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ORIGINAL ARTICLE

Synthesis and microbial degradation of azopolymers for possible applications for colon specific drug delivery I

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Escherichia coli;
Candida

Abstract For the purpose of colonic drug targeting, poly(ester) and poly(amide) azopolymers were synthesized and characterized. The polymers were based on azobenzene-4, 4\-dicarboxylic acid. It was polycondensated either with specific diamines to yield polyamides or with specific diols to yield polyesters. The polymers were characterized by infrared spectroscopy, elemental analyses, ^{1}H NMR and Thermogravimetric analysis (TGA). The molecular weights were measured by Gel Permeation Chromatography (GPC). The synthesized polymers showed a good film forming.

Degradation of poly azobenzene diacid-*co*-ethlene glycol 4000 and polyazo benzene diacid-*co*-jeffamine 4000 as azopolymer by microorganisms was investigated and the results demonstrated that *Aspergillus* fungi have produced almost the highest value, however, *Ganoderma resencium* and *Pleurotus ostreatus* came in the last rank. The highest poly azobenzene diacid-*co*-jeffamine 4000 degradation rate constant (μmol/ml/h) was performed by *Aspergillus fumigatus* which produced 5.73 ± 0.23 μmol/ml/h at 228 nm; whereas *Aspergillus ochraceous* produced the lowest poly azobenzene diacid-*co*-jeffamine 4000 degradation rate constant of 0.94 ± 0.72 μmol/ml/h at 332 nm.

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1. Introduction

In the last few years, much attention has been paid to the selective oral delivery of drugs to the colon (Schacht et al., 1996; Friend, 2005). The reasons for this concern are summarized in the following points: (1) the need to reduce GI side effects by medical treatments for colon inflammation and colon motility disorders by local delivery of drugs (Rubinstein., 2000); (2) the discovery of new anti-inflammatory drugs that could be used for the treatment of typical inflammatory diseases of the large bowel (Lang and Peppercorn, 1999); (3) the disclosure of the mechanism of action of some nonsteroidal anti-inflammatory drugs (NSAID) such as sulindac which were found to be active

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against colon polyps (first stage in colon carcinoma) (Qiao et al., 1997); (4) the recognition that, in some cases, the colon is capable of absorbing drugs efficiently; (5) accumulated evidence that drug absorption enhancement is feasible, primarily in the colon (Shiga et al., 1987); (6) recent findings that protein drugs are absorbed into systemic compartments from the large intestine better than from the small intestine (Antonin et al., 1996); (7) the unique metabolic activity of the colon, which makes it an attractive organ for drug delivery systems designers; and (8) like any other organ specific targeting, only a small dose of the drug is required, which subsequently results in fewer adverse drug reactions. Colon targeting can be achieved by different approaches. Several systems are currently being investigated as potential means for colonic drug delivery. The factors exploited are the pH gradient between the stomach and the colon and the high concentration of the intestinal microflora residing in the colon; this has been reported to be up to five-times greater than concentrations in other parts of the gastrointestinal tract (Kakoulides et al., 1998).

It was shown that polymeric azo dyes, designed for application as food dyes, can be degraded in the colon (Dubru and Wright, 1975; Hanohan et al., 1977; Brown et al., 1981). This approach was first described by Saffran and co-workers who cross-linked copolymers of styrene and 2-hydroxyethyl methacrylate with specific azo compounds and used these for the oral delivery of insulin (Saffran et al., 1986; Saffran et al., 1990). An example of polyester and one of a polyurethane containing azo linkages along the polymer backbone have also been reported (Samyn et al., 1995; Kimura et al., 1992). Consequently, the use of coating materials that are degraded selectively in the colon is of obvious importance for designing oral delivery of the drugs to the colon.

Many bacteria and fungi species including (*Colstridia*, *Eubacter*, *Streptococcus*, *Bacteroides*, and *Aspergillus*) could be good candidates to degrade the dye as reflected from the in vivo performance studies to achieving the reduction of azo groups.

The present paper describes our approach to design degradable polymer systems for the delivery of drugs to the colon. Azo-containing polyamides and polyesters were prepared by polycondensation of azobenzene diacid chloride with specific diamines and diols. The degradation of azo polymers was studied by recording the decrease of the absorbance of azo dye solution in phosphate buffer salt (PBS), at pH 7.2, containing tested organism, vs. time.

2. Experimental

2.1. Materials

p-Nitrobenzoic acid, thionyl chloride, diols (1,4 butanediol, 1,6 hexanediol, 1,12 dodecanediol, triethylene glycol, polurinic F68 and polyethylene glycol 4000) were purchased from Aldrich; USA and were used as received. 1,7-diaminoheptane, polytetrahydrofuran diamine 750 (PTHF750), polytetrahydrofuran diamine 1000 (PTHF 1000), polytetrahydrofuran diamine 2000 (PTHF 2000), jeffamine 148 (JF 148), jeffamine 2001 (JF 2001) and jeffamine 4000 (JF 4000) were obtained from Texaco Chemical Co. (Austin, TX, USA) and were dried by isotropic distillation with toluene. Triethylamine (TEA) was purchased from Aldrich, USA and was purified by reacting with p-toluenesulfonyl chloride, dried over calcium hydride and distilled prior to use. Chloroform was washed with sulfuric acid and water and was dried over calcium hydride.

2.2. Microorganisms

The following microorganisms were chosen to evaluate its activity toward the reduction or (degradation) of the synthesized polymers: *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceous* and *Fusarium oxysporum* were isolated from the Egyptian soil and identified. *Pleurotus ostreatus Hngary* (HAR17) was obtained from the Agricultural Research Center, Cairo, Egypt, and *Ganoderma resencium* mycelial hyphae were isolated and purified from their fruiting bodies, *Candida albicans* was used as yeast model. *Escherichia coli* were used as bacterial organisms.

2.2.1. Media

Nutrient agar and Nutrient broth medium were used for growing *E. coli* as bacterial cultures. Sabouraud dextrose agar was used for growing of *C. albicans*. Malt extract medium was used for growing *P. ostreatus*, *G. resencium*, *A. flavus*, *A. fumigatus*, *A. niger*, *F. oxysporum*, and *A. ochraceous*.

2.2.2. Evaluation of the microbial biodegradation of the azopolymers

For each organism, a series of test tubes have been prepared to obtain active vegetative microbial growth before the process of biodegradation. Stock cultures were used to inoculate 5 ml of broth medium specific for organism in number of test tubes. After incubation for 24 h at specific temperature for each organism, the cells were harvested by centrifugation (4000 rpm) for 10 min, suspended in 0.5 ml of PBS buffer, pH 7.2 (2 mM KH_2PO_4, 3 mM Na_2HPO_4. H_2O, 167 mM NaCl) containing 0.125 mM benzylviologen and 6.3 mM D-glucose monohydrate. A certain volume was added to proper solid medium and the colony forming unit was determined by spread plate technique.

Approximately 107 colony forming unit per ml of the selected microorganisms was used to inoculate the proper medium containing 33 μM of the azopolymers and 0.125 mM benzylviologen in test tube. Then, the test tubes were closed with rubber stopper, and incubated at 37 °C in a horizontal shaking water bath, set at 100 rpm/min. At regular time intervals, one tube was withdrawn from the water bath, opened and 0.5 ml of 30% trichloroacetic acid aqueous solution was added to stop the reaction, the absorbance of the clear supernatant was measured at the maximum wavelength of absorbance (λ_{max} = 332 and 228 nm for polymer IX and 320 and 230 nm for polymer XV. A calibration graph for each azo polymer was carried out by measuring the absorbance of PBS buffer solution pH 7.2, solution containing 3% (w/w) of trichloroacetic acid and a known concentration of the azo polymer. From the calibration graph, the azopolymer concentration was determined and plotted against the time. The rate of azo polymer degradation (K, the slope of the linear part of the degradation curve) was calculated as micromoles of azopolymer degraded per hour and per ml of inoculum (μ mol/h/ml).

2.2.3. Instrumentation

FTIR spectra were recorded on a Perkin-Elmer 1430 Ratio Recording Infrared Spectrophotometer apparatus from KBr

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