Mucoadhesion of Copolymers and Mixtures Containing Polyacrylic Acid

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Water-soluble polymers were synthesized from dextran and polyacrylic acid and their ocular mucoadhesion was evaluated. One series had polyacrylic acid grafted onto the polysaccharide backbone of dextran, and another series had dextran grafted onto the polyacrylic acid backbone. Mucoadhesion of these copolymers was investigated using a tensile apparatus and compared with that of polyacrylic acid/dextran mixtures prepared in different proportions. Whatever the copolymer structure, no synergistic effects were seen and mucoadhesion was not markedly increased compared to dextran. The adhesion of copolymers was the same as that of mixtures having a similar polyacrylic acid content and was always less than that of polyacrylic acid alone. Formation of an interpolymer complex occurred at concentrations up to 60% polyacrylic acid, and only above this value did bioadhesion increase above that of dextran. When this complex was dissociated by neutralization of the carboxyl groups of polyacrylic acid, the mucoadhesion of the copolymers and the mixtures was improved. These experiments demonstrated that copolymers and mixtures of dextran and polyacrylic acid did not produce polymers with improved ocular mucoadhesion.

KEY WORDS: mucoadhesion; polyacrylic acid; dextran; copolymer.

INTRODUCTION

Bioadhesion, in particular mucoadhesion, has been of interest for the development of controlled drug delivery systems to improve buccal, nasal, and oral administration of drugs (1–4). In ophthalmic therapy one of the major problems is to produce and maintain an adequate concentration of the drug at the site of action for a prolonged period of time (5–7). Bioadhesive polymers may remain attached to the mucus layer in the eye until they are removed spontaneously from the surface by tear production and natural mucin turnover. During ocular drug delivery, bioadhesion of a polymer vehicle could improve drug bioavailability and prolong drug residence time and, thereby, allow once-daily dosing. Moreover, localization in specified regions coupled with optimum contact with the absorbing surface could permit better drug penetration.

Mucoadhesion may be accounted for by two major phenomena. The first is the formation of electrostatic, hydrophobic, or hydrogen bonds at the interface between the polymer and the mucins; the second is the diffusion of the polymer chains in the mucus layer. In the latter case, the polymer should be glycoprotein compatible and, consequently, may exhibit structural analogy. Mucins have a polypeptide backbone and contain hundreds of oligosaccharide grafts. On average, each graft consists of 8 to 10 monosaccharide residues (8), with L-fucose or sialic acids located at the terminal ends (9).

Among various candidate bioadhesive polymers, polyacrylic acid (PAA) has been reported to interact via carboxyl groups and functional groups of the mucus glycoproteins (10,11). Moreover, oligo- or polysaccharide chains in the polymer may improve interdiffusion of mucin and polymer (12,13). Therefore, we synthesized polymers containing both carboxyl groups and saccharide units, such as dextran–PAA copolymers. Dextran, a polysaccharide currently used in pharmaceutical formulations, is less bioadhesive than PAA. In the case of copolymers, both polyacid physical interactions and polysaccharide interdiffusion could act simultaneously and enhance the overall adhesion. Two synthetic routes were investigated: (i) grafting of polyacrylamide onto dextran, followed by hydrolysis of dextran-g-polyacrylamide copolymers, and (ii) direct copolymerization of acrylic acid with a functionalized dextran.

The polymers were evaluated for their potential as ocular bioadhesives. Their mucoadhesive strength was compared with that of polymer mixtures of dextran and PAA. In addition, the influence of the degree of neutralization of PAA on adhesive strength was investigated.

MATERIALS AND METHODS

Materials

Dextran (DEXT; M_w = 6000 and 70,000) was purchased from Fluka, polyacrylic acid (PAA; M_w = 250,000) from Aldrich—France, and porcine stomach mucin from Sigma—France. Acrylamide (AM) was recrystallized twice from acetone. Isocyanatoethylmethacrylate (IEM) and acrylic acid (AA) were distilled under reduced pressure before use. Ceric ammonium nitrate, potassium persulfate ($K_2S_2O_8$), potassium metabisulfite ($K_2S_2O_5$), and dibutyltin dilaurate were used without further purification.

^{1}H NMR spectra were recorded at 25°C in D_2O solution on a Bruker AC 200 spectrometer operating at 200 MHz.

Tensile strength measurements were made using a Serie T5K apparatus (J. J. Lloyd Instruments, Paris, France) equipped with a 5-N load cell.

Method I: Synthesis of DEXT-g-Poly(AM-co-AA)

Grafting of Poly(AM) on DEXT

Dextran 6000 (2 g) and acrylamide (6 or 10 g) were dissolved in 200 ml of water and the solution was purged with nitrogen for 2 hr at 25°C. Ceric ammonium nitrate (0.022 g) was dissolved in 0.5 ml of a deoxygenated 1 *M* nitric acid solution and immediately added to the solution. The reaction mixture was stirred for 4 hr at 25°C under nitrogen and then poured into 200 ml of acetone. The precipitate was dissolved in 200 ml of water and purified by fractional precipitation

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with acetone (14) to remove most of the homopolyacrylamide. Then the last fraction was dialyzed (Spectrapor 7; cutoff, 50,000) against water to remove ungrafted dextran and freeze-dried.

Conversion of DEXT-g-Poly (AM) to DEXT-g-Poly(AM-co-AA) by Hydrolysis

Fifty milliliters of 8 *M* NaOH was added to 150 ml of a DEXT-g-poly (AM) copolymer solution (13.3 g/l). Hydrolysis was carried out for 24 hr at 25°C. The reaction mixture was poured into methanol, then the precipitate was filtered and dissolved in water. Acidic copolymers were obtained after elution from an Amberlyst A-15 ion-exchange resin (Fluka). The hydrolytic yield was measured by titration of a copolymer solution with NaOH.

Method II: Copolymerization of AA and the Methacrylic Derivative of Dextran

Formation of Functionalized Dextran

Figures 1a and b outline the chemical steps in the reaction. Dextran (10 g) was dissolved in 200 ml of dry dimethyl sulfoxide (DMSO; freshly distilled over calcium hydride). Dibutyltin dilaurate (0.01 ml) was added as catalyst with IEM (0.42 g). The mixture was stirred for 3 hr at 25°C and the methacrylic derivative of dextran was obtained after precipitation with acetone.

Copolymerization with AA

The following conditions were typically employed. A solution of the functionalized dextran (4 g) and acrylic acid (12 g) in 110 ml of water was purged with nitrogen for 2 hr. $K_2S_2O_8$ (0.152 g) and $K_2S_2O_5$ (0.125 g) were added as initiator and the reaction mixture was stirred under nitrogen for 4 hr at 30°C. The reaction mixture was dialyzed against water for several days, in order to remove the initiator and unreacted dextran, and freeze-dried. Then the polymer was washed with methanol to eliminate the homopolyacrylic acid, and the PAA-g-DEXT copolymer was dissolved in water and freeze-dried.

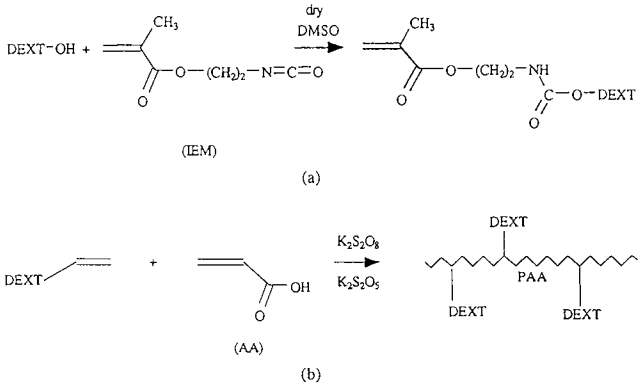

Fig. 1. Method II: (a) formation of functionalized (methacrylic derivative) dextran; (b) copolymerization with AA.

Polymer Mixtures (PAA/DEXT Mix) Prepared with Different Degrees of Neutralization

A stock solution of PAA (approximately 4%, w/v) was prepared and a titration curve was established with 1 *M* NaOH. Polymers with various percentages of neutralization were obtained by adding appropriate amounts of NaOH. The neutralized solutions were then freeze-dried.

Mixtures of PAA (with different percentages of neutralization) and dextran 70,000 were prepared in the proportions (w/w) 2/1, 1/1, and 1/2.

Preparation of Tablets for Measurement of Bioadhesive Strength

The day before the experiment, circular tablets (13-mm diameter, 2-mm thickness, 200-mg average weight) were prepared by compressing polymer powders with a hydraulic press, i.e., using a force of 8 tons during 2 min (15). Six tablets were made for each polymer.

The particle size of the powders was evaluated with a light microscope and found to be about 20 μm for all polymers.

Measurement of Bioadhesive Strength

Evaluation of bioadhesion was performed with a classical tensile apparatus, which was modified as previously described (16,17).

Briefly, instead of the two clamps of the tensiometer, two cylindrical brass holders were connected to the crosspiece and to the lower support of the tensile tester. The diameter of the upper support was the same as that of the polymer tablet (13 mm), which was affixed to it with a cyanoacrylate adhesive (Loctite 401, Breda, The Netherlands). The lower support had a circular surface of 25-mm diameter. A cellulose paper disk (Whatman 5) was glued on the lower support and was impregnated with a uniform layer of 100 μl of mucin gel (consisting of a 15%, w/w, dispersion of porcine stomach mucin in water). This method is a modification of a procedure described by Saettone *et al.* (18).

In all experiments, the initial contact pressure was maintained at 0.2 N. After a contact time of 3 min, the upper support was raised at a constant speed (1.5 mm/min) and the tensile strength was recorded as a function of elongation until the break point. In general, all values reported hereafter are the averages from tests on six polymer tablets.

RESULTS AND DISCUSSION

Copolymer Synthesis

Two methods of copolymer synthesis were used, which led to copolymers with different structures. In Method I, PAA was grafted onto the polysaccharide backbone (dextran). In Method II, functionalized dextran was grafted to the PAA backbone. Dextran of low molecular weight (M_w = 6000) was used in order to eliminate the unreacted dextran by dialysis.

Method I

The usual way to obtain copolymers of polysaccharide

and acrylic monomers is the cerium IV grafting method (19). Few examples of grafting PAA to starch (20,21) or cellulose (22,23) have been reported. Our attempts to graft directly PAA to dextran were unsuccessful, since only mixtures of dextran and homopolyacrylic acid were obtained. We therefore used a two-step reaction incorporating hydrolysis of DEXT-g-poly (AM) copolymers.

Grafting of polyacrylamide to dextran is well-known (14,24–26). The polymerization is initiated by a polysaccharide–cerium complex. Grafting takes place predominantly at the end of the dextran chains because of the greater reactivity of *cis*-glycol end groups (13,24), and thus, most of the synthesized copolymers are in fact biblock copolymers. Assuming that there is only one PAM graft per dextran chain in the purified copolymers, the number-average molecular weight (M_n) of the acrylic side chain (13) can be estimated (Table I).

DEXT-g-poly (AM) copolymers were then hydrolyzed. Alkaline hydrolysis (with sodium hydroxide) is more suitable than acid hydrolysis because the latter cleaves the dextran backbone and leads to imide formation in the poly(AM) side chains (26). Under alkaline conditions, only 66% of acrylamide units can be hydrolyzed into sodium acrylate. In our case, the hydrolytic percentage was about 60%. After elution from a cationic ion-exchange column, DEXT-g-poly(AM-co-AA) copolymers were obtained. Characteristics of these copolymers are reported in Table I.

Method II

We developed a method for copolymerization of AA with a methacrylic derivative of dextran (Fig. 1). Dextran was functionalized with IEM (Fig. 1a). The average functionality can be defined as the number of IEM grafted to dextran chains. It was estimated from the ^{1}H NMR spectra as the ratio of ethylenic protons (assigned to grafted IEM) and dextran protons. A functionality of about 0.5, which assumes that most dextran molecules bear no more than one methacrylic group, prevented cross-linking and thus led to water-soluble copolymers. Characteristics of these polymers are presented in Table II.

Bioadhesion

In previous experiments to evaluate ocular bioadhesion we used bovine conjunctiva. However, it was demonstrated that a cellulose paper, impregnated with porcine stomach mucins, gave more reproducible results (15). The rank order of bioadhesion for a series of polymers was the same as bovine conjunctiva. Therefore, cellulose paper disks impregnated with mucin were used throughout this study.

Table I. Composition and Bioadhesion of DEXT-g-Poly(AM-co-AA) Copolymers (Method I)

% DEXT[a]	% PAA[a]	% PAM[a]	M_n poly(AA-co-AM)[b]	Bioadhesive strength F_{max} (N)
100	0	0	6,000	1.11 ± 0.12
12	56.3	31.7	29,300	1.17 ± 0.27[c]
9.4	52.5	38.1	38,500	1.15 ± 0.41[c]
				4.60 ± 0.96[d]
0	100	0	250,000	4.98 ± 0.31

[a] Weight fractions (from ^{1}H NMR spectra).
[b] Average molecular weight of poly(AA-co-AM) side chains.
[c] Polymer in acid form.
[d] Polymer in alkaline form.

Table II. Composition and Bioadhesion of PAA-g-DEXT Copolymers (Method II)

% PAA[a]	Bioadhesive strength F_{max} (N)
55.5	1.72 ± 0.36
66.3	1.73 ± 0.32
76.5	2.06 ± 0.74

[a] Weight fraction (from ^{1}H NMR spectra).

Mucoadhesive Properties of Copolymers and Mixtures

In synthesizing copolymers of dextran and PAA we had hoped to have a synergistic effect resulting from the mechanisms of physical interactions and interdiffusion.

The polymers made using Method I, DEXT-g-poly (AM-co-AA), were found to have an adhesion (1.15–1.17 N) only slightly above that of dextran (1.11 N). In contrast, when the polymer was in the alkaline form, the bioadhesion increased to 4.6 N (Table I).

Polymers made by grafting dextran on a PAA backbone also gave values of low adhesion. Thus, the polymers having a fraction of PAA of 56, 66, and 77% (w/w) had bioadhesive strengths of 1.72, 1.73 and 2.06 N, respectively (Table II).

When mixtures of dextran and PAA were examined, the bioadhesion of the tablets ranged from 1.1 to 1.8 N (Table III).

Mucoadhesion of PAA (4.9 N) was roughly fourfold higher than that of dextran (1.1 N), in agreement with a previous report (24). It was clear from the present results that, rather than a synergistic effect between the constituents, the mixtures and copolymers exhibited less bioadhesion than expected. For, starting from dextran, increasing the amount of PAA in the mixture (or copolymer), would have been predicted to produce concomitant increases in mucoadhesion. In fact, mucoadhesion remained approximately the same order as that of the dextran. Only above 60% of PAA was there a trend for the tensile strength to increase toward that of PAA alone.

A possible explanation may be the formation of an in-

Table III. Composition and Bioadhesion of PAA/DEXT Mixtures

PAA/DEXT (w/w) mixture	Bioadhesive strength (N)
PAA	4.98 ± 0.31
2/1	1.70 ± 0.49
1/1	1.13 ± 0.33
1/2	1.72 ± 0.15
DEXT	1.79 ± 0.23

Table IV. Effect of Neutralization on Bioadhesion of PAA and PAA/DEXT Mixtures[a]

% neutralization	PAA	PAA/DEXT (2:1)	PAA/DEXT (1:1)	PAA/DEXT (1:2)
0	4.98 ± 0.14 (5)	1.70 ± 0.15 (7)	1.70 ± 0.20 (6)	1.13 ± 0.14 (6)
50	2.28 ± 0.14 (5)	1.67 ± 0.04 (6)	2.29 ± 0.16 (6)	2.40 ± 0.15 (5)
80	4.15 ± 0.27 (5)	2.83 ± 0.22 (4)	3.48 ± 0.21 (4)	2.48 ± 0.20 (5)
100	3.62 ± 0.36 (4)	2.69 ± 0.33 (8)	3.23 ± 0.51 (6)	3.11 ± 0.14 (4)

[a] Values are expressed as mean newtons ± SE (*n*).

terpolymer complex between dextran and PAA chains. Each polymer contains functional groups (ether and hydroxyl groups for dextran, carboxyl groups for PAA) which can lead to hydrogen bonds. The formation of such a complex with PAA has already been described (28). In addition, Satoh *et al.* (29) have observed a similar interpolymer complex between PAA (carboxyvinyl polymer) and hydroxypropyl cellulose following penetration of water. Below a critical value of PAA, all of the polyacid is involved in the interpolymer complex, and hence, bioadhesion remains constant. Above this value, "free" PAA is mainly responsible for the bioadhesion and the tensile strength increases with the PAA content. Assuming a critical value of approximately 60% (weight fraction) of PAA, the complex would require three PAA units for one dextran.

It would appear to be necessary to dissociate this interpolymer complex in order to allow interactions between homopolymer mixtures (or copolymers) and mucus glycoproteins.

Effect of Neutralization on Mucoadhesion

The influence of pH on mucoadhesive properties has not been clearly established. Several studies have shown that bioadhesion of PAA decreased when the pH was increased. This decrease is attended by the progressive disappearance of the undissociated carboxyl groups which link to the mucus by hydrogen bonding (10,18,30). In contrast, Duchêne *et al.* (31), using polymeric tablets of the same PAA polymer, found no effect of pH on the bioadhesive power.

We investigated the effect of the degree of neutralization on the bioadhesive strength of PAA homopolymer alone and of PAA/dextran mixtures. Results are shown in Table IV. As observed previously (32), there was a trend toward a decrease in bioadhesion of the PAA homopolymer as the degree of neutralization increased. In contrast, the mucoadhesion of the three copolymer mixtures had a tendency to increase with the degree of neutralization.

The interpolymer complex of the mixtures dissociates when the degree of neutralization is increased, and bioadhesion reaches a limiting value intermediate between that of sodium polyacrylate and that of dextran homopolymers. As noted above, the effect of the neutralization also was observed with a DEXT-PAA copolymer. Thus, our results provide support for an increase in mucoadhesion with an increase in pH for PAA polymers, copolymers, and mixtures.

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