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Synthesis and biological study of some amino acid functionalized starch-*graft*-polyacrylamide

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Abstract

The biological activities of the amino acid modified starch-*co*-polyacrylamid were screened for their antimicrobial activities against some Gram positive/negative bacteria in addition to some pathogenic yeasts and fungi in solid media using cut plug method. The polymers GP3, GP5 and GP6 showed some activities were recorded against the pathogenis yeasts. The MIC ranged from 150 to 200 μg/ml. No toxicity was observed for all the tested polymers using the biochemical induction. The effect of these polymers on respiration or flow of potassium was also recorded.

Keywords: Biopolymers; Starch; Graft copolymers; Modified polyacrylamide; Antimicrobial activities

1. Introduction

Synthetic macromolecules with antimicrobial and bacterium adsorbing activities are often used in medical applications, hygienic and food packaging (Tashiro, 2001). Biopolymers as starch, cellulose, and soy proteins are preferred over synthetic polymers for the development of various materials for two reasons: (a) they are based on annually renewable sources and abundantly available; (b) they are biodegradable, and their production, use and disposal do not present environmental problems (Kaplan, 1998). Vinyl graft copolymerization onto these naturally occurring macromolecules is one of the best methods to improve the performance properties of these polymers without sacrificing their biodegradable nature (Fanta, 1973). A wide variety of carbohydrate graft copolymers have been investigated as potential biomaterials in the field of controlled delivery systems (Bajpai & Giri, 2002; Heller, 1993), biomedical engineering, and biotechnology (Hashimoto, Okada, & Honjou, 1990; Wang et al., 2002). Although much attention has been focused on the synthesis and study of the reaction conditions for grafting starch with a variety of monomers such as acrylamide (Athawale & Lele, 1998a,b; Singh, Sandle, & Varma, 1984), methacrylamide (Athawale, Rathi, & Lele, 1998), acrylonitrile, methcrylonitrile (Athawale & Lele, 2000a,b) and acrylic acid (Athawale & Lele, 1998a,b), but almost none of these studies did go further to modify or carry out chemical reactions on the new grafts. Few studies were carried out on some modified starch-*g*-copolymers and their use in heavy metal removal (Khalil & A-Halim, 2001; Lutfor et al., 2000, 2001), removal of some basic dyes from aqueous solutions (A-Gaffar, 2002), and recently crosslinked starch-*g*-polyacrylamide modified with some amino acids was used as support for catalytic decomposition of hydrogen peroxide (El-Hamshary & El-Seginy, 2004). Antimicrobial polymers are of great importance in the biomedical field. Polymeric materials recently gained interest in the biomedical fields for use in such devices as catheters, pacemakers, implants and wound dressing and medical textiles. However, these polymers could be contaminated or infected by microorganisms such as bacteria if they are exposed to the atmosphere (Jayakumar, Rajkumar, Nagendran, & Nanjundan, 2002). The use of antimicrobial polymers could alleviate these problems.

The objective of this work is to extend the modification of grafted starch copolymer with different amino acids, and to investigate their biological activities against some bacteria and fungi.

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2. Materials and methods

2.1. Experimental

2.1.1. Materials

Starch was supplied by the Cairo Co. for Starch and Glucose. Acrylamide (Merck), ceric (IV) ammonium nitrate and divinylbenzene (Aldrich) were used as received. Amino acids: glycine, β-alanine and histidine (BDH) were used as received.

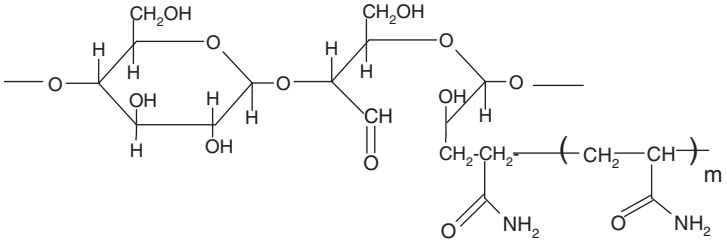
2.1.2. Measurements

Elemental microanalysis, IR and XRD were done at The Central Laboratory of Microanalysis at Tanta University. IR spectra were recorded on Perkin–Elmer 1430 ratio recording infrared spectrophotometer from KBr pellets. The wide-angle X-ray diffraction (XRD) studies were obtained by using a Phillips diffractometer (PW 1840). The pure starch and graft copolymer were scanned at 2θ between 5 and 70°. Thermo gravimetric analysis was carried out at The Central Laboratory of Microanalysis at Cairo University on Shimadzo TGA-50H in the temperature range of 25–500 °C.

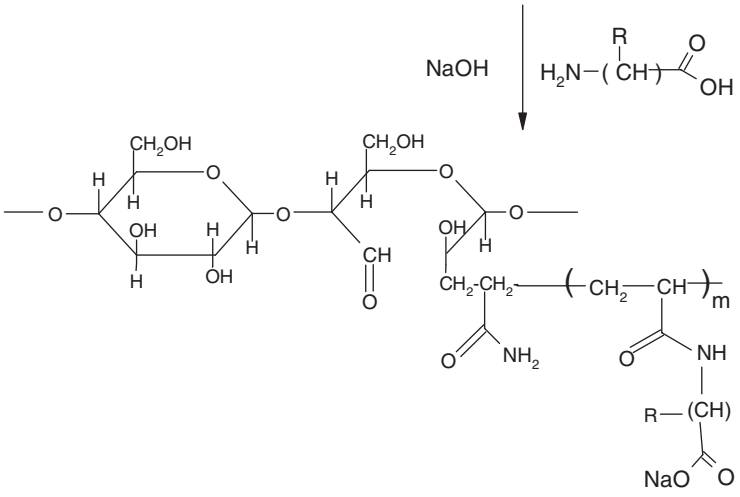
2.1.3. Preparation of polyacrylamide–starch graft copolymer

The synthetic procedures used to obtain samples of uncrosslinked and crosslinked St-*g*-PAM are outlined in Scheme 1. The modification of the grafted copolymers with amino acids, glycine, β-alanine and histidine were achieved by transamidation reaction with about 10-fold excess of the sodium salt of the amino acid of interest. The reactions were carried out at 80 °C for 12 h. The products were filtered and washed thoroughly with distilled water, ethanol and acetone to remove unreacted amino acid and sodium hydroxide (El-Hamshary & Al-Sigeny, 2004).

The resulting products were identified by elemental microanalysis (Table 1) and IR spectra that gave absorptions at 1590–1625 cm^{-1} due to carboxyl anions. The carboxyl



GP_1: St-*g*-PAM



$GP_{2\text{-}6}$

GP_2= Glycine modified St-*g*-PAM
GP_3 = Fe^{III} complex of GP_2
GP_4= β-Alanine modified St-*g*-PAM
GP_5= Histidine modified St-*g*-PAM
GP_6= Crosslinked St-g-PAM

Scheme 1. Modification of starch-*g*-polyacrylamide with amino acids.

Table 1
Characteristics of the amino acids-modified starch-*g*-acrylamide polymer

Polymer	Functional groups pattern		
	N-content (%)		Carboxyl content (mmol/100 g)
	Calcd	Found	
GP_1	5.65	5.89	–
GP_2	5.11	15.75	240
GP_3[a]	5.65	5.89	–
GP_4	4.86	12.67	304
GP_5	12.35	7.92	152
GP_6[b]	6.03	3.74	–

[a] GP3 is the Fe^{III} complex of the sample GP2:glycine modified St-*g*-PAM (El-Hamshary & El-Seginy, 2004).
[b] St-*g*-PAM crosslinked with 2% divinylbenzene (El-Hamshary & El-Seginy 2004).

contents of the modified grafted materials were determined by equilibrating 0.25 g of the modified grafted material with HCl (0.2 N, 10 mL) for 24 h with magnetic stirring, and was titrated against standard NaOH.

2.1.4. Antimicrobial assay

The prepared polymers were tested against different kinds of gram positive and gram negative bacteria, which included: *Escherichia coli*, *Pseudomonas spp*., *Bacillus subtilis*, *Corynebacterium spp*. and *Mycobacterium spp*. Some other medically important yeasts were also examined. They included *Candida albicans*, *Candida tropicalis*, *Aspergillus niger* and *Penicillium italicum*, which are animal and plant pathogens. The media used were either nutrient agar (for bacteria), Sabouraud agar (for yeast) or Czapeks agar for fungi. Since the prepared polymers were insoluble in water, their antimicrobial activities were carried out using cut plug method according to Pridhame, Lindenfeser, and Mitchell (1956). The assay plates were seeded with the test organisms ($3–5\times10^5$ c.f.u./ml). After agar solidification, the wells were filled with 20 mg of powdery polymers as described before (Kenawy, Abdel-Hay, El-Shanshoury, & El-Newehy, 1998). Plates were incubated at 30 °C for 2 days in case of bacteria, at 27 °C for 7 days for fungi and at 37 °C for yeasts, respectively. The antimicrobial activities were represented by the diameters of the inhibition zone (cm).

The minimal inhibitory concentrations (MICs) were determined using agar dilution method according to Laak, Pijpers, Noordergraab, Schoerers, and Verheijd (1990). The tested organisms were either *E. coli*, *Mycobacterium spp.* or *C. albicans*. The tested polymers were incorporated into agar plates containing either nutrient agar or Sabouraud agar with different concentrations (50–500 μg/ml). Each concentration was added under aseptic condition into known volume of testing media. The plates were rotated carefully after pouring the media and inoculated with tested organisms. After plates incubation the MICs were measured as soon as colonies developed. The MICs were recorded as the lowest concentration at, which no growth was observed; only a single colony or a layer of very small colonies.

The effect of the polymers on cell respiration or leakage of potassium was determined according to Mahmoud and Ali (2004).

The cytotoxicity of the polymers was determined using biochemical indicator assay (BIA) as described by Elespuru and Yarmolinsky (1979). Agar plates spotted with polymer drops in dimethylsulfoxide (DMSO) were covered with a layer of agar containing 6-bromo-2-naphthyl β-galactopyranoside as indicator. The indicator turned red upon hydrolysis with β-galactosidase, which was produced in response to DNA damaging as mentioned by Elespuru and White (1983).

3. Results and discussion

3.1. Starch grafting

Acrylamide was grafted onto cornstarch using ceric (IV) ammonium nitrate (CAN) as initiator. CAN is preferred as initiator since it produces free radicals on the starch backbone itself thus minimizing the formation of homopolymer and lead to pure graft copolymer with increasing the grafting efficiency (Athawale & Lele, 2000a,b). Evidence for grafting was indicated from the weight increase of the resulting grafted material, which gave PG of 40.20% and GE 16.10%, respectively, the nitrogen microanalysis (Table 1) and from the IR spectra of the grafted material showed a broad band at 3406 cm^{-1} due to overlapping of OH stretching and amide NH. Another additional band appeared at 1656 cm^{-1} due to amide C=O stretching vibration. The signal at 2906 cm^{-1} was due to methylene groups.

Thermo gravimetric analysis of the graft copolymer was performed to study its thermal stability compared with pure starch. A careful comparison of the degradation data for starch and St-*g*-PAM (Table 2) shows that the graft copolymer has higher onset temperature for every stage of decomposition, which shows that the graft copolymer has lower percentage weight loss as compared with pure starch for relevant stages. Hence, it can be said that grafting of acrylamide has considerably delayed the degradation of starch (Athawale & Lele, 1998a,b). Singh et al. have reported similar results for St-*g*-PAM copolymers (Singh et al., 1984)

3.2. X-ray diffraction

The X-ray powder diffraction pattern of pure starch and starch-*g*-polyacrylamide were recorded at 2θ values between 5

Table 2
Thermogravimetric data of starch and its graft copolymers

Sample	Decomposition stages	$T_{initial}$ (°C)	T_{max} (°C)	% Weight loss	% Residue at 495 (°C)
Starch	1	37	56.6	8.5	
	2	28	332.8	75.6	4.25
	3	416.5	479	11.6	
St-*g*-PAM	1	24.2	59.3	6.1	
	2	148.5	262	38.6	
	3	262	334	13.8	27.5
	4	412	494	13.5	

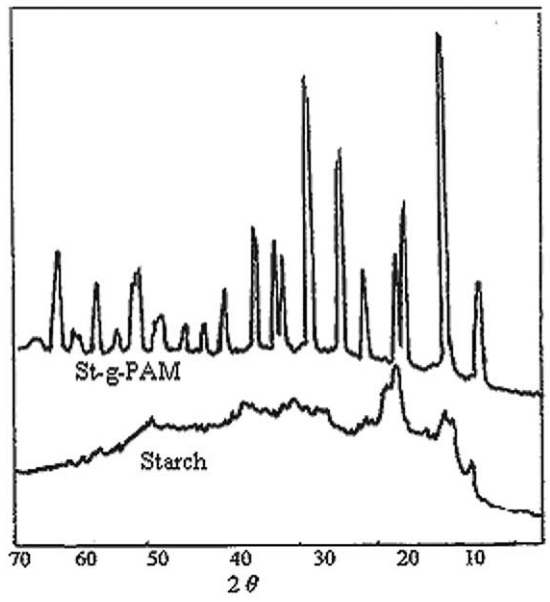


Fig. 1. Wide angle X-ray diffraction of starch and starch-*g*-polyacrylamide.

and 70°. Pure starch shows ~ four peeks between 2θ of 10 and 30° (Fig. 1), which on grafting appear to have altered and sharpened in addition to the appearance of new peaks in the amorphous region. It can therefore be inferred that both the amorphous and crystalline regions are involved in grafting.

3.3. Modification with amino acids

The amino acid functions: glycine, β-alanine and histidine were introduced into the starch-*g*-polyacrylamide by transamidation reaction with ~ 10-fold excess of the sodium salt of the amino acid of interest (Scheme 1). The carboxyl content of the modified grafted copolymer is presented in Table 1.

3.4. Biological activities

One of the objectives of our study was to develop new and active polymer having broad spectrum against pathogenic bacteria, yeasts and/or fungi. All prepared polymers were examined for their antimicrobial activities. The reported results are only for the most active polymeric materials.

The data in Table 3 represent the activities of the polymers using cut plug method against different test organisms. It was found that polymers GP3, GP5 and GP6 were the most active compared to GP1, GP2, and GP4. *E. Coli*, *Mycobacterium* and *Pseudomonas* were largely affected by polymers GP3, GP5 and GP6 where the diameter of inhibition zone was about 0.9 cm on solid medium. All of the tested yeasts or fungi did not affect by the polymeric materials.

The minimal inhibitory concentrations of the GP3, GP5 and GP6 compared to ampicillin after a fixed period of time were summarized in Table 4. The MICs ranged between 150 and 200 μ/ml.

It is clear that start material GP1 has a limited effect on bacterial growth. The structural modification of GP1 resulted in enhanced activities as observed in samples GP3, GP5 and GP6, or reduced the activities as obvious from samples GP2, and GP4. The activities of the most active polymers were compared with that recorded for ampicillin as one of the most active antibacterial agents.

No toxicity was recorded against bacterial DNA by three tested polymer samples. The polymers GP3, GP5 and GP8 may affect or damage cell membrane of the *E. coli* and enhanced K^+ flow to outside the cell. On the other hand polymer GP8 clearly decreased cell respiration of *E. coli* in addition to enhancement of K^+ flow, which leads to cell death (Table 5).

From the above results, it can be concluded that polymers GP3, GP5 and GP8 can act as antimicrobial agents. They have no toxicity and their MICs were small in comparison with standard antibacterial agent. Modifications on similar structure are currently underway to enhance water solubilities and biological activities. The mode of action of samples GP3, and GP5 may be on cell membrane and/or respiration in addition to other effects on cell organales.

Table 3
Antimicrobial activity of modified St-*g*-PAM against different organisms

Tested organisms	Diameter of inhibition zone (cm) produced by polymers					
	GP1	GP2	GP3	GP4	GP5	GP6
Escherichia coli	0.5	0.5	0.9	0.6	0.7	0.9
Pseudomonas spp	0.5	0.0[a]	0.9	0.6	0.9	0.9
Corynebacterium spp	0.5	0.0	0.6	0.6	0.8	0.7
Staphylococcus spp	0.5	0.5	0.7	0.0	0.7	0.8
Shigella sonnei	0.6	0.6	0.6	0.0	0.7	0.8
Klebsiella pneumoniae	0.5	0.5	0.8	0.5	0.9	0.7
Mycobacterium spp	0.5	0.5	0.9	0.5	0.9	0.9

The test was carried out by using cut plug technique (Pridham et al., 1956).
[a] Zeros mean that no inhibition zone.

Table 4
Minimal inhibitory concentrations (MICs) of Polymers (μg/ml)

Test organisms	Polymers			
	GP_3	GP_5	GP_6	Ampicillin
Escherichia coli	200	150	150	5
Pseudomonas spp	150	200	200	10
Corynebacterium spp	150	200	150	5
Staphylococcus spp	175	200	150	10
Shigella sonnei	150	200	150	5
Klebsiella pneumoniae	175	150	175	15
Mycobacterium spp	150	150	150	150

The test was carried out by using agar technique with different types of bacteria and the most active polymers and *Ampicillin* as a standard.

Table 5
Cell toxicity, flow of K^+ and cell respiration

Test material	[a]Cell toxicity (DNA damage)	[b]flow of K^+	Oxygen consumption μl O_2/mg dry cell/h
Control (water)	−ve	0.46×10^{-6}	29.0
GP3			
200	−ve	0.6×10^{-6}	28.7
250	−ve	0.7×10^{-6}	28.7
GP5			
150	−ve	0.42×10^{-6}	27.9
200	−ve	0.43×10^{-6}	29.0
GP6			
150	−ve	0.67×10^{-6}	0.24
200	−ve	0.9×10^{-6}	0.20

[a] Cell toxicity was done using biochemical induction assay.
[b] Flow of K^+ and cell respiration were done using different doses of the tested polymers, water as control and *E. coli* as test organism.

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