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Chemically Modified Natural Polymers as Biomaterials

1. Polysaccharide-Gelatin Conjugates

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Summary

Natural biopolymers such as carboxymethyl cellulose and alginic acid which contain carboxyl functional groups were converted to the corresponding azides. These azides were then reacted by coupling-grafting method with the ε-amino groups of gelatin. Positive proof of grafting of these natural polymers to gelatin was obtained based on the results of nitrogen analysis and IR spectra. These derivatives were found to be of wide interest in the field of biomedical polymers.

Introduction

Collagen and its degraded form, gelatin, can be crosslinked and modified to make them blood compatible and mechanically useful for prosthetic applications. Collagen/gelatin-muccopolysaccharide composite materials has recently shown much promise as biomaterials (YANNAS, 1975). This report forms part of our systematic studies for the preparation of biomaterials based on natural polymers, particularly collagen and gelatin. Polysaccharides such as alginic acid (AA) and carboxymethyl cellulose (CMC) are selected for our initial studies. These polysaccharides are somewhat related to several of the normal physiological polysaccharides such as chondroitin sulphate, hyaluronic acid and heparin.

Experimental

Materials

Gelatin: A pure powdered, Merck grade gelatin was used.
Polysaccharides: Alginic acid (Wilcox and Beckman) and Carboxymethyl cellulose (Burgoyne) were used without further purification.
Chemicals: Hydrazine hydrate 80% (Burgoyne) was used as obtained. All other chemicals used were of reagent grade.

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Esterification of polysaccharides

1. Methylation of alginic acid (AA)

The esterification of alginic acid (AA) has been carried out essentially using the method of JANSEN, JANG (1946) with slight modification. 20 gms of AA was suspended in water for swelling and made acidic with HCl. The sample was left overnight, filtered and washed with distilled water, acetone and then with methyl alcohol. Then the alginic acid was suspended in excess methanol and made to 0.1 N with hydrochloric acid. The contents were left at room temperature for about one week for methylation. After the reaction period the contents were filtered, washed with methanol and dried well.

2. Methylation of carboxymethyl cellulose (CMC)

About 20 gm of sodium salt of CMC was suspended in water and converted to acid as explained in the case of alginic acid. The methylation of CMC was carried out according to the procedure of CROOK et al (1970).

Preparation of hydrazide and azide of AA and CMC

The procedure for the preparation of hydrazide and azide varied little from that of MITZ and SUMMARIA (1961). About 2 gms of AA ester/CMC ester was treated with 20 ml of 80% hydrazine hydrate. The contents were left at room temperature for overnight. The hydrazide was then precipitated with methanol, filtered, washed and dried over $CaCl_2$ in vacuum desicator.

The above hydrazide was suspended in 80 ml of 0.6 N HCl, stirred well and cooled to 0° - 5 °C. 10 ml of 5% solution of sodium nitrite was added drop by drop at 0 °C and the mixture is stirred for 20 minutes. The azide obtained was in the form of a solution. This was used as such after adjusting to the pH 9 by adding sodium hydroxide.

Coupling-grafting procedure

A 5% gelatin solution was prepared and added slowly to the azide solution maintained at pH 9 0° - 5 °C for 30 min. and then left at room temperature overnight. The gelatin-polysaccharide graft copolymers were precipitated after the reaction using the different procedures which are given below:

a) By adding a 10% solution of calcium chloride. The grafted product obtained using calcium chloride method was further purified by thoroughly washing with dil. HCl and then distilled water. This process removed the calcium ions bound on the grafted substrate.

b) By lowering the pH using conc. HCl. The grafted product was allowed to stand overnight and then separated by centrifugation, washed thoroughly with distilled water and dried.

In another procedure, the azide was precipitated by adding methanol, filtered at 5 °C and dried. The above is treated with 5% gelatin solution while stirring at 0° - 5 °C for 30 min. and then left overnight at room temperature. The polysaccharide-gelatin graft copolymers were then filtered, washed and dried.

Analysis of the graft copolymers

Total nitrogen content of the graft copolymers were calculated by the estimation of nitrogen using micro kjeldahl method. The gelatin content of the grafted polysaccharides were then calculated by multiplying the nitrogen content by 5.6.

Percent grafting

The percent grafting was calculated as follows:

$$\frac{\text{Total weight of the graft copolymer} - \text{wt. of gelatin}}{\text{Weight of gelatin}} \times 100$$

IR spectra

The IR spectra of the grafts were measured with a Perkin Elmer model 337 IR spectrophotometer in the form of KBr pellets.

Results and Discussion

Since AA and CMC contains free carboxyl groups on every monomeric unit, advantage was taken of this functional group to bind it to gelatin molecule through covalent bonds. Before coupling the gelatin, the AA and CMC had to be converted into their corresponding reactive acid azides by using the method of MITZ and SUMMARIA (1961) with slight modification.

Coupling-grafting of these two natural polymers to gelatin is expected to take place in a peptide like manner to the ε-amino groups of the lysine residues in gelatin by means of AA and CMC azides. The analysis of the grafted products is shown in Table 1. The percent grafting was varied with the method of precipitation of grafted products. Maximum percent grafting was found to be 29% and the variation in percent grafting noticed can be attributed to incomplete removal of Ca^{++} and other cations by washing. The grafted products isolated by lowering the pH using concentrated hydrochloric acid showed an increase in the percent grafting(Table1) The coupling-grafting reaction using the solid azides of polysaccharides gave the percent grafting values similar to those obtained in the case of acid precipitation method. From the results (Table 1) it can be seen that the acid precipitation and solid azide methods gave higher percentage of grafting. However, in the case of calcium chloride precipitation, it is necessary to remove the bound calcium ions by washing with dil-HCl. In all these procedures, it was possible to keep unreacted gelatin in solution form and precipitate only in the polysaccharide-gelatin grafted products.

TABLE 1

Composition of Polysaccharide-gelatin graft copolymers

Sample	Nitrogen %	Gelantin %	Polysaccharide %	Grafting %
AA-G_1	2.06	12.17	87.83	13.01
AA-G_2	3.06	35.30	64.70	20.76
AA-G_3	3.47	26.38	73.62	24.21
AA-G_4	4.02	28.62	71.38	29.18
CMC-G_1	2.41	13.55	86.45	15.68
CMC-G_2	2.84	25.26	74.74	18.93
CMC-G_3	3.29	37.57	62.43	22.68
CMC-G_4	3.13	26.69	70.31	21.34

AA-G_1 and CMC-G_1: Alginic acid - gelatin and carboxy methyl cellulose - gelatin graft copolymers (precipitated with 10% calcium chloride solution).

AA-G_2 and CMC-G_2: Alginic acid - gelatin and carboxy methyl cellulose - gelatin graft copolymers (precipitated with 10% calcium chloride solution and washed with dilute HCl).

AA-G_3 and CMC-G_3: Alginic acid - gelatin and carboxy methyl cellulose - gelatin graft copolymers (precipitated with concentrated HCl).

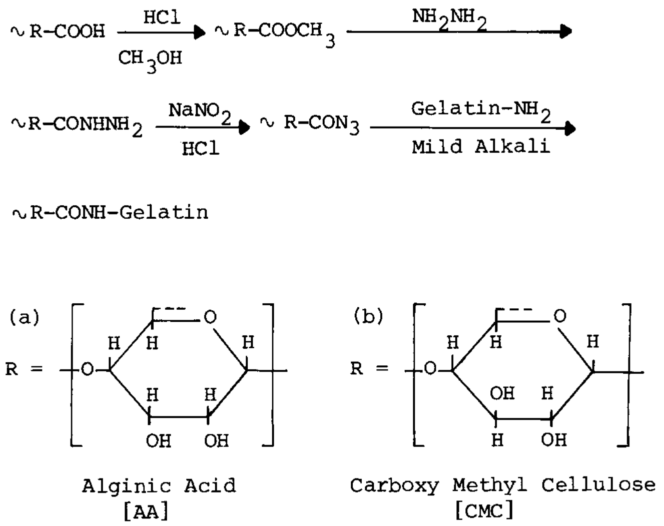
AA-G_4 and CMC-G_4: Alginic acid - gelatin in carboxy methyl cellulose-gelatin graft copolymers (solid azides of polysaccharides are used as backbone substrates).

The reactions involved in the preparation of polysaccharide-protein grafted copolymers are given in Scheme 1.

Infrared spectra of the polysaccharide-gelatin graft copolymers

The IR spectra of the azides of polysaccharides (AA and CMC) showed the characteristic azide absorption bands at 2250 cm^{-1} (Figs.1a and 2a).

In the polysaccharide-gelatin graft copolymers, the azide peaks have disappeared and new secondary amide bands in the range of 600-700 cm^{-1} (620 and 680 cm^{-1}) (FREEMAN et al., 1968 and CROSS, 1960) appeared in addition to the characteristic amide bands at 1625 cm^{-1} and 1680 cm^{-1} (Figs.1b and 2b).



$$\sim R\text{-}COOH \xrightarrow[CH_3OH]{HCl} \sim R\text{-}COOCH_3 \xrightarrow{NH_2NH_2}$$

$$\sim R\text{-}CONHNH_2 \xrightarrow[HCl]{NaNO_2} \sim R\text{-}CON_3 \xrightarrow[\text{Mild Alkali}]{\text{Gelatin-}NH_2}$$

$$\sim R\text{-}CONH\text{-}Gelatin$$

Scheme-1 Coupling-Grafting of Gelatin to AA and CMC

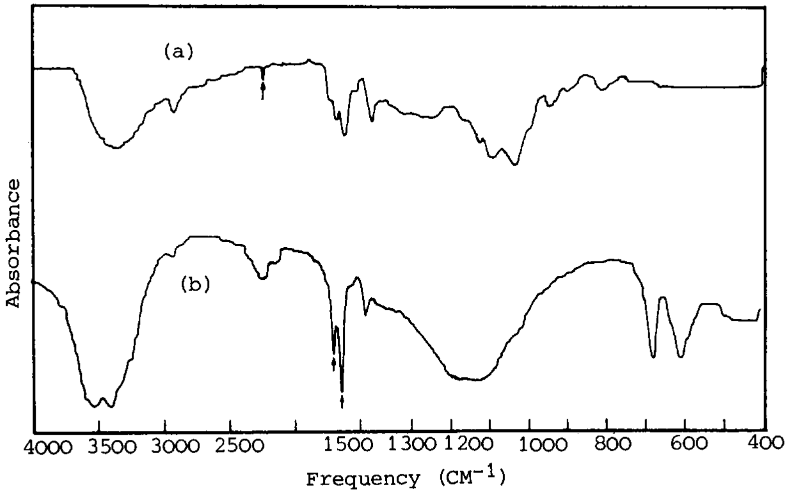


Fig. 1(a) Infrared Spectrum of Alginic Acid Azide
(b) Infrared Spectrum of Alginic Acid-Gelatin Graft Copolymer

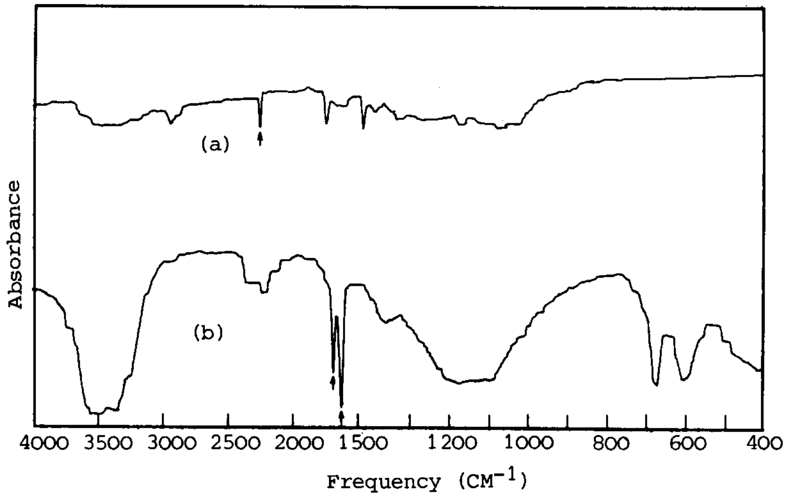


Fig. 2(a) Infrared Spectrum of Carboxy Methylcellulose Azide
(b) Infrared Spectrum of Carboxy Methylcellulose-Gelatin Graft Copolymer

This showed evidently that the coupling-grafting of gelatin has actually taken place in a peptide like manner through the ε-amino groups of lysine residues by means of AA and CMC azides.

The grafted samples are in the powdered form and are being tried for their application as biocompatible materials. The rationale behind these studies is the fact that collagen fibres of vascular wall are imbeded in a polysaccharide matrix that normally prevents contact between blood components and collagen; however, as a result of injury to the vascular wall, the polysaccharide matrix may be disturbed, thus enabling direct contact between collagen and blood components, including the platelets. Further the connective tissue is a complex of collagenous protein with small amounts of polysaccharides notably chondroitin sulfate and hyaluronic acid. The biocompatability and other properties of these polysaccharide-protein composites will be reported elsewhere.

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