A colorimetric assay for mucous glycoproteins using Alcian Blue

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The present paper describes a simple, sensitive technique for measurement of respiratory mucous glycoproteins that involves their precipitation with the cationic dye Alcian Blue. The assay was adapted from that described by Whiteman (1973) for measurement of urinary glycosaminoglycans. This method is useful for measurement of mucins secreted in response to stimulating agents in isolated airway preparations of animals, and of human airway secretions obtained by bronchoscopy. The Alcian Blue assay was developed as an alternative to the usual methods of glycoprotein determination, which measure total hexose content (phenol/sulphuric acid, anthrone, orcinol), which are of limited sensitivity (Mantle, Allen, 1978) and are hazardous to operate, requiring the use of large amounts of sulphuric acid. Mucous glycoproteins are polyanionic, having both sialic acid and sulphate residues attached to their oligosaccharide structures. It is these groups that interact with Alcian Blue to form insoluble complexes. A 0.1% (w/v) solution of Alcian Blue in 0.1 M-sodium acetate/acetic acid buffer, pH 5.8, containing 25 mM-MgCl$_2$ was clarified by sedimentation at 1870 g for 30 min at 20°C in an MSE Mistral 2L centrifuge. To 3.0 ml portions of tracheal mucus samples in plastic test tubes was added 1 ml of the purified Alcian Blue solution. After at least 2 h equilibration at room temperature, mucus–Alcian Blue complexes were sedimented at 1870 g for 30 min at 20°C. Supernatant solutions were discarded, and each pellet was washed twice by successive resuspension in 40% (v/v) ethanol/0.1 M-sodium acetate buffer, pH 5.8, containing 25 mM-MgCl$_2$, and sedimentation at 1870 g for 10 min at 20°C. A 40% (w/v) Manoxol 1B (sodium dibutylsulphosuccinate) solution was clarified by filtration. Mucus–dye complexes were dissociated by addition of 1 ml of the Manoxol 1B solution and ultrasonication at 50 W for 10 s with a Dawes soniprobe. Samples were sedimented for 1 min as described above to eliminate the foam generated during sonication. Absorbance at 620 nm was measured in a Cecil CE272 spectrophotometer.

A calibration curve for Alcian Blue precipitation was determined for a cat tracheal mucus preparation (Fig. 1) obtained as described by Gallagher et al. (1975). The protein content of this preparation was determined by alkaline hydrolysis followed by ninhydrin reaction, and the carbohydrate content was determined by g.l.c. (Hall, 1978). The carbohydrates present were those typical of cat tracheal mucins (Gallagher et al., 1975) and contained no deoxyribose, ribose or uronic acids. This mucus preparation was not contaminated by nucleic acids or proteoglycans. The resultant absorbance at 620 nm of the Alcian Blue dye complexed by mucous glycoproteins was linear over the range 0–50 μg (Fig. 1). Precipitation of Alcian Blue was much greater with cat tracheal mucus than with bovine serum albumin (O), a non-glycosylated, non-sulphated protein. By the use of mucus that had been radiolabelled with D-[$^3$H]glucose and sodium [35S]sulphate precursors, it was observed that less than 4% of radiolabelled glycoprotein remained in the supernatant solution after incubation with Alcian Blue. Precipitation with this cationic dye is therefore an efficient method for measurement of respiratory mucus.

The Alcian Blue assay and the periodate/Schiff-stain method of Mantle & Allen (1978) were used to determine the rate of secretion of cat tracheal mucins in response to the α-adrenoceptor-stimulating-agent phenylephrine (R. L. Hall, I. LAMPERT, R. J. Miller, A. C. PEATFIELD & P. S. Richardson, unpublished work). Good correlation was observed between the results for the two methods, which measure different chemical properties of the mucin carbohydrates. However, the Alcian Blue method was found to be slightly more sensitive than the periodate/Schiff-stain technique for the determination of tracheal mucins, since a 2-fold higher colour yield was obtained.

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Fig. 1. Standard curve for Alcian Blue assay

The resultant absorbance at 620 nm of Alcian Blue dye complexed by increasing amounts of cat tracheal mucous glycoproteins (●), and bovine serum albumin (O). Each result is shown as the mean ± s.e. for three determinations. The weight of the cat tracheal mucous glycoprotein is the sum of carbohydrate and protein content.