Interaction between sulphated macromolecules and hydroxyapatite studied by infrared spectroscopy

GRAHAM EMBERY & GUNNAR RØLLA

Department of Dental Science, University of Liverpool, U.K. and Department of Pedodontics and Caries Prophylaxis, Faculty of Dentistry, University of Oslo, Norway

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Transmission infrared spectroscopy has been used to study some aspects of the mechanism of binding of chondroitin-4-sulphate to hydroxyapatite. The disappearance of absorption maxima characteristic of the covalently bound sulphate groups indicates that these groups may be involved in bridging the chondroitin sulphate molecule to the crystal lattice of the hydroxyapatite. The technique may be valuable, both in studying the formation of organic films on tooth surfaces and the mechanisms of endogenous mineralisation.

Key-words: Glycosaminoglycan; mineralisation, tooth integuments

Graham Embery, Department of Dental Sciences, University of Liverpool, P. O. Box 147, Liverpool, U.K.

Many workers have used hydroxyapatite as the model support medium in attempts to study the formation of organic films on tooth surfaces. Recently, attention has been directed at the initial preference of the hydroxyapatite surface for anionic substances and it has been shown that acidic molecules such as casein, polyglutamate and polyphosphates have a high affinity for hydroxyapatite (2, 5, 11). It has been reported that the ability of acidic glycosaminoglycans to interact with hydroxyapatite is primarily dependent upon the negative charge of these molecules conferred by the constituent carboxyl and ester sulphate groups (8). Acidic glycosaminoglycans have been demonstrated biochemically in human calculus extracts (7, 9, 14), and a sulphated glycoprotein which exhibited blood-group activity has been detected in the 2 h acquired pellicle (16, 19) and in dental plaque (18).

The presence of covalently bound sulphate groups in these compounds confers special infrared spectral properties on the molecules concerned. Using the structurally defined glycosaminoglycan chondroitin-4-sulphate and hydroxyapatite as the model reactive system, the following report provides details of the molecular events involved in the binding of sulphated heteropolysaccharides to mineralised surfaces measured by using infrared spectral data. Such information may be directly implicated in the intermolecular events concerned in the formation of the tooth organic integuments. The information may also be of relevance to investigators studying endogenous mineralis-

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ation since glycosaminoglycans have often been considered important to this process (4).

MATERIALS AND METHODS

A sample of chondroitin-4-sulphate (whole cartilage) was obtained from the Sigma Chemical Co. Ltd., U.K. and was electrophoretically pure on cellulose acetate electrophoresis in 0.2 M calcium acetate (pH 7.2) for 6h at 0.6 mA per cm width (20). Analysis yielded 32.0% hexuronic acid (3), 32.0% total hexosamine (10), and 12.5% ester sulphate (6). The infrared spectrum is given in Fig. 1 and shows a strong absorption band between 1230–1250 cm^{-1} due to the S=O stretching vibrations of the sulphate groups in the molecule (13). Further absorption bands were present at 725, 850 and 928 cm^{-1} characteristic of the C-O-S stretching vibrations of sulphate groups spaced axially in the 4' position of the D-galactopyranose ring (12). No major absorption bands were evident at 1000, 828 and 775 cm^{-1} indicating the lack of 6 isomer sulphates in the preparation. The infrared spectra was well defined and served as an indicator of the degree of purity of the chondroitin-4-sulphate preparation.

Hydroxyapatite (Biogel-HTP+) was obtained from Biorad Laboratories, Richmond, U.S.A. and was washed exhaustively to remove free inorganic phosphate. The preparation possessed a surface area of 50–75 m^2/g. The infrared spectra is shown in Fig. 1 and was identical to those of two other hydroxyapatite samples (supplied by Dr. G. Ingram, Unilever, U.K.) which yielded 36 m^2/g (Ca: P, 1.63) and 26 m^2/g (Ca: P, 1.45) respectively. It was noteworthy that the Biogel preparations possessed no absorption throughs in the areas of interest corresponding to the spectrum of chondroitin 4 sulphate.

The reaction between chondroitin-4-sulphate and hydroxyapatite was achieved by dissolving 5 mg of chondroitin-4-sulphate in 5 ml of distilled water followed by the addition of 50 mg of hydroxyapatite. The suspension was gently shaken at 37°C for 5 min and the solid material was recovered by centrifugation at 5000 g for 10 min. The solid material was washed three times in distilled water (20 ml) by resuspension and centrifugation at 5000 g for 10 min to remove unbound chondroitin-4-sulphate. Previous studies (8) had shown that in the order of 200 μg of chondroitin-4-sulphate bound to 50 mg of hydroxyapatite under the conditions stated. The hydroxyapatite-chondroitin sulphate complex was finally recovered by lyophilisation and termed the *reacted* mixture. In order to establish that the infrared absorption pattern of the hydroxyapatite was not simply 'masking' the characteristic sulphate bands of the chondroitin-4-sulphate, an *unreacted* mixture was prepared in which the hydroxyapatite and chondroitin-4-sulphate were added together in the solid form, i.e. non-solution, immediately prior to examination by infrared spectroscopy. The *reacted* and *unreacted* preparations were added to solid KBr (150 mg) and examined as minimum thickness discs on a Perkin-Elmer Infrared Spectrophotometer 577.

RESULTS

The infrared spectra obtained are shown in Fig. 2. In the unreacted mixture the presence of sulphate bands at 1250, 850 725 cm^{-1}, and the suggestion of a shoulder at 928 cm^{-1}, are clearly apparent. In contrast the infrared spectrum of the reacted mixture is characterised by the disappearance of absorption troughs at 1250, 928, 850 and 725 cm^{-1} which we attribute to the interaction of the sulphate groups with the crystal lattice of the hydroxyapatite, presumably via the calcium sites present in the hydration shell (15).

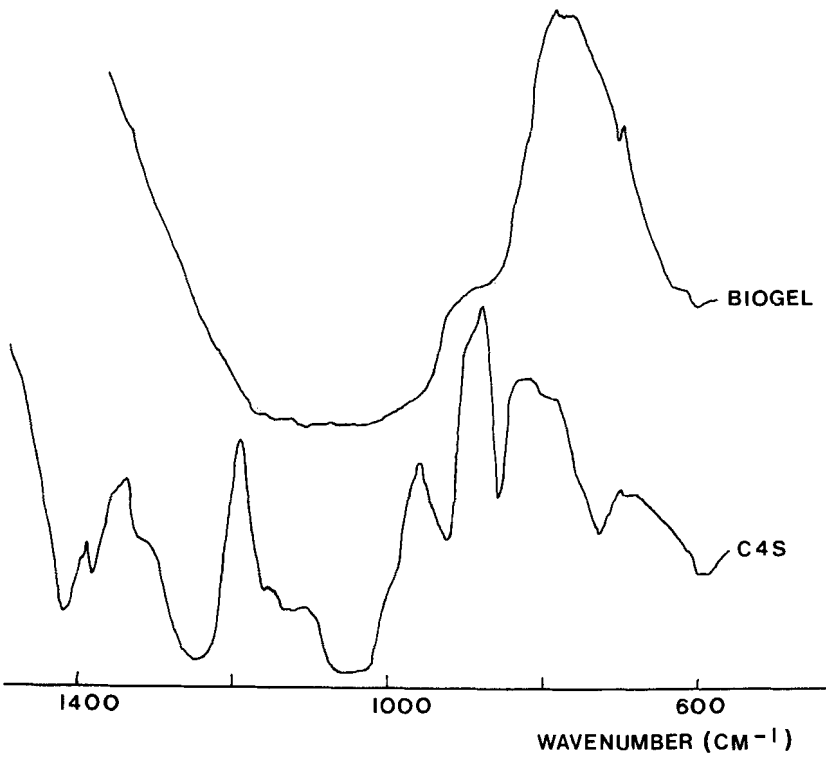

Fig. 1. Infrared spectra of hydroxyapatite (Biogel HTP+) and chondroitin-4-sulphate. Spectra are displaced vertically to facilitate comparison.

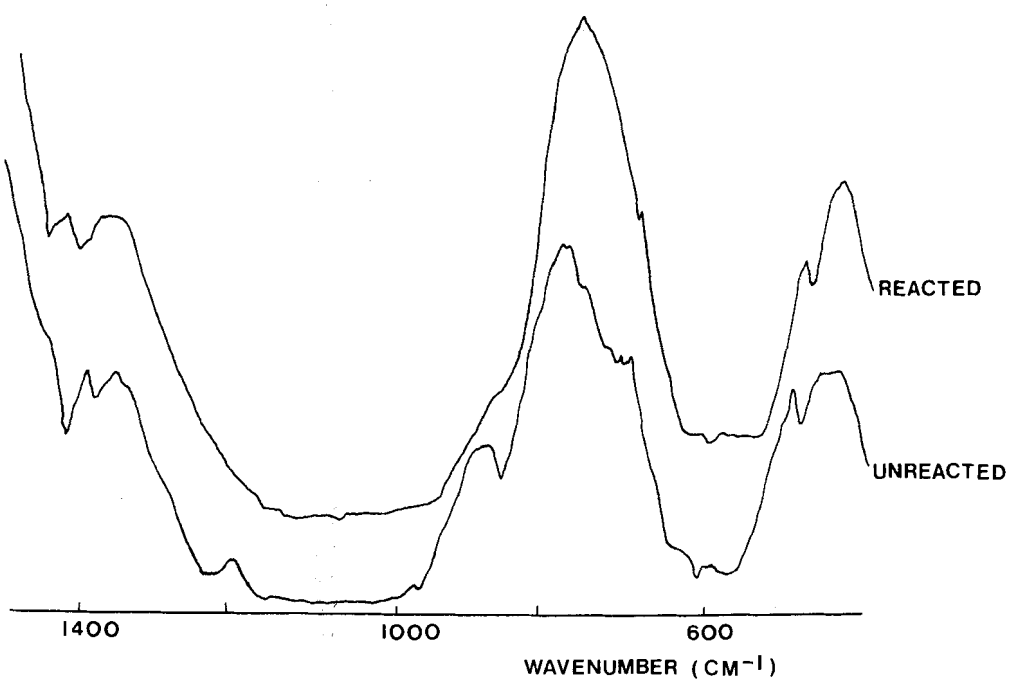

Fig. 2. Infrared spectra of reacted and unreacted mixture. Spectra are displaced vertically to facilitate comparison.

DISCUSSION

This type of mechanism has previously been discussed in relation to both pellicle formation and interaction between salivary agglutination factors and oral bacteria (17). It is contended that infrared spectroscopy may prove a valuable tool in studying the absorption of sulphated macromolecules to solid surfaces and yield defined evidence on the specific mechanisms of interaction. Direct transmission infrared spectroscopy has been used by Termine and Conn (21) to study apatite formation in the presence of various phosphorylated derivatives including nucleotide phosphates and hexose phosphates. More recently, Baier & Glantz (1) have used internal reflection infrared spectroscopy to study the formation of salivary *in vivo* films on different types of solid surfaces including siliconised germanium. The physical approach to the study of organic film formation on tooth surfaces may prove of great value in dental research.

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