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Escherichia coli K99 pili are composed of one subunit species

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1. INTRODUCTION

K99 is a pilus found on most, if not all, enterotoxigenic *Escherichia coli* (ETEC) that cause diarrhea in neonatal calves [1–6] and on up to one third of all ETEC that cause diarrhea in pigs [7,8]. In vivo, K99 promotes colonization of the small intestine by facilitating adhesion of ETEC to the mucosa of the small intestine [9,10]. K99 facilitates adhesion by bridging the space between bacterium and the putative mucosal receptor.

K99 has been purified and shown to be a protein polymer composed of subunits of two different M_rs: 22500 and 29500 [11]. A small amount of lipid may also be associated with K99. The objective of this manuscript is to report that K99 is composed of only a single subunit species even though two different molecular weight species are observed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

2. MATERIALS AND METHODS

2.1. Purification of K99

K99 was purified from *E. coli* K12 strain 1474 as previously described [11] except that the pili were extracted from whole cells using 2 M urea in 0.05 M sodium phosphate (pH 7.2) instead of 1 M NaCl in the same buffer.

2.2. Electrophoretic procedures

SDS-5% polyacrylamide continuous gels were prepared and used according to the procedure of Weber and Osborn [12]. SDS-11, 15 and 20% polyacrylamide discontinuous gels were prepared and used according to the procedure of Lugtenberg [13]. Samples were denatured by boiling 5 min in 2% SDS. When required, 1% 2-mercaptoethanol (MetOH) was included in the denaturation solution. Also when required, 1% MetOH was included in the upper electrophoresis buffer. All gels were stained with Coomassie brilliant blue by the procedure of Fairbanks [14].

2.3. Amino-terminal amino acid determination

The procedure of Gros and Labousse [15] was used to dansylate purified K99 with the following modifications. Dansyl chloride was dissolved in acetone and the reaction mixture was adjusted to a final concentration of 8 M urea and 1% SDS. The dansylation reaction was allowed to proceed for 16 h at room temperature after which the protein was precipitated and washed twice with 10% trichloroacetic acid. The dansylated K99 was hydrolyzed for 4 h at 100°C in vacuo with 6 N HCl. The hydrolysate was dried over NaOH, extracted with water (pH 3.5):ether (1:1) and both phases were dried. The residue from each phase was dissolved in pyridine and chromatographed in two dimensions on silica gel-thin layer plates [15].

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3. RESULTS

3.1. Electrophoresis of K99

Previously it was shown by SDS-5% polyacrylamide gel electrophoresis that purified K99 was composed of protein subunits with M_rs of 22500 and 29500 [11]. K99 obtained after isoelectric focusing also had the same two subunit species (K99 focuses at pH 10). However, when K99 is stored at 4°C for longer than a month it appears, by SDS-5% polyacrylamide gel electrophoresis, to be composed of only the 22500 subunit. McMichael and Ou [16] observed the same phenomenon for the type I pili of *E. coli* and Isaacson and Richter [17] for 987P pili of *E. coli* and associated it with the breakage and reformation of disulfide bonds.

Therefore, we examined the effect of the reducing agent MetOH on the conformation of K99 in SDS-polyacrylamide gels. Fig. 1 shows K99 separated on an SDS-15% polyacrylamide gel. The K99 in track A was denatured in the presence of MetOH while the K99 in track B was denatured in the absence of MetOH. The calculated molecular weights for K99 (Fig. 2) in track A are 18200 ± 58 and 19500 ± 100 and in track B is 17600 ± 170. When K99 is denatured in the presence of MetOH is included in the electrophoresis buffer only one subunit species is observed having an $M_r = 19500$ (not shown).

Since the calculated M_rs of K99 were so different from the originally published weights we electrophoresed K99 on SDS-5% polyacrylamide gels as previously [11]. When the K99 was denatured with MetOH two subunit species were obtained with M_rs of 22500 and 29500 as before. K99 denatured in the absence of MetOH appeared as a single subunit species with an M_r of 22000.

The M_rs of K99 subunits electrophoresed in SDS-11% polyacrylamide gels were the same as obtained in 15% gels whereas the M_rs obtained in 20% gels were slightly lower than in the 15% gels (approx. M_r 500 less for each M_r subunit species when denatured in the presence of MetOH; without MetOH denaturation was not examined).

Fig. 1. SDS-15% polyacrylamide gel of K99. Sample in track A was denatured in the presence of MetOH while the sample in track B was denatured in the absence of MetOH. A minor, MetOH-sensitive, contaminant can be seen in both tracks.

3.2. Amino-terminal amino acid

The only dansylated amino acids obtained from K99 were identified as aspartic acid and lysine (dansylated in the ϵ-amino position). Since the isoelectric point of K99 is 10 [11] it is assumed that all the aspartic acid residues of K99 are present as the amides and therefore asparagine is the amino-terminal amino acid of K99.

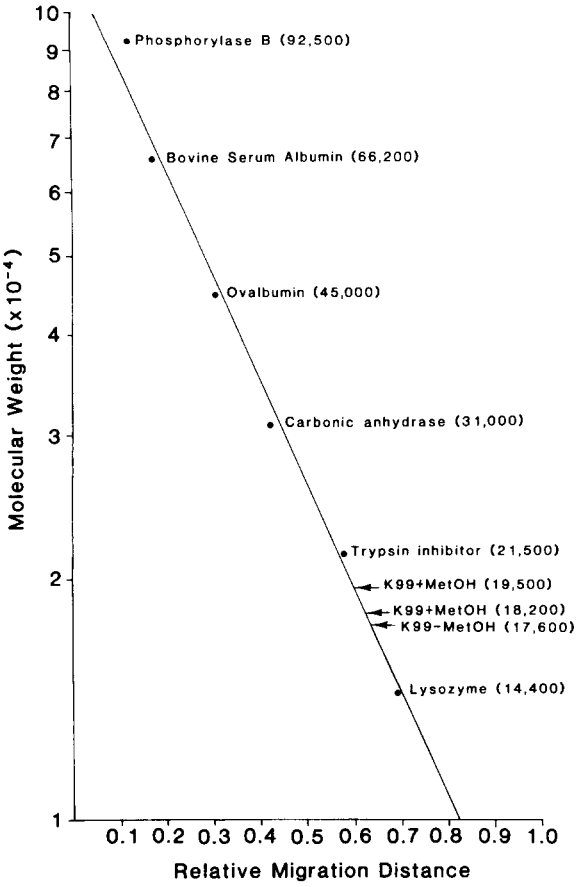

Fig. 2. Plot of the M_r of some known proteins vs. their relative distance of migration.

4. DISCUSSION

The results of SDS-polyacrylamide gel electrophoresis are consistent with the hypothesis that K99 is composed of a single subunit species, contrary to what we previously published [11]. Amino-terminal amino acid analysis resulted in the detection of a single amino acid: dansyl aspartic acid which is also consistent with this hypothesis. The confusion about subunit species stems from the effect of MetOH on the subunits. Denaturation in the presence of MetOH yields what appears to be two differently sized subunits. This result can now be interpreted as follows. K99 subunits contain a single intrachain disulfide bond (amino acid analysis of K99 shows two cysteine residues [11]) that rapidly re-forms after cleavage with and the subsequent removal of MetOH. Thus, the subunits may assume one of two conformations that appear to have had different M_rs in SDS polyacrylamide gels depending upon whether the disulfide bond reforms. A third conformation of K99 subunits occurs when the disulfide bond is not cleaved with MetOH. This is demonstrated by the differences in the apparent M_rs of the untreated K99 compared to the lower band in the MetOH treated sample (17600 vs. 18200). We assume that the upper band in the MetOH-treated sample (Fig. 1, track A) is the completely denatured form of K99 since it is the only band observed when K99 is denatured in the presence of MetOH and MetOH is also included in the electrophoresis buffer.

In SDS-15% polyacrylamide gels the most probable M_r for K99 therefore is 19500. This number compares favorably with the value obtained by amino acid analysis: 21300 [11]. However, in SDS-5% polyacrylamide gels, the most probable M_r for K99 is 29500. The reason for the observed difference between the M_rs determined in the two gel systems is not understood. Other *E. coli* pili (type I and 987P pili) that respond to MetOH like K99 [16,17] do not vary in apparent M_rs in the two gel systems. Some preparations or purified K99 contain the phospholipid phosphatidyl ethanolamine [18]. Whether the phospholipid is a contaminant of the preparation or whether it could account for the M_r differences in the two gel systems is currently under investigation.

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REFERENCES

[1] Guinée, P.A.M., Jansen, W.H. and Agterberg, C.M. (1976) Infect. Immun. 13, 1369–1377.
[2] Isaacson, R.E., Moon, H.W. and Schneider, R.A. (1978) Am. J. Vet. Res. 39, 1750–1755.

[3] Meyers, L.L. and Guinée, P.A.M. (1976) Infect. Immun. 13, 1117–1119.
[4] Moon, H.W., Whipp, S.C. and Skartvedt, S.M. (1976) Am. J. Vet. Res. 37, 1025–1029.
[5] Morin, M., Larivière, S. and Lallier, R. (1976) Can. J. Comp. Med. 40, 228–240.
[6] Sivaswamy, G. and Gyles, C.L. (1976) Can. J. Comp. Med. 40, 247–251.
[7] Moon, H.W., Kohler, E.M., Schneider, R.A. and Whipp, S.C. (1980) Infect. Immun. 27, 222–230.
[8] Moon, H.W., Nagy, B., Isaacson, R.E. and Ørskov, I. (1977) Infect. Immun. 15, 614–620.
[9] Isaacson, R.E., Fusco, P., Brinton, C.C. and Moon, H.W. (1978) Infect. Immun. 21, 392–397.
[10] Nagy, B., Moon, H.W. and Isaacson, R.E. (1976) Infect. Immun. 13, 1214–1220.
[11] Isaacson, R.E. (1977) Infect. Immun. 15, 272–279.
[12] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[13] Lugtenberg, B., Meijers, J., Peters, R., Van der Hoek, P. and Van Alphen, L. (1975) FEBS Lett. 58, 254–258.
[14] Fairbanks, G., Steck, L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617.
[15] Gros, C. and Labousse, B. (1969) Eur. J. Biochem. 7, 463–470.
[16] McMichael, J.C. and Ou, J.T. (1979) J. Bacteriol. 138, 969–975.
[17] Isaacson, R.E. and Richter, P. (1981) J. Bacteriol. 146, 784–789.
[18] Isaacson, R.E. (1981) In: Proceedings of the Third International Symposium on Neonatal Diarrhea (Acres, S.D., ed.), pp. 213–236. Veterinary Infectious Disease Organization, Saskatoon, Sask.