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Synthesis of starch-based drug carrier for the control/release of estrone hormone

Chee-Youb Won,[a] Chih-Chang Chu[a]* and Tarng-Jenn Yu[b]

[a]*Fiber and Polymer Science Program, Department of Textiles and Apparel, Cornell University, Ithaca, NY 14853-4401, USA*
[b]*Department of Cardiovascular Surgery Veterans General Hospitals–Taipei and National Yang-Ming University, Taipei, Taiwan, 11217, Republic of China*

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The objective of this study was to provide new synthetic route to prepare starch as a potential carrier for controlled release of drugs. A starch was modified with bromoacetyl bromide in order to provide more reactive sites for coupling of bioactive estrone and a suitable spacer between the drug carrier and the hormone. The degree of substitution (D.S.) per anhydroglucose (AHG) unit was calculated from the bromine content and ranged from 0.11 to 2.29, depending on the ratio of bromoacetyl bromide to starch. The starch–estrone conjugate was then synthesized by reacting bromoacetylated starch with the sodium salt of estrone. The structures of bromoacetylated starch and starch–estrone conjugate were determined by means of FTIR, ^{1}H NMR, ^{13}C NMR and UV. Additionally, X-ray diffraction patterns showed the amorphous character of the bromoacetylated starches. © 1997 Elsevier Science Ltd

INTRODUCTION

The attachment of biologically active compounds to synthetic and natural polymers has been frequently investigated as a means of improving the efficacy of drug control/release devices through a constant but prolonged release of bioactive compounds with minimum side effects. The controlled release of the bioactive compounds, which are covalently coupled to a polymeric carrier via hydrolyzable linkages, can be achieved by hydrolytic or enzymatic cleavage of the linking bonds.

One of the main challenges in designing drug control/release devices is to construct a biocompatible carrier, which is easily metabolized and eliminated, does not adversely affect any system inside the human body and is inexpensive so reducing health care costs. A class of biomaterials that may be a potential candidate as a drug carrier is natural polymers, such as carbohydrates and proteins. Starch, long-chain polymers of D-glucose, is one of the most abundant naturally occurring polysaccharides. Due to its biocompatibility, biodegradability, non-toxicity, and abundant sources, it has been experimentally used as a drug carrier, e.g. for phenethylamines (Zilkha *et al.*, 1972) and acetylsalicylic acid (Kratzl & Kaufmann, 1961; Kratzl *et al.*, 1961). In those studies, starch was first converted to the chlorocarbonate derivatives by reacting with phosgene and then chemically reacted with drugs. The main disadvantage of that approach is the use of phosgene which is extremely toxic. Sjoholm *et al.* (1987) reported the possibilities of using polyacryl starch microparticles as a carrier for two antiparastic drugs, primaquine and trimethoprim. These two drugs containing primary amino groups were covalently coupled to the starch microparticles via tri-, tetra- and pentapeptide spacer arms.

Although a variety of drugs have been tried with starch carrier, the delivery of hormones via starch has not been reported in the literature to our knowledge. Due to the inherent nature of the minute amounts of hormone released inside the human body upon activation, a more precise mode of hormone release via drug carriers is essential.

The objective of this paper is to report a better chemical synthesis than that using phosgene to modify starch and a new means to attach hormone, in particular estrone, onto the modified starch. Estrogens promote the growth of the reproductive organs in females. They promote growth and cornification of the vaginal epithelium and stimulate cervical mucous secretion (Goth, 1972).

*To whom correspondence should be addressed.

MATERIALS AND METHODS

In order to prepare the starch–estrone conjugate, the hydroxy groups of starch were first modified with bromoacetyl bromide and then treated with the sodium salt of estrone for attaching the hormone onto the bromoacetylated starch.

Materials

A commercial soluble starch (A.C.S. reagent) was purchased from Aldrich Chemical (Milwaukee, WI, USA) and dried to constant weight at 90°C under reduced pressure. Estrone with the chemical structure shown was purchased from Sigma Chemical (St. Louis, MO, USA) and used without further purification. Bromoacetyl bromide, lithium chloride and sodium hydride (from Aldrich) were used as received. Tetrahydrofuran (THF) was distilled from purple sodium/benzophenone under nitrogen. Pyridine was purified by distilling from calcium hydride onto 4 Å molecular sieves. *N*,*N*′-Dimethylformamide (DMF) and *N*,*N*′-dimethylacetamide (DMAc) were purified by a conventional method (Riddick *et al.*, 1986).

Preparation of solvent-exchanged starch

The extensive inter- and intramolecular hydrogen bondings among hydroxy groups in starch make it difficult to dissolve, even in the LiCl/DMAc solvent system at low temperature (McCormick *et al.*, 1985). Therefore, the starch was pretreated with distilled water, methanol and DMAc in order to facilitate dissolution with minimum degradation. The resulting solvent-exchanged swollen starch was readily dissolved in LiCl/DMAc solvent at 40°C.

Starch (10 g) was stirred in 100 ml of distilled water at room temperature for 24 h. The mixture was filtered under reduced pressure, added to 100 ml of methanol and then stirred for 3 h. The starch was filtered again and added to 100 ml of DMAc. Finally, the mixture was stirred for 12 h and filtered. To determine the concentration of starch per gram of swollen starch, five samples of 1 g each were dried *in vacuo* at 90°C for 48 h. The average starch content for this swollen starch was determined to be 0.61 g/g of sample.

Reaction of swollen starch with bromoacetyl bromide

In order to provide reactive sites on starch for subsequent coupling of estrone, as well as to introduce a suitable spacer between the carrier and the estrone, solvent-exchanged swollen starch was converted into a suitable reactive derivative with bromoacetyl bromide.

Swollen starch (2.59 g: 1.98 g of actual starch weight, 0.0367 mol OH) in LiCl/DMAc solvent system (9 wt%, 50 ml) was placed in a three-necked flask equipped with a nitrogen inlet and outlet, heating mantle, thermometer and magnetic stirrer. The mixture was heated to 40°C for 1 h until the starch was completely dissolved. The solution was then cooled to room temperature. To initiate bromoacetylation, stoichiometric quantities of bromoacetyl bromide and pyridine were added. Pyridine was added in a 1:1 molar ratio with respect to the bromoacetyl bromide in each case. After stirring the mixture at room temperature for 12 h, the solution was poured into a large amount of cold 2 M HCl to precipitate the product. The precipitated product was filtered and washed several times with cold distilled water. It was then purified by reprecipitation using DMF as solvent and cold distilled water as precipitant. This purification was repeated twice. Finally, the bromoacetylated starch was filtered using an aspirator and then dried *in vacuo* at 40°C for 48 h in the presence of phosphorus pentoxide.

Preparation of estrone salt

Estrone (1 g, 0.0037 mol) dissolved in 60 ml of dry THF was added dropwise to a suspension of sodium hydride (0.89 g, 0.037 mol) in 150 ml of dry THF under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4 h. The excess of sodium was then filtered under nitrogen. The solvent was removed with a rotary evaporator, and the product was dried *in vacuo* at 30°C for 24 h.

Reaction of bromoacetylated starch with the sodium salt of estrone

Under nitrogen, the bromoacetylated starch was dissolved in 60 ml of DMF, and the sodium salt of estrone was added dropwise while stirring at room temperature. After stirring at room temperature for 8 h, the reaction mixture was poured into an excess of distilled water to precipitate the product. The product was purified by reprecipitation, using DMF as solvent and distilled water as precipitant, and then dried *in vacuo* at 40°C for 48 h in the presence of phosphorus pentoxide.

Characterization

Elemental analysis (Br) was carried out on a Carlo Erba 1106 EA-instrument. The degree of substitution (D.S.) of the bromoacetylated starch was calculated from the bromine content using the following formula. A similar formula was used by Hebeish and Khalil (1988) for their calculation of the D.S. of carboxymethyl starch during its preparation using chloroacetic acid and sodium hydroxide.

$$\text{D.S.} = \frac{[\text{mol. wt of starch repeating unit}] \times [\%\text{Br}]}{[\text{atomic wt of bromine} \times 100] - [(\text{mol. wt of the ester substituent} - 1) \times \%\text{Br}]}$$

where mol. wt of starch repeating unit = 162; atomic wt of bromine = 80; mol. wt of the ester substituent ($-COCH_2Br$) − 1 = 121; %Br = bromine per-cent.

Fourier transform infrared spectra were recorded on a Mattson Galerxy Series 5000 spectrophotometer with a resolution of $2\,cm^{-1}$. The FTIR spectrum of the original solid starch was taken as a dispersion in Nujol cast between two KBr plates. All other samples were cast on KBr discs from DMF solutions. ^1H NMR spectra were recorded with a Varian Unity 500 operating at 400 MHz for proton. ^{13}C NMR spectra were obtained at 100 MHz with a Varian VXR 400s spectrometer. All of the chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard for both ^1H and ^{13}C NMR spectra. The sample tube size was 5 mm with sample concentrations of $10\,mg\,ml^{-1}$ in DMSO-d_6. A Cary 5 spectrophotometer with 1 cm quartz cuvettes was used to obtain UV spectra at a scanning rate of 1000 nm/min. Spectrophotometric grade solvent (DMF) was used for calibration and background subtraction. X-ray powder diffraction measurements were performed at room temperature with a Scintag PADX diffractometer, using graphite filtered CuK_α radiation (45 kV, 40 mA). The scanning rate was 3°/min over a range of $2\theta = 0-30°$.

RESULTS AND DISCUSSION

Bromoacetylated starch

The reaction of swollen starch with bromoacetyl bromide produces modified starches for subsequent coupling with estrone. In general, the reactions between acid halides and polymers containing hydroxylic groups in the presence of pyridine as a catalyst and hydrogen chloride acceptor were the common methods for the esterification of polymers (Arranz *et al.*, 1980). Thus, the modification of starch with bromoacetyl bromide may be an approach for the coupling of bioactive compounds to this biopolymer.

The effect of reaction conditions on the degree of substitution (D.S.) is summarized in Table 1. The starch molecule contains three hydroxyl groups (one primary, OH-6 and two secondary, OH-2 and OH-3) per anhydroglucose (AHG) residue in the polymer chain. Each of these can react with bromoacetyl bromide in the same manner; however, their relative reactivities vary considerably. In general, the 1° hydroxy group reacts much more readily than the two 2° hydroxy groups. The extent of modification was controlled by the amount of bromoacetyl bromide used. It is clear that the D.S. increases by increasing the bromoacetyl bromide/starch ratio. The highest D.S. (2.29) was obtained when an equimolar concentration of two reactants was used. The production of starch ester through pyridine–bromoacetyl bromide chemistry appeared to be limited to the production of a maximum D.S. of 2.3. This limitation resulted from the one-step esterification procedure. A complete esterification (D.S. = 3.0) of cellulose has been noted to require a several-step esterification procedure (Buchanan *et al.*, 1987). Yields of up to 90% of the bromoacetylated starch were obtained.

Table 1. Effect of reaction conditions on the degree of substitution (D.S.) for the reaction of starch with bromoacetyl bromide

Run no.	Reaction conditions [OH][a] ($mol\,l^{-1}$)	Reaction conditions [BAB][b] ($mol\,l^{-1}$)	Yield (%)	Br (%)	D.S.[c]
1	0.018	0.001	86	5.05	0.11
2	0.018	0.006	83	11.52	0.28
3	0.018	0.012	90	36.29	1.63
4	0.018	0.018	85	41.76	2.29

[a] Concentration of hydroxy groups in starch.
[b] Bromoacetyl bromide.

The structure of the bromoacetylated starches was characterized by FTIR, ^1H and ^{13}C NMR spectrometers. Figure 1(b) shows the FTIR spectrum of the bromoacetylated starch. The residual OH groups show its stretching vibration at around $3400\,cm^{-1}$. A new absorption peak at $1746\,cm^{-1}$ can be assigned to the characteristic carbonyl groups in $-COO-CH_2-Br$. The relative intensities of these bands seem to depend on the modification extent in the biopolymer.

The ^1H NMR spectrum of the same modified starch (Fig. 2) shows a band at $\delta = 4.27$, which corresponds to the methylene protons of bromoacetate groups (Srinivasan *et al.*, 1982). There are also bands at $\delta = 1.24$ due to the methylene protons in the main backbone chain and a band at $\delta = 5.09$ which can be assigned to the resonance of the methine protons of modified starch (Moritani & Fujiwara, 1977).

The ^{13}C NMR spectrum, Fig. 3(a), shows the characteristic chemical shifts at $\delta = 167.26$ correspond to the carbonyl carbon atom of ester groups. The peak $\delta = 40.9$ can be assigned to methylene carbon atom of bromoacetyl groups. The signals between $\delta = 64.07$ and 100.95 are due to sugar carbon atoms.

Figure 4 presents the X-ray diffraction scans for the modified starch samples. The original starch (A) showed peaks at 14.2°, 16.9°, and 22.3° and the modified starch (B) and (C) displayed broad peaks at 20.3°

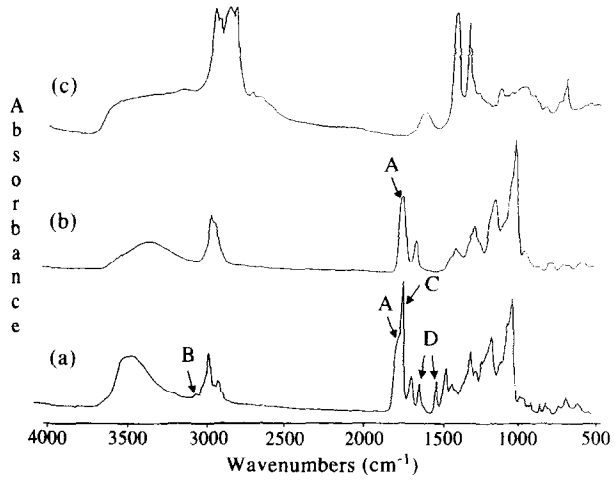


Fig. 1. FTIR spectra of (a) unmodified starch, (b) bromoacetylated starch (D.S. = 2.3), and (c) starch–estrone conjugate. (A) Ester C = O stretch; (B) aromatic C—H stretch; (C) ketone C = O stretch in estrone; (D) ring C = C stretch.

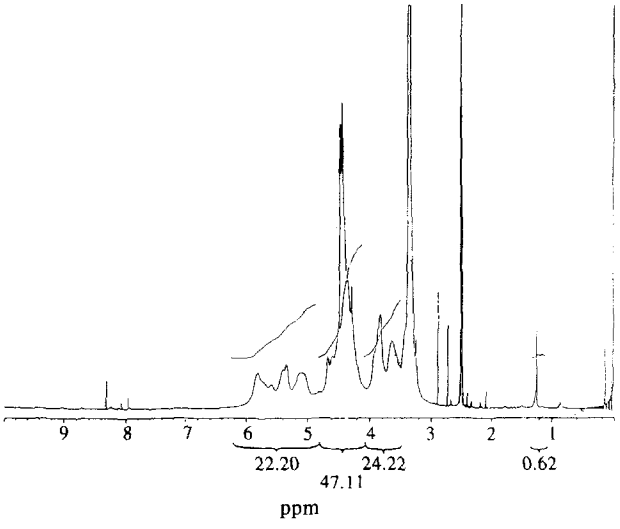


Fig. 2. ^{1}H NMR spectrum of bromoacetylated starch (D.S. = 2.3) in DMSO-d_6.

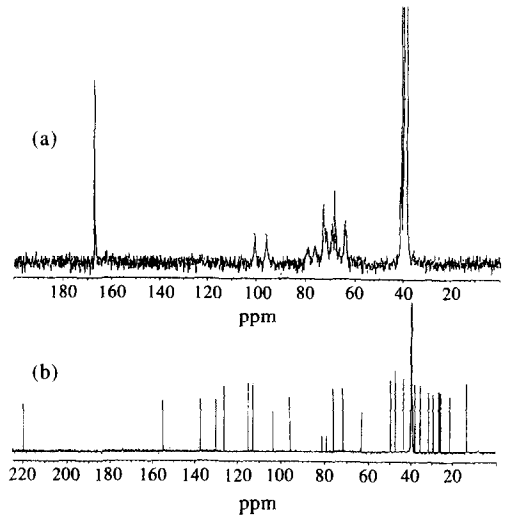


Fig. 3. ^{13}C NMR spectra of (a) bromoacetylated starch (D.S. = 2.3); (b) starch–estrone conjugate in DMSO-d_6.

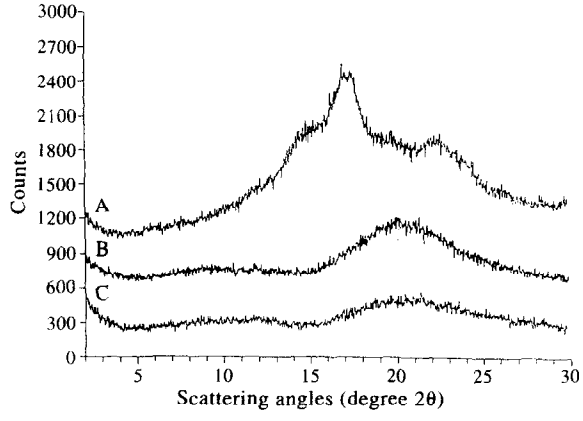


Fig. 4. X-Ray powder diffraction patterns of (A) unmodified starch; (B) bromoacetylated starch (D.S. = 1.63); and (C) bromoacetylated starch (D.S. = 2.3).

and 20.5°, respectively. The substitution of the -OH groups in starch by bromoacetyl groups leads to a gradual loss of crystalline characteristic as evident in the disappearance of the relatively sharp peak at 16.9°. Thus, an increase in D.S. results in a flatten X-ray diffraction curve. The pendent bromoacetyl groups of the modified starches are believed to disrupt the chain packing and contribute to their amorphous character. Jarowenko (1986) reported a similar loss of crystalline characteristic of starch upon chemical modification. They suggested that the recrystallization of gelatinized starch was suppressed.

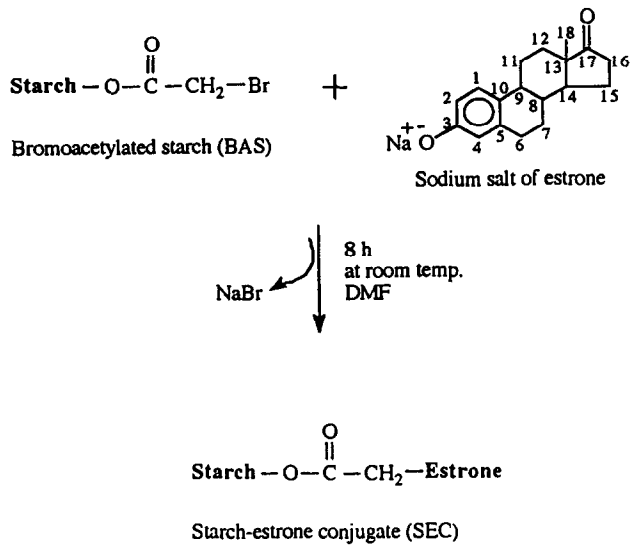
The finding of an increase in amorphous contents of the modified starch is also in agreement with the observed better solubility of the modified biopolymer in comparison to that of the corresponding unmodified one (see Table 2).

Table 2. ^{13}C chemical shifts of estrone in starch–estrone conjugate

Carbon positions	δ (ppm)	Carbon positions	δ (ppm)
1	125.95	10	129.79
2	112.68	11	25.44
3	154.89	12	31.25
4	114.83	13	47.22
5	136.99	14	49.48
6	28.93	15	21.02
7	26.02	16	35.27
8	39.19	17	219.58
9	43.34	18	13.41

Starch–estrone coupling

The coupling of pharmacologically active estrone hormone to the bromoacetylated starch was carried out by using the sodium salt of estrone via nucleophilic substitution as shown in Scheme 1.



Scheme 1.

The estrone was readily converted to its sodium salt by treatment with sodium hydride at room temperature in THF. The ease of sodium salt formation depends on the relative acidities of alcohols. The aromatic alcohol of estrone (3-position) reacted immediately with sodium hydride under mild conditions (Allcock & Fuller, 1980).

The FTIR spectrum of the starch–estrone adduct is shown in Fig. 1(c). The small peak at around 3005 cm^{-1} corresponds to the aromatic C—H bonds in estrone. The 17-carbonyl group of estrone is observed at 1726 cm^{-1}. It shows new absorption associated with the C=C stretching band of the aromatic ring system in estrone at 1503 cm^{-1}. Three new bands at 1258, 1246, and 1218 cm^{-1} are observed, which were assigned to the characteristic C—O stretching in 3-benzoate ester of estrone (Jones & Herling, 1954).

Figure 5 shows the UV spectrum of the same starch–estrone conjugate. The UV maximum for the starch–estrone conjugate occurs at 279 nm. According to the literature (Oehlschlager & Johnson, 1989), steroids containing a phenolic A-ring (single aromatic chromophores) exhibit a band at 280 nm.

Figure 3(b) presents the ^{13}C NMR spectrum of starch–estrone conjugate (DS = 2.3) in DMSO-d_6 solution, and the chemical shifts of estrone of starch–estrone conjugate are given in Table 2. This ^{13}C NMR spectrum shows new chemical shifts due to estrone moiety, as well as sugar carbon atoms (δ = 63.67–110.15).

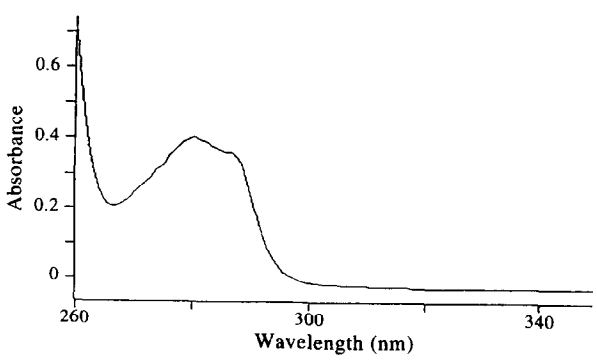


Fig. 5. UV spectrum of starch–estrone conjugate in DMF.

Table 3. Solubilities of unmodified, bromoacetylated starch and starch–estrone conjugate

Solvents	Samples[a]				
	BAS1	BAS2	BAS3	SEC	Unmodified starch
DMF	±	++	++	++	—
NMP	±	++	++	++	—
DMSO	±	++	++	++	—
DMAc	—	+	±	±	—
$CHCl_3$	—	—	—	—	—
THF	—	—	—	—	—

Solubility: ++, soluble at room temperature; +, soluble in hot solvent; ±, partially soluble; —, insoluble.
[a] BAS1, bromoacetylated starch (D.S. = 0.11); BAS2, (D.S. = 1.63); BAS3, (D.S. = 2.29); SEC, starch–estrone conjugate.

The solubility data of the unmodified, bromoacetylated and estrone–conjugated starches are given in Table 3. The bromoacetylated and estrone–conjugated starches were soluble at room temperature in polar aprotic solvents, such as DMF, DMSO, and NMP, whereas the unmodified starch did not dissolve in these solvents.

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