Synthesis of peptide derivative as bio adhesive

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Abstract. Bio adhesive material is a protein adhesive, which has recently been highlighted as an attractive biologically based and highly functional adhesive as a new chemo enzymatic synthesis of co polypeptide. The bio adhesive polymer was prepared by modification of pepsin structure with vinyl monomer such as maleic anhydride using ceric ammonium nitrate as an initiator, then the grafted copolymer was substituted with amino drugs. This design carries controlled delivery over an extended period of time due to its digesting nature. The new drug copolymer was investigated using common spectroscopy methods, such as FTIR, 1H-NMR and UV Spectroscopy. The physical properties for the prepared polymers were tested. Our results show that deprotonated is required for drug copolymers when analyzed in different pH values at 37 °C in vitro study and controlled drug release was compared at zero time and after three days. The rate of hydrolysis in basic medium was higher than acidic medium. It was concluded there are several advantages of sustain release by modified drug with slow release and in vivo performance was noted to be promising using for applications of polymer used different mice and rabbits infected with bacteria and wounds.

Keywords: Pepsin, chemo enzymatic , Maleic anhydride, , Graft Drug Copolymer.

1-Introduction

Pepsin is an enzyme that breaks down proteins into smaller peptides (that is a protease). It is produced in the stomach and is one of the main digestive enzymes in the digestive of humans and many other animals, where it helps digest the proteins in food[1]. Pepsin has a three dimensional structure, of which one or more polypeptide chains twist and fold, bringing together a small number of amino acids to form the active site, or the location on the enzyme where the substrate binds and the reaction takes place. Pepsin is an aspartic protease, using a catalytic aspartate in its active site [2].

It is one of three principal proteases in the human digestive system, the other two being chymotrypsin and trypsin. During the process of digestion, these enzymes, each of which is specialized in severing links between particular types of amino acids, collaborate to break down dietary proteins into their components, i.e., peptides and amino acids, which can be readily absorbed by the small intestine. Pepsin is most efficient in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.[1] Pepsin's proenzyme, pepsinogen, is released by the chief cells in the stomach wall, and upon mixing with the hydrochloric acid of the gastric juice, pepsinogen activates to become pepsin[2, 3].

Pepsin was one of the first enzymes to be discovered. It was discovered in 1836 by Theodor Schwann. Schwann coined its name from the Greek word πέψις pepsis, meaning "digestion" (from πέπτειν peptein "to digest").[4][5][6][7] Scientists around this time began discovering many biochemical compounds that play a significant role in biological processes, and pepsin was one of
them. An acidic substance that was able to convert nitrogen-based foods into water-soluble material was determined to be pepsin.[8]

It became one of the first enzymes to be crystallized, it was using dialysis, filtration, and cooling.[9] Pepsin is most active in acidic environments between 37 °C and 42 °C.[10][11] Accordingly, its primary site of synthesis and activity is in the stomach (pH 1.5 to 2). Pepsin will digest up to 20% of ingested amide bonds by cleaving preferentially at the N-terminal side[12] of aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.[13] Pepsin exhibits preferential cleavage for hydrophobic, preferably aromatic, residues.

Increased susceptibility to hydrolysis occurs if there is a sulfur-containing amino acid close to the peptide bond, which has an aromatic amino acid. Pepsin remains in the larynx following a gastric reflux event.[14][15] At the mean pH of the laryngopharynx (pH = 6.8) pepsin would be inactive but could be reactivated upon subsequent acid reflux events resulting in damage to local tissues[16]. The bond between the polymer and the drug if stable in gastric juice and is slowly hydrolyzed in the presence of a pancreatic enzyme in an alkaline medium, the drug might not be released until it reaches the small intestine for example if the drug-polymer conjugate by ester bond, the resulting ester will be hydrolyzed specifically in the presence of α-chymotrypsin at an alkaline pH. Compared to conventional oral dosage forms they offer several advantages including[17-20].

2. Experimental

2.1. Materials

Pepsin was purchased from Fluka and dried at 110°C for about 2 h to remove absorbed moisture. Cerium ammonium nitrate (CAN), Maleic anhydride (MA) and Amoxicillin were purchased from Sigma Chemicals, all the available chemical reagents were used without further purification.

2.2 Instrumentation

Melting point was measured using Thermal Microscope (Kofler-method), and Reichert thermovar, Stuart SMP 30. Infrared spectrophotometer measurements were performed using Shimadzu FT-IR 8400 series Fourier Transform. 1H-NMR spectra were measured with a Bruker spectrophotometer model ultra-shield at 300.13 MHz in DMSO-d6 and CDCl3 as solvents with TMS as internal standard, Al-Albyt University-Jordan

UV-Visible double beam scanning spectrophotometer VARIAN (UV-Vis)-100 Conc, at room temperature. The modification of natural polymers is a promising technique for the making a new materials. The productive method can change absorbent polymers[1]. Natural polymers are modified as a means to overcome their viscosity, microbial degradation, and partial or low solubility.

2.3.A- Preparation of pepsin graft maleic anhydride (R1)

(1Gm) of pepsin was dissolved in (10mL) of acetone, (0.5ml) of ceric ammonium nitrate (CAN), (1Gm) of maleic anhydride was added, the mixture was introduced in polymerization bottle, and heated about (30) minutes at (60 °C). The nutty color product was produced (85%) conversion ratio. (CAN was prepared by adding 1 Gm from it in 10 ml from 5% HNO3)

2.3.B- Substitution of (R1) with amino drugs (R1A)
(0.30 Gm) of pepsin g-maleic anhydride was dispersed in (5ml) of dioxin, (0.30Gm) of amoxicillin dissolved in (5ml) of dioxin, (0.3 ml) of DMF was added to the mixture. then was refluxed with stirring about 1 hour at (90°C), the colored solution was filtered, the filtrate was isolated and the solvent was evaporated, the black product (A) of pepsin-g-[N-Amoxicillin maleic amic acid] was washed with ether two times and dried at (50°C), conversion ratio (82 %), S. p. (130-150°C), all physical properties were listed in Table (1).

Table (1) Physical properties of prepared polymer (R1A)

| Pol. | -Drug | Color | Softening point °C | Conversion ratio % |
|------|-------|-------|--------------------|--------------------|
| R1   | Amoxicillin | Black | 130-150           | 82                 |
3.4. Applications of polymer used different mice and rabbits infected with bacteria and wound

The appropriate amount of prepared polymer (R1A) on the site of infection on different infected mice and rabbits with different bacterial infection or different wounds, for several days, the results showed that gave a good promising using.

4. Results And Discussion

Modification of pepsin by grafting with maleic anhydride. Pepsin can be grafted as a main chain of the backbone of a polymer, it was initiated by redox initiators, ceric ion. When (Ce4+) salts such as cerium ammonium nitrate, at first a ceric ion–complex occurs, and pepsin radicals created by hydrogen abstraction from pepsin. Thus, the radical formation on the pepsin backbone occurs on the oxygen atom [22].

Graft copolymerization of unsaturated monomer [20] maleic anhydride on pepsin backbone could added new properties and more attention production, pepsin-g-maleic anhydride was modified with Amoxicillin as amino drug which acted as ring opening of maleic anhydride by using (CAN) as initiator, as illustrated below [21].

Scheme (1) Mechanism of ring opening reaction of pepsin-g-maleic anhydride by nucleophile reaction.

Figure (1). FTIR Spectrum of pepsin
Figure(1). FTIR spectrum of pepsin showed a stretching band at 3300 cm⁻¹ which is assigned for the NH group stretching vibration and the appearance of two carbonyl groups C=O at 1620 cm⁻¹ and 1700 cm⁻¹ which belong to amide groups vibration, (−CH) absorption appeared at 2960 cm⁻¹, another band appeared at 1438 cm⁻¹ due to the (C-N), band at (3055 cm⁻¹), band appeared at (3344) cm⁻¹ due to u(NH) stretching vibration, band (1649) cm⁻¹ due the (amide) stretching, carboxylate absorption at (1570) cm⁻¹, Figure(3) showed absorption at (3290) cm⁻¹ due to u(NH) stretching. band appeared at (1708) cm⁻¹ due to (C=O) stretching, carboxamide absorption at 1631, carboxylate anion band appeared at 1527 cm⁻¹, −OH absorption band appeared at 3365 cm⁻¹ OH proved the amic acid/male between drug and pepsin which compared with the FTIR spectrum of pepsin maleic anhydride -g- Figure(2).
Figure 4. $^1$H-NMR Spectrum of prepared polymer R1A

Figure 4. $^1$H-NMR Spectrum of prepared polymer pepsin-g-[N-drug Maleic acid] showed the following signals:

1.2 ppm (Triplet, 3H, CH3), 6.2 ppm (Singlet, 1H, CO-NH amide), 7.8–7.9 ppm (4H, Aromatic ring), 4.5 ppm (Singlet, OH for pepsin), 12.0 ppm (Singlet, 1H, COOH).

5. Controlled drug release [23,24,25]

Studying Release of (R1A) was studied. 100 mg was added continuously in (100 ml) buffer solution at (37 °C). The wavelength of $\lambda_{\text{max}}$ was measured at different periods and different pH values (1.1–7.4) by using UV spectrometer. The sample was analyzed by UV-spectroscopes periodically withdrawn the sustained release was measured by the mole fraction constructed from UV spectra.

Figure 5. UV Spectra absorptions through hydrolysis of (RA1) in pH 7.4 and pH 1.1

6. Conclusions

The prepared drug copolymer was analyzed in different pH values at (37 °C) as in vitro study and controlled drug release was compared at zero time and after three days, indicated the rate of hydrolysis in basic medium is higher than acidic medium. It was observed that modified drug release with extended drug action via slow release and in vivo performance was noted to be promising.
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