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Synthesis of PEG hydrogel with dityrosine for multi-functionality and pH-dependent fluorescence

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ABSTRACT

A poly(ethylene glycol) (PEG)-based hydrogel was synthesized by the copolymerization of PEG diacrylate (PEGDA) and PEG methacrylate (PEGMA) linked with *N*,*N*′-di(*tert*-butoxycarbonyl)-dityrosine (DBDY). Owing to the presence of the dityrosine derivative DBDY, the resulting hydrogel exhibited pH-dependent fluorescence and contained multi-functional groups (1 carboxylic acid group and 2 amine groups protected by *tert*-butoxycarbonyl groups). A high molecular mass chemical can be bound to DBDY in the hydrogel through one of these functional groups (i.e. the formation of amide bonding). Then the porosity of the resulting hydrogel is increased by the action of an enzyme such as proteinase. Also, if a drug is bound directly to DBDY, the enzyme can control its detachment. Hence, the PEGMA–DBDY hydrogel could be useful for drug release near the site of the disease where the expression level of a certain enzyme is especially high (e.g. matrix metalloproteinases in cancers). Binding of a UV-absorbing compound to DBDY was monitored by measuring the change in fluorescence intensity, which is known as the phenomenon of fluorescence resonance energy transfer (FRET).

1. Introduction

Hydrogels are three-dimensional networks (cross-linked structures) of hydrophilic homopolymers or copolymers that swell dramatically when contacted with water or a physiological liquid. Hydrogels have structures similar to those of living tissues and have attracted interest in the fields of biosensors, cell culture, tissue engineering and controlled drug release [1–5]. Poly(ethylene glycol) (PEG)-based hydrogels have been widely used because of their strength, processability, low fouling potential, good thermal and pH stability and minimal adhesion of cells and proteins [6].

'Intelligent' hydrogels with different degrees of swelling in response to external stimuli, such as temperature [7,8], pH [9] and electric field [10], have gained most attention because they are potentially able to release a drug at a specific site. The expression level of certain enzymes is especially high near the site of disease (e.g. matrix metalloproteinases (MMPs) in cancers [11–15]). If a hydrogel becomes more porous as the result of the action of that enzyme, the release rate of a drug inside the hydrogel will be increased at the site of the disease. If a drug is chemically bound to the surface of a hydrogel and that enzyme catalyzes breakage of the chemical bonding, the drug will be released specifically at the site of the disease. These two types of drug release are illustrated in Fig. 1. When hydrogels are made with PEG, a linker can be connected to one end of the PEG to generate chemical bonding that can be attacked by an enzyme (i.e. bonding with S in Fig. 1(A), and a drug in Fig. 1(B)). This study was designed to examine the suitability of dityrosine (DY) as such a linker.

DY, which is formed by C–C coupling of two tyrosine molecules (see Fig. 2(A) for the structure), has been found in proteins exposed to reactive oxidizing agents (e.g. hydrogen peroxide, hydroxyl radical and superoxide radical), ionizing radiation or peroxidase-catalyzed reaction and has been used as a biomarker for oxidative protein damage and selective proteolysis [16,17]. However, DY occurs naturally in fungal cell-wall proteins, insect eggs and sea-urchin envelopes and in extensible proteins, including elastin, resilin and calmodulin [18–22]. Owing to the presence of a biphenyl ring, DY has intense fluorescence at a wavelength of 420 nm, measurable upon excitation with either 315 nm (alkaline solutions) or 284 nm (acidic solutions) absorption bands [16].

The objective of this study was to synthesize a PEG-based hydrogel conjugated with DY. The resulting hydrogel is expected to exhibit pH-dependent fluorescence and to contain multi-functional groups where other molecules, such as a drug or a protein, can be bound. As shown in Fig. 2(A), DY has 2 amine and 2 carboxylic acid group that could prove useful for binding a drug or a protein. If the drug has the property of UV absorbance, both binding and detachment of the drug from PEG-DY can be detected because of the phenomenon of fluorescence resonance energy transfer (FRET).

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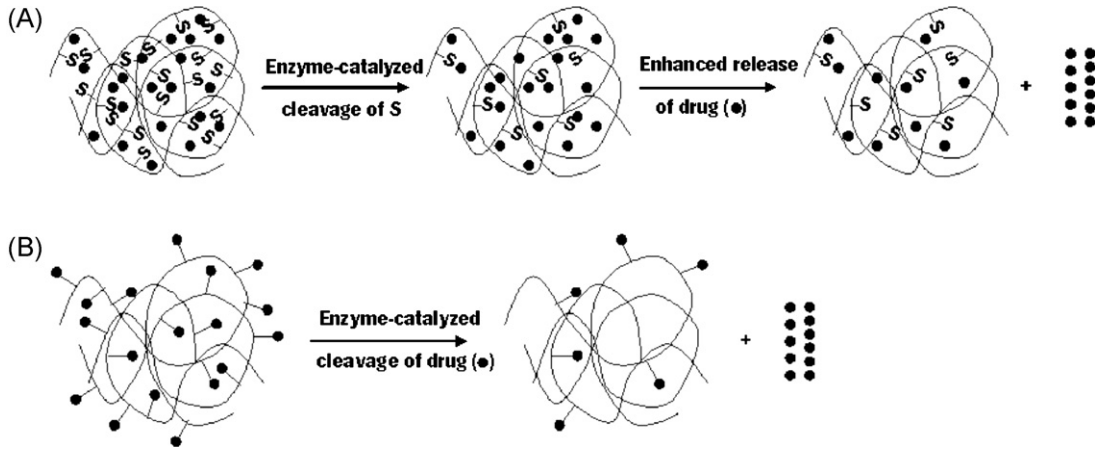

Fig. 1. Two different modes of the drug release in a hydrogel catalyzed by a specific enzyme: the increase in the porosity by the enzyme-catalyzed cleavage of a substrate (A) and the breakage of the chemical bonding between the hydrogel and the drug (B). S denotes the substrate of the enzyme.

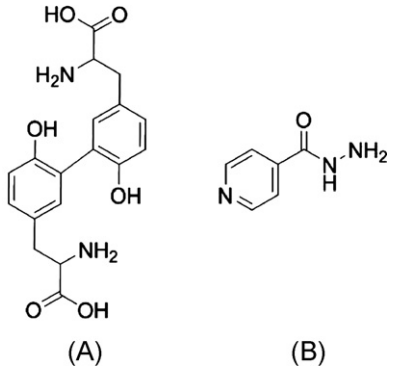

Fig. 2. Structures of dityrosine (A) and isonicotinyl hydrazine (B).

For this purpose, the DY derivative *N*,*N*′-di(*tert*-butoxycarbonyl)-dityrosine (DBDY) was first synthesized from *N*-(*tert*-butoxycarbonyl)-tyrosine using horseradish peroxidase (HRP). DBDY was then covalently linked to poly(ethylene glycol) methacrylate (PEGMA) by an esterification reaction. PEGMA–DBDY and poly(ethylene glycol) diacrylate (PEG-DA) were copolymerized to hydrogels by exposure to UV radiation. Isonicotinyl hydrazine (INH; see Fig. 2(B) for the structure), which has been used as first-line antituberculosis medication, has the property of UV absorbance and was chosen as a model drug linked to DBDY to detect the presence of FRET.

2. Experimental

2.1. Materials

Boric acid (99%), *N*-(*tert*-butoxycarbonyl)-tyrosine (BY) (98%), D_2O, *N*,*N*-dicyclohexylcarbodiimide (DCC) (99%), 2,2-dimethoxy-2-phenylacetophenone (DMPA), HRP, *N*-hydroxysuccinimide (NHS) (98%), INH, β-mercaptoethanol (98%), anhydrous methylene chloride (MC), *N*,*N*-dimethylformamide (DMF) (99.8%), sodium tetraborate decahydrate (99.5%), trifluoroacetic acid (TFA) (99%),

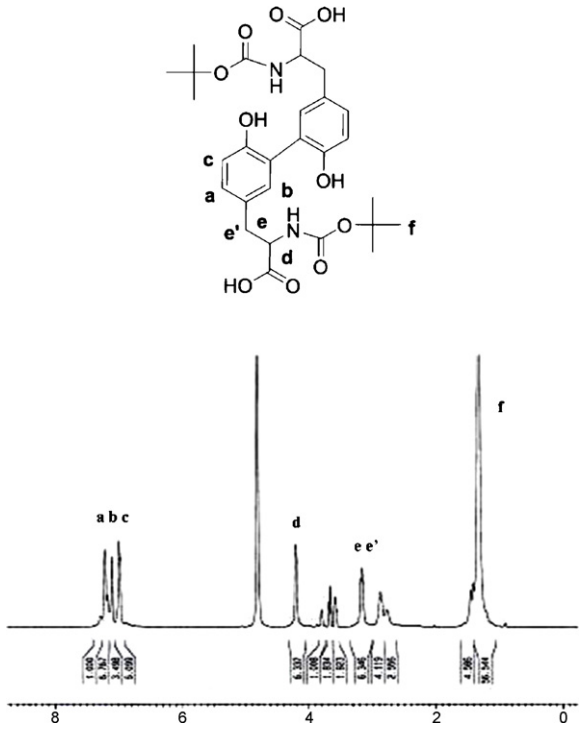

Fig. 4. ^{1}H NMR spectrum of DBDY.

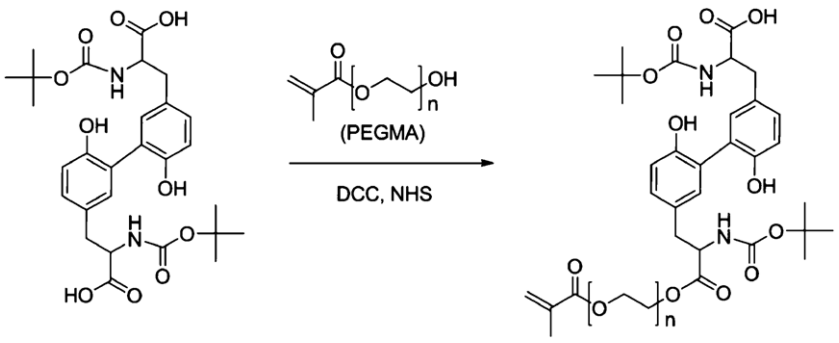

Fig. 3. Synthesis of PEGMA–DBDY.