CHROM. 3467

Acrylamide gel electrophoresis of purified submaxillary mucins

In a comparative study on the composition and properties of mucins isolated from the bovine, dog, cat[1] and rat[2] submaxillary glands, the electrophoretic analysis of the purified mucins by the moving boundary method indicated the material to be homogeneous.

Some recent evidence in the literature[3,4] indicates that mucins can serve as a matrix for nucleation and crystal growth.

Because of this implication, as a preliminary to studying the mechanism by which calcification could occur, gel electrophoretic studies were carried out on the four mucins to determine if in fact the isolated mucins were homogeneous or if any minor contaminants could be detected which could affect the proposed studies.

Experimental

Electrophoretic analyses were carried out in a Canalco disc electrophoresis apparatus (Model 6). A modification of the method described by DAVIS[5] was used for the analysis of purified mucins.

Gel columns with an inner diameter of 5 × 80 mm were cut from the same piece of glass tubing to minimize the difference in electrical resistance between columns.

The separating gel was 15% acrylamide at pH 8.9. The concentrating and sample gels were 2.5% acrylamide at pH 6.5. The sample to be analyzed, 0.5 mg, was directly dissolved in the sample gel as was 10 μl of 0.1% methyl green used as a tracking dye.

The final electrophoresis buffer was β-alanine–tris buffer, pH 8.7; prepared by dissolving 17.5 g β-alanine and 3.0 g tris in distilled water and diluted to 1 l.

Electrophoresis was carried out for 1.5 h and the current applied was according to the following scheme:

(a) at 1.0 mA per column for 15 min, then

(b) at 3.0 mA per column for 75 min.

The staining procedure was a modification of the method of DABBOUS[6]. The gels were immersed in a 10% solution of 5-sulfosalicylic acid for 18 h, then stained in a 0.25% solution of coomassie brilliant blue R250 for 20 h. Excess stain was removed by shaking the gels in 7% acetic acid solution containing 5% trichloroacetic acid. This staining technique was much more sensitive to microquantities of the components separated than the amido black staining technique originally described.

Results

In Fig. 1 are presented the results from the analysis of the submaxillary mucins. As indicated, only the cat mucin (2) migrates as a single component. The bovine (1), dog (3) and rat (4) mucins indicate some minor components plus a doublet of approximately equal staining intensity.

It is interesting to note that of the animal mucins isolated only the cat does not readily form calculus. Perhaps the two components are necessary in order for calcification to take place. In view of this, we believe, that the doublet, in the mucins isolated from the cow, dog and rat, is an indication that the native mucin exists as a conjugate. The appearance of the two bands is the result of the separation of the

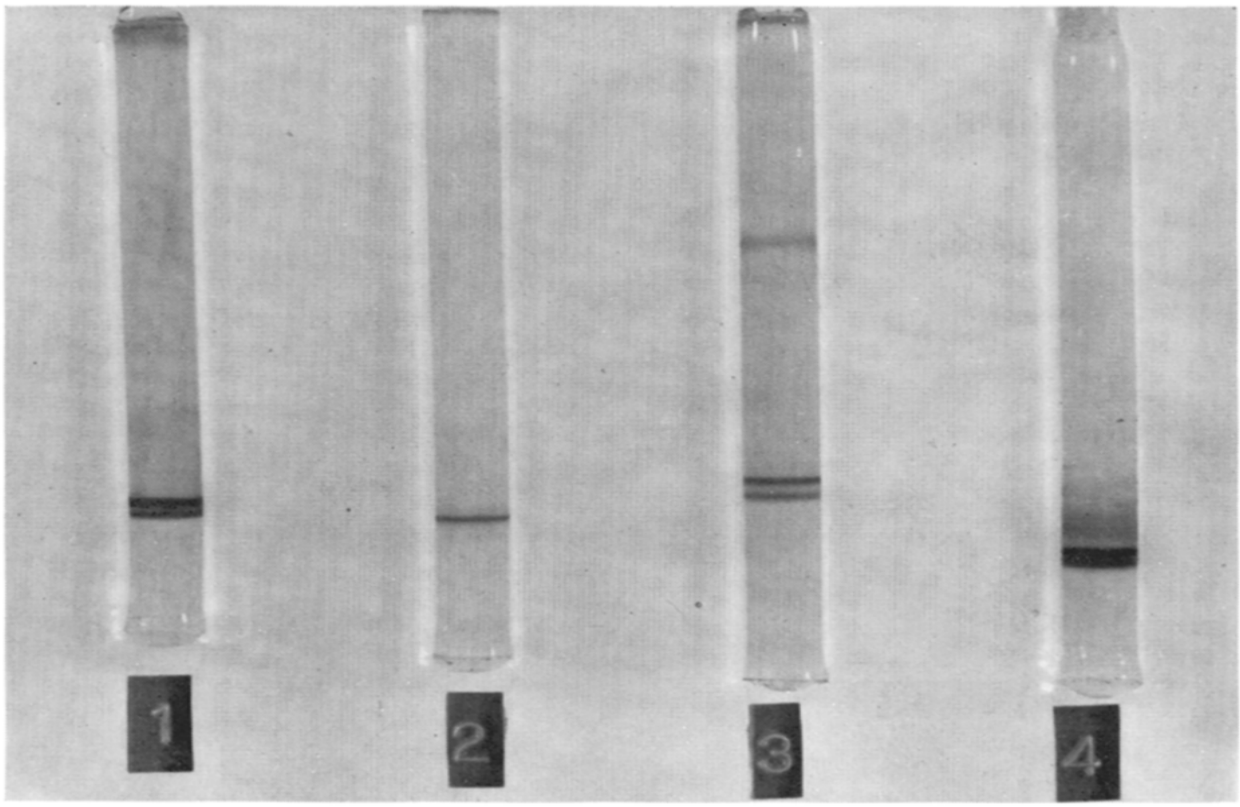

Fig. 1. Gel electrophoresis of submaxillary mucins: (1) bovine; (2) cat; (3) dog; (4) rat. Stained with sulfosalicylic acid–coomassie blue. Time of run: 90 min.

conjugate under the experimental conditions employed. This is further based on the fact that the four mucins migrated as a single component in moving boundary electrophoresis.

Acknowledgement

This work was supported by a PHS Research Grant AM-DE-05338.

University of Pittsburgh,
School of Dental Medicine,
Pittsburgh, Pa. 15213 (U.S.A.)

M. Kh. Dabbous
F. J. Draus

1 F. J. Draus, *Arch. Oral Biol.*, 10 (1965) 719.
2 F. J. Draus, *Arch. Oral Biol.*, 9 (1964) 223.
3 F. J. Draus, F. L. Miklos and B. West, *Arch. Oral Biol.*, 11 (1966) 521.
4 R. G. Eilberg, D. Gould and A. E. Sobel, *Nature*, 207 (1965) 481.
5 B. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
6 Kh. M. Dabbous, *Ph. D. Dissertation*, Univ. of Tennessee, 1967, p. 61.

Received February 19th, 1968