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Isoelectric Point of Cell-Free K99 Antigen Exhibiting Hemagglutinating Properties

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The isoelectric point of the K99 antigen in partially purified preparations isolated from *Escherichia coli* B41 was 4.2. Electrofocused K99 antigen hemagglutinated guinea pig and sheep erythrocytes and gave a single precipitin line on diffusion against antisera to *E. coli* B41 and absorbed factor K99 antisera.

Many *Escherichia coli* strains enteropathogenic for calves and lambs contain the K99 antigen (3, 4, 6), a plasmid-determined surface structure that appears to facilitate adhesion to the epithelium of the small intestine, thereby promoting its colonization and subsequent production of diarrhea (7). The properties of cell-free K99 partially purified by isoelectric precipitation at pH 4.0 have been described before its evaluation as a vaccine for controlling colibacillosis in farm animals (5). Isaacson (2) has recently reported an isolation procedure for K99 that yields highly purified material with an isoelectric point (pI) greater than 10.0. The present communication establishes the pI of the K99 antigen used at this laboratory.

Cell-free K99 was partially purified by isoelectric precipitation at pH 4.9 (5) from heated suspensions of *E. coli* B41 (O101:K99), the established K99 reference strain (6). The material gave a single precipitin line in gel diffusion against absorbed factor K99 antiserum, prepared by absorbing antiserum to *E. coli* B117 (O8:K85 ab,K99) with *E. coli* G7 (O8:K87,K88ab) and E6811 (O141:K85ab), and a single line with OK B41 antiserum (8). The antigen hemagglutinated (5) both guinea pig (9 wells, doubling dilutions) and sheep erythrocytes (10 wells). The hemagglutinating activity was not affected by overnight absorption at 37°C with O101 antiserum (8), but it was completely abolished by heating at 100°C for 15 min.

Isoelectric focusing experiments were performed at 4°C in an LKB column (110 ml; LKB Instruments Ltd.) using a glycerol gradient containing 1 or 2% carrier ampholytes as described in LKB Instruments application note 219. As it was possible the K99 isolated by isoelectric precipitation might have co-precipitated either by occlusion or adsorption to contaminant precipitates, the pI of K99 was originally examined by applying the antigen to preformed wide-range pH gradients (pH 3.5 to 10). Sample volumes of 1 ml containing 16 mg of protein were applied at the position of pH 9.0, which was well above the pI of the K99 precipitate. For the accurate determination of pI, 1% solutions of narrow-range ampholytes (pH 3.5 to 5.0) were employed by using 2-ml samples containing 32 mg of protein, which were added to the light gradient solution. The pH gradients were formed overnight at 15-W constant power, with the lower electrode serving as the anode.

Samples examined in preformed wide-range pH gradients focused to a single sharp band of precipitate near the anodic pole of the column, which was eluted stepwise from above, and the eluate was monitored for absorption at 280 nm. Fractions containing precipitates were clarified by dilution in 100 mM phosphate buffer pH 7.4. A single peak was observed corresponding to a pI of about 4.0. Isoelectric focusing in narrow-range ampholytes confirmed these findings and identified the pI of K99 as 4.2 (Fig. 1). After dialysis against phosphate buffer, focused K99 hemagglutinated sheep and guinea pig erythrocytes (8 wells each) and gave a single precipitin in gel diffusion tests against antiserum to K99 and strain B41.

Isaacson (2) reported that K99 isolated by ammonium sulfate precipitation and ion exchange chromatography has a pI greater than 10 and does not hemagglutinate guinea pig erythrocytes. Furthermore, preliminary studies with a sample of purified K99 sent to this laboratory by Isaacson have shown that it also fails to hemagglutinate sheep erythrocytes (unpublished data). The K99 antigen isolated at this laboratory hemagglutinates both guinea pig and sheep erythrocytes, and the antigen isolated by Burrows et al. (1) agglutinates sheep erythrocytes (it was not reported whether agglutination occurred with guinea pig erythrocytes). The difference in properties of the K99 antigen isolated by Isaacson and that isolated by others might be due to the presence or absence of this hemag-

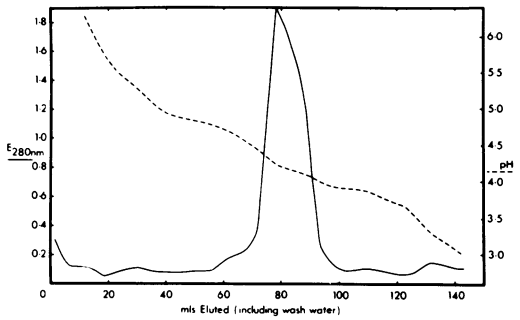

FIG. 1. *Elution profile of isoelectrically focused K99.*

glutinin, and the question arises whether the hemagglutinin is an integral component of K99.

The present study shows that absorption of cell-free K99 with somatic antisera did not reduce the hemagglutinin titer, although earlier investigations showed that the hemagglutinating activity of K99 was reduced by absorption with antisera to K99 (5). Burrows et al. (1) found that antisera produced against their K12 (K99+) strain inhibited the hemagglutination of heterologous K99+ strains and, conversely, OK antisera to these inhibited hemagglutination of the K12 (K99+) *E. coli*. Furthermore, K99 isolated from different *E. coli* strains grown under different conditions possesses hemagglutinating activity (1, 5). Thus, it is unlikely that hemagglutinating activity is associated with contaminating bacterial components or medium constituents. The genetic recombination experiments of Burrows et al. (1) clearly demonstrated that the determinants for K99 and hemagglutinin are located on the same plasmid and that culturing K99+ bacteria at 18°C suppresses the expression of both of these characters. The balance of experimental evidence, therefore, favors K99 possessing hemagglutinating properties. In view of the reported differences in pI and hemagglutinating properties of K99 preparations, further work is necessary to fully understand the nature of the material encoded by the K99 plasmid.

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LITERATURE CITED

1. **Burrows, M. R., R. Sellwood, and R. A. Gibbons.** 1976. Haemagglutinating and adhesive properties associated with the K99 antigen of bovine strains of *Escherichia coli*. J. Gen. Microbiol. **96**:269–275.
2. **Isaacson, R. E.** 1977. K99 surface antigen of *Escherichia coli*: purification and partial characterization. Infect. Immun. **15**:272–279.
3. **Meyers, L. L., and P. A. M. Guinee.** 1976. Occurrence and characteristics of enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. Infect. Immun. **13**:1117–1119.
4. **Moon, H. W., S. C. Whipp, and S. M. Skartvedt.** 1976. Etiologic diagnosis of diarrhoeal disease of calves. Incidence and methods for detecting enterotoxin and K99 antigen production by *Escherichia coli*. Am. J. Vet. Res. **37**:1025–1029.
5. **Morris, J. A., A. E. Stevens, and W. J. Sojka.** 1977. Preliminary characterization of cell-free K99 antigen isolated from *Escherichia coli* B41. J. Gen. Microbiol. **99**:353–357.
6. **Orskov, I., F. Orskov, H. W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile, transmissable *Escherichia coli* K antigen, previously called "Kco", possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. B **88**:31–36.
7. **Smith, H. W., and M. A. Linggood.** 1972. Further observations on *Escherichia coli* enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains: the transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Microbiol. **5**:243–250.
8. **Sojka, W. J.** 1965. *Escherichia coli* in domestic animals and poultry. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.