,K88ab), v50 (O10:K'V50') and v113 (O119:K'V113').

Isoelectric focusing. K99 antigen was focused in pre-formed pH gradients (pH 3.5 to 10.0) using 1% (v/v) ampholytes (Morris *et al.*, 1978). After determining the pH of the column fractions, those containing a precipitate were clarified by dilution in 100 mM-phosphate buffer pH 7.4 and all the fractions were examined for absorption at 280 nm. Fractions containing 280 nm-absorbing material were dialysed against 10 mM-phosphate buffer pH 7.4 and concentrated to the original volume of K99.

Ion exchange chromatography. Columns (25 × 450 mm) of the anionic gel DEAE-Sephadex A50 and the cationic exchanger SP Sephadex C50 were employed. The conditions used for chromatography were those described by Isaacson (1977) except that the SP Sephadex C50 column was equilibrated and eluted with 50 mM-phosphate buffer pH 6.0. The material which eluted in the void volume of each column was collected, dialysed and concentrated in the same way as fractions from the isoelectric focusing experiments.

Haemagglutination tests. The direct haemagglutination test using 3% (pcv/v) sheep r.b.c. has been described previously (Morris *et al.*, 1977). The effect of OK antisera on the haemagglutinating properties of K99 and its components was investigated by incubating equal volumes of sample and antiserum at 37 °C overnight. Control tubes contained 100 mM-phosphate buffer pH 7.4 in place of antiserum. Precipitates were

Fig. 1. Anionic and cationic forms of K99 antigen from *E. coli* strain B41 demonstrated by (a) immunoelectrophoresis and (b) Ouchterlony double diffusion. Well 1 contained unfractionated K99; well 2 contained the anionic fraction of K99 from SP Sephadex C50; well 3 contained the cationic fraction of K99 from DEAE-Sephadex A50; troughs and well 4 contained OK antiserum to strain B44.

removed by centrifugation when necessary and the absorbed samples were examined for haemagglutinating activity.

Gel diffusion and immunoelectrophoresis. Ouchterlony double diffusion tests were performed in agar gels (Ørskov & Ørskov, 1970) using 50 µl samples. Immunoelectrophoresis was as described by Morris & Hussaini (1974). Samples from the ion exchange columns were concentrated a further fivefold before use in each of these tests.

RESULTS AND DISCUSSION

Material from the ammonium sulphate precipitated K99 antigen isolated from the reference strain *E. coli* B41 (O101:K99) focused to a single band of precipitate in the pH 4 to 5 region of the electrofocusing column. After clarifying fractions containing precipitate, absorption of the eluate at 280 nm revealed a single peak of anionic material with a pI of 4.5. This was in close agreement with the pI of 4.2 reported for acetic acid precipitated K99 (Morris *et al.*, 1978). The focused material haemagglutinated sheep r.b.c. (5 wells, doubling dilutions) but no such activity was detected when the antigen was absorbed individually with OK antisera to strains B41 (O101:K99), B44 (O9:K30,K99) or B85 (O9:K99).

Since Guinée, Jansen & Agterberg (1976) reported that crude K99 migrated towards the cathode in immunoelectrophoresis and Isaacson (1977) found that highly purified K99 has a strong positive charge, the focused K99 was examined by immunoelectrophoresis. A long arc of precipitation extending from the antigen well towards the cathode was observed on diffusion against OK antiserum to strain B44 together with a short precipitation arc extending from the well towards the anode. In order to confirm this finding new batches of K99 were isolated and examined by immunoelectrophoresis with OK antisera to each of the following strains: B41 (O101:K99), B42 (O9:K35,K99), B44 (O9:K30,K99), B79 (O101:K32,K99), B85 (O9:K99), B111 (O101:K99), RVC118 (O9:K30?,K99) and RVC1616 (O101:K30?,K99). Antisera to four organisms lacking K99, namely G7 (O8:K87, K88ab), Abbotstown (O149:K91,K88ac), v50 (O10:K'V50') and v113 (O119:K'V113'), were also used. No precipitin lines developed on diffusion against antisera to strains G7, Abbotstown, v50 or v113, but the anionic and cationic components were seen with OK antisera to strain B41 and each of the seven heterologous strains possessing the K99 antigen.

Ion exchange chromatography separated the K99 antigen into two components. The anionic fraction eluted with the void volume of the SP Sephadex C50 column, while the cationic component eluted with the void volume of the DEAE-Sephadex A50 column. Immunoelectrophoresis of the separated K99 components indicated that each was free

from contamination by the other (Fig. 1*a*). The anionic component consistently exhibited a strong haemagglutination reaction (7 to 8 wells) which was stable for at least 6 weeks at 4 °C in 50 mM-phosphate buffer pH 7.4. The haemagglutinating activity (3 to 5 wells) of the cationic component was lost within 3 weeks when stored under identical conditions. Absorption of the anionic and cationic components with OK antisera to strains B41, B42 or B85 totally removed their haemagglutinating activity.

Agar gel double diffusion experiments with unfractionated K99 gave two precipitin lines on diffusion against heterologous OK antiserum to strain B44. The slowly diffusing component gave a reaction of identity with the anionic fraction of K99 and the cationic fraction was identified as the faster diffusing component. Furthermore, the crossing of the precipitin lines from the anionic and cationic components showed that each was antigenically distinct (Fig. 1*b*).

The findings suggest that cell-free K99 from the reference strain B41 is composed of two antigenically distinct components; a slowly diffusing anionic component with stable haemagglutinating activity, and a faster diffusing cationic component with a labile haemagglutination reaction. Evidently the K99 preparations isolated in this laboratory contain a predominance of the anionic component. Variations in the relative proportions of each component, however, may explain the differences in gross immunochemical properties of other K99 preparations.

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