carbohydrate moieties of ovomucoid variant O1 are consistent with this composition, but that a third moiety has a lower, and variable, carbohydrate content.

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Biosynthesis of Ovomucoid *in vitro*

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The capacity of hen oviduct for the synthesis of ovalbumin *in vitro* was demonstrated by Anfinsen & Steinberg (1951), and subsequently the formation of other specific proteins has been examined in this tissue (Canfield & Anfinsen, 1963). The present report describes a modified system that has been used to follow the incorporation *in vitro* of labelled lysine and glucosamine into ovomucoid, a glycoprotein of fairly well-defined structure.

Isolation of ovomucoid from homogenates was achieved as described previously (Beeley, 1970). The product was homogeneous on immunoelectrophoresis. Radioisotopic purity was indicated by the lack of change in specific radioactivity when additional purification steps (ethanol precipitation and gel filtration) were included in the isolation of ovomucoid from labelled homogenates.

Portions of the magnum of hen oviduct (1 g), finely chopped, were incubated at 37°C in 5 ml of salts-containing medium (Krebs & Henseleit, 1932) supplemented with amino acids, glucose and antibiotics. The gas phase was O₂+CO₂ (95:5). [³H]Lysine (40 µCi) and [¹⁴C]glucosamine (4 µCi) were added to the medium. Samples of tissue were incubated for times of up to 8 h, and ovomucoid was isolated from the incubation medium and from the 105000g supernatant from homogenates of washed oviduct tissue.

Incorporation (d.p.m./g of tissue) of labelled lysine into intracellular ovomucoid increased linearly over a 6 h period. A small proportion of the newly synthesized ovomucoid began to appear in the incubation medium with a lag period of approx. 1 h.

In the presence of cycloheximide (100 µg/ml) incorporation of lysine was completely inhibited over a 7 h incubation period, whereas glucosamine incorporation into intracellular ovomucoid was unaltered. This finding indicates that addition of glucosamine residues to ovomucoid can occur in the absence of

protein synthesis. It also suggests that oviduct tissue may contain a substantial pool of precursors with completed ovomucoid peptide chains but incomplete carbohydrate moieties. However, attempts to isolate membrane-bound precursors of ovomucoid from this tissue have not proved fruitful.

Ovomucoid isolated from egg-white or from hen oviduct homogenate contains five or more variants differing in charge and carbohydrate composition (Beeley, 1971). A preliminary study indicates that the most basic, sialic acid-free component O1, is the first of the ovomucoid species to become labelled with lysine.

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Solubilization of Cartilage Protein-Polysaccharide with Lanthanum Chloride

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Recent work has shown that protein-polysaccharide can be extracted in high yields from shredded cartilage by using concentrated solutions of univalent and bivalent salts and subsequently purified as a proteoglycan fraction by equilibrium density-gradient centrifugation (Hascall & Sajdera, 1969). In the present study it has been found that the trivalent lanthanide salts can also be used to extract protein-polysaccharides in high yield from bovine nasal cartilage, but at appreciably lower ionic strength than solutions of salts of lower valency.

Cartilage slices were shaken in salt solutions (1:20, w/v) for 20 h. The cartilage residue was recovered on a sintered-glass disc and solubilized by proteolytic digestion (Mason & Wusteman, 1970). The amount of protein-polysaccharide extracted was estimated from the uronic acid content (Bitter & Muir, 1962) of the extracting solution and the solubilized residue. Protein-polysaccharides extracted with lanthanide chlorides were first precipitated quantitatively by diluting the extract with water (1:9, v/v) and then converted into the soluble potassium salt by the method of Doganges & Schubert (1964).

Protein-polysaccharides obtained by salt extraction were purified by equilibrium density-gradient

centrifugation to give the proteoglycan subunit fraction (Hascall & Sajdera, 1969).

Dilute solutions of LaCl_3 (0.5M, 13.0) solubilized 80% of the total cartilage protein-polysaccharide. CeCl_3 gave a similar result. MgCl_2 (3.0M, 19.0), CaCl_2 (2.0M, 16.0), LiCl (6.0M, 16.0) and guanidinium chloride (4.0M, 14.0) also extracted about 80% of the protein-polysaccharide present, but at higher ionic strength.

Analysis of the proteoglycan subunit fraction obtained from the LaCl_3 extract showed that its protein content, uronic acid content and amino acid composition were very similar to those of the proteoglycan obtained by extraction with the other salts mentioned above. It also showed similar behaviour on electrophoresis at pH 3.0 and 7.0 in a sucrose density gradient (R. M. Mason & R. W. Mayes, unpublished work).

Recent histochemical evidence has suggested that La^{3+} may be used at low concentration (23 mM) as an electron stain for demonstrating acidic protein-polysaccharides in tissues (Shea, 1971). It is noteworthy therefore that, although cartilage chondroitin sulphate protein-polysaccharides are solubilized at one concentration of LaCl_3 , they are completely precipitated from solution when the salt concentration is lowered to 50 mM by either dilution or dialysis. This suggests that the basis for histochemical staining of acidic protein-polysaccharides in the tissues is the formation of an insoluble complex between La^{3+} and the anionic polysaccharides at low concentrations of La^{3+} .

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Aggregation of Cartilage Proteoglycans

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Proteoglycans of bovine nasal septum have been shown to undergo a reversible aggregation that was induced by a 'glycoprotein link fraction', which was separated from the proteoglycan by equilibrium density-gradient centrifugation in 4M-guanidinium chloride (Hascall & Sajdera, 1969).

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Previous work (Tsiganos & Muir, 1970; Tsiganos *et al.*, 1971) has shown that the disaggregation of proteoglycans was accompanied by a large decrease in apparent size on gel chromatography. The reversible aggregation of proteoglycan has now been examined further.

Proteoglycans were extracted from pig laryngeal cartilage with 4M-guanidinium chloride and purified by equilibrium density-gradient centrifugation. Samples were further fractionated by equilibrium density-gradient centrifugation in 4M-guanidinium chloride in an MSE 65 centrifuge (8×25 ml angle rotor) at 95000 g_{av} . for 48 h at 20°C (Tsiganos *et al.*, 1971).

Three fractions were taken, and the distribution of the total uronic acid and protein respectively was as follows: bottom fraction (B), 97.4 and 57.4%; middle fraction (M), 1.0 and 5.2%; top fraction (T), 1.6 and 37.4%.

Gel chromatography on Sepharose 2B showed that half the starting material was excluded from the gel, whereas the disaggregated proteoglycan in fraction B was of much smaller average size and only 14.4% was excluded from the gel. To find in which fraction the component inducing aggregation might be, fractions were remixed under disaggregating conditions and in the same relative proportions in which they occurred in the extract. After dialysis, when reaggregation should occur, they were again compared by gel chromatography. The mixing of fraction M or fractions M and T with fraction B produced a large change in size to give a profile similar to that of starting material. In contrast, when fractions B and T were mixed there was little change in elution profile, although the protein in fraction T became bound to the proteoglycan. Thus fraction M and not T promoted aggregation, and this was confirmed in proteoglycans from a variety of sources including bovine nasal cartilage. Hascall & Sajdera (1969), however, found that aggregation was promoted by the 'glycoprotein link fraction', which corresponds to fraction T. This discrepancy may be due to different conditions of centrifugation. The present results showed that the protein-rich fraction T was not essential for aggregation. The aggregating component was present in a fraction that was more than half carbohydrate in composition and accounted for less than 1.5% of the proteoglycan starting material.

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