Polyacrylamide Gel Electrophoretic Comparison of the Polypeptides from Ureaplasmas Isolated from Cattle and Humans

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Eleven *Ureaplasma* isolates from cattle, chosen to represent the serological diversity of these strains, were compared with the eight serovars (serotypes) of *Ureaplasma urealyticum* (ureaplasmas from humans) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of polypeptides labeled by growing organisms in the presence of [³⁵S]methionine. The bovine isolates had many common polypeptides, as did the human isolates. Furthermore, the bovine isolates could be distinguished from the human ones. Within the bovine strains, there appeared to be a relationship between the polypeptides present and the serological structure. The results further support the view that ureaplasmas isolated from cattle should be regarded as a species different from *U. urealyticum*.

Those mycoplasmas that possess urease activity comprise the genus *Ureaplasma* (10). A single species has been proposed, *U. urealyticum*, which is serologically heterogeneous, and eight strains have been suggested as representing eight serovars (serotypes), I to VIII, within this species. Some of these serovars are quite distinct, whereas cross-reactions are evident between others. All of these strains are of human origin. Subsequent studies on other *Ureaplasma* isolates from humans have indicated that other serovars also exist (8).

Bovine ureaplasmas are also serologically heterogeneous, and it has been suggested (3, 7) that they exist as clusters of serologically similar, but not identical, strains, some of which show no cross-reactions, whereas others share many antigens. Strains that could be regarded as serovars representing the range of antigens synthesized by bovine ureaplasmas have been proposed (3, 4, 7).

Classically, *Mycoplasma* species are defined by biochemical and serological criteria, with considerable weight being given to the results of the serological tests (1). However, although ureaplasmas isolated from cattle do not cross-react to a significant degree with isolates from humans by various serological tests (3, 4, 7), the serological heterogeneity within bovine or human strains is such that differences in serology alone cannot be considered sufficient evidence for proposing a second species within the genus *Ureaplasma* to accommodate isolates from cattle.

In a previous study, the guanine plus cytosine contents of the deoxyribonucleic acids from bovine strains were shown to be different from

those of the eight serovars of *U. urealyticum*, indicating that the bovine and human isolates existed as two distinct populations and that the two groups of organisms should not be regarded as belonging to the same species (5, 6).

The study reported here was designed to make further comparisons among the eight serovars of *U. urealyticum* and the strains considered representative of those found in cattle. The technique was to compare proteins, labeled by growing organisms in the presence of [³⁵S]methionine to avoid making comparisons of medium components, by polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

***Ureaplasma* strains.** Strains representing serovars I to VIII of *U. urealyticum* (10) were obtained from F. T. Black, Aarhus, Denmark. The bovine *Ureaplasma* strains (A417, Bu2, Mmb167, T95, T288, T45, D48, T315, T44, T74, and T71) were those proposed previously as representing the serological diversity found among isolates from cattle (4).

Preparation of extracts of organisms. The growth from 10-ml volumes of overnight cultures at 37°C of strains grown in U4 broth (4) containing 250 µCi of [³⁵S]methionine (Radiochemical Centre, Amersham, United Kingdom) was harvested by centrifugation at 10,000 × g, and the pellet was washed twice in 0.15 M NaCl. Finally, 100 µl of a solution containing 0.0625 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 6.8), 2% sodium dodecyl sulfate, 2% 2-mercaptoethanol, 0.5 M urea, and 10% glycerol was added to the pellet, and the suspension was incubated at 37°C for 1 h, overnight at room temperature, and for 2 min at 100°C in a water bath. This preparation was stored at -20°C until required.

Polyacrylamide gel electrophoresis. Proteins and polypeptides were analyzed by slab gel electro-

phoresis; 10% polyacrylamide running gels with 3% polyacrylamide stacking gels were employed by the method of Harris and Brown (2). Samples stored at -20°C were heated at 100°C for 2 min before electrophoresis. Protein bands were visualized by fluorography with Enhance (New England Nuclear, Southampton, United Kingdom) as the fluorescent intermediate and Fuji RX as the X-ray film.

RESULTS AND DISCUSSION

A comparison of the polypeptides of the 8 human and 11 bovine strains is presented in Fig. 1A and B. Between 30 and 40 polypeptides could be distinguished in the samples. In general, the patterns obtained with the bovine strains appeared similar to each other and distinct from the patterns obtained with the human strains, and vice versa.

More specifically, polypeptide bands common to the bovine strains but not present in the human strains can be seen in areas labeled a (three bands), b, c, d, f, and g in Fig. 1A and B. Polypeptides common to the human strains and not present in bovine isolates can be seen in areas 1 (two bands), 2, 3, 4, 5, and 6 (Fig. 1A and B).

Within the bovine strains, major bands that are not common are apparent in area h, i.e., among the smaller polypeptides, and the differences relate to previous findings by serological tests. Thus, in this area, strains A417, Bu2, Mmb167, T95, T288, and T45 appear to have two polypeptides with similar sizes, and these strains are all in the same serological cluster (4). Also in this area, strains D48, T315, and T71 appear to have two major polypeptides which are of similar size but which are distinct from

those of the previously mentioned group; strains D48 and T315 are in a second serological cluster. Finally, strains T74 and T44, which are serologically distinct from the previously mentioned nine strains by the growth inhibition test, appear to have two similar major bands which also distinguish them from the previously mentioned nine strains. The polypeptides from the human strains appear similar to each other in this region, except that strains II, IV, V, VII, and VIII appear to have a band not seen with the other three strains. A major polypeptide band was present in area e for all human and bovine isolates. This polypeptide varied slightly in size for the different strains, but it did not seem useful in distinguishing between bovine and human isolates. Previously, Texier et al. (J. Texier, C. Mouches, J. M. Bove, D. Taylor-Robinson, C. J. Howard, R. N. Gourlay, and J. Latrille, in *Proceedings of the 3rd Conference of the International Organization for Mycoplasmaology*, in press) noted differences in this area among the polypeptides from human strains.

The results with the human ureaplasmas are essentially consistent with those published previously with media with either a low protein content (9) or labeled proteins (Texier et al., in press); i.e., the human ureaplasmas have many common polypeptides but also some specific ones.

The bovine strains are also similar to each other, but are distinct from the human strains. To some extent the differences within the bovine group can be related to serological differences reported previously. These gel electrophoresis results confirm by a third method (the other two

FIG. 1. Analysis of whole-cell lysates of ureaplasmas, grown in the presence of $[^{35}\text{S}]$ methionine, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Numbers across the top indicate strains.

being serological structure and guanine plus cytosine content of deoxyribonucleic acid) that the bovine and human *Ureaplasma* isolates belong to two distinct populations and that the bovine ureaplasmas should be regarded as constituting a species distinct from *U. urealyticum*. Also, the technique may provide a useful method for identifying isolates.

REPRINT REQUESTS

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LITERATURE CITED

1. Freundt, E. A., and D. G. ff. Edward. 1979. Classification and taxonomy, p. 1-41. In M. F. Barile and S. Razin (ed.), *The mycoplasmas*, vol. 1. Academic Press, Inc., New York.
2. Harris, T. J. R., and F. Brown. 1975. Correlation of polypeptide composition with antigenic variation in the swine vesicular disease and coxsackie B₅ viruses. *Nature* (London) **258**:758-760.
3. Howard, C. J., R. N. Gourlay, and J. Collins. 1975. Serological comparison between twenty-five bovine *Ureaplasma* (T-mycoplasma) strains by immunofluorescence. *Int. J. Syst. Bacteriol.* **25**:155-159.
4. Howard, C. J., R. N. Gourlay, and J. Collins. 1978. Serological studies with bovine ureaplasmas (T-mycoplasmas). *Int. J. Syst. Bacteriol.* **28**:473-477.
5. Howard, C. J., R. N. Gourlay, D. J. Garwes, D. H. Pocock, and J. Collins. 1974. Base composition of deoxyribonucleic acid from bovine T-mycoplasmas. *Int. J. Syst. Bacteriol.* **24**:373-374.
6. Howard, C. J., D. H. Pocock, and R. N. Gourlay. 1978. Base composition of deoxyribonucleic acid from ureaplasmas isolated from various animal species. *Int. J. Syst. Bacteriol.* **28**:599-601.
7. Ogata, M., H. Kotani, and K. Yamamoto. 1979. Serological comparison of bovine ureaplasmas. *Jpn. J. Vet. Sci.* **41**:629-637.
8. Robertson, J. A., and G. W. Stenke. 1979. Modified metabolic inhibition test for serotyping strains of *Ureaplasma urealyticum* (T-strain mycoplasma). *J. Clin. Microbiol.* **9**:673-676.
9. Sayed, I. A., and G. E. Kenny. 1980. Comparison of the proteins and polypeptides of the eight serotypes of *Ureaplasma urealyticum* by isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Int. J. Syst. Bacteriol.* **30**:33-41.
10. Shepard, M. C., C. D. Lunceford, D. K. Ford, R. H. Purcell, D. Taylor-Robinson, S. Razin, and F. T. Black. 1974. *Ureaplasma urealyticum* gen. nov., sp. nov.: proposed nomenclature for the human T (T-strain) mycoplasmas. *Int. J. Syst. Bacteriol.* **24**:160-171.