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Preparation of thermally denatured albumin gel and its pH-sensitive swelling

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Abstract

Heat induced albumin gel was prepared as a candidate for a drug carrier of a pH sensitive drug delivery system. A given amount of albumin was dissolved in phosphate buffer solutions of known pH. Albumin solution was heated in a water bath and thermally denatured albumin was obtained. Before heating, the absorbance of albumin solution was measured spectrophotometically at 600 nm. The structure of thermally denatured albumin was analyzed by Fourier transform infrared (FTIR). Heat induced albumin gels were swelled to their equilibrium state in aqueous swelling mediums of given pH. The structure of thermally denatured albumin depends on the pH of albumin solution, i.e. the charge distribution of albumin molecule in solution. The structure of heat induced albumin affected the equilibrium swelling ratio and pH sensitivity in swelling. The equilibrium swelling ratio of heat induced albumin increased with the increase of pH of swelling medium on the right side of isoelectric point of albumin. On the other hand, the swelling ratio decreased as the pH of swelling medium increased, on the left side of isoelectric point. At the isoelectric point, the swelling ratio showed a minimum value. Gel, which was made from albumin solution whose pH was adjusted to high value, was found to have high pH sensitivity in swelling.

Keywords: Albumin; pH; Swelling; Thermally denatured protein

1. Introduction

Crosslinking of protein can be achieved either by direct reaction between functional groups (usually carboxyl group and amino group) in the polypeptide side chains (self crosslinking) (Clarke and Courts, 1977), or by chemically crosslinking agents such as glutaraldehyde and 2,3-butaione (Yapel, 1979). Usually, self crosslinking is accomplished by heating relatively concentrated protein solution over 50°C. To produce insoluble protein gel, a number of workers choose the thermal denaturation method (self crosslinking). Albumin

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microspheres, containing 5-fluorouacil (Morimoto and Fujimoto, 1985), cetylpyridinium chloride (Egbaria and Friedman, 1990a) and metrondiazol (Egbaria and Friedman, 1990b) were prepared and their sustained release was studied. Magnetic albumin particles containing Fe_2O_3 and anti-cancer agents were prepared and used for treatment of cancer (Gupta et al., 1988).

Protein has strong functionality and reactivity because of the presence of carboxyl groups and amino groups. In addition, most protein has high water solubility, excellent emulsification capacity and heat coagulability (Ma and Holme, 1982; Kwon et al., 1992). These properties promise the application of protein to stimuli-response system area such as drug delivery system. However previously reported researches treated mainly the preparation of microparticle or microcapsule, or the application of protein bead as a carrier for sustained release of drug (Gupta et al., 1988; Egbaria and Friedman, 1990b; Kwon et al., 1992).

The structure of thermally denatured proteins reflects the aggregation pattern of the protein molecules, which is strongly affected by the denaturation temperature, the heating period, and the conditions of protein solution such as pH and ionic strength (Nakamura et al., 1978; Woodward and Cotterill, 1986). The interaction between each protein molecule denatured on heating is mainly governed by the surface net charge and the hydrophobic area exposed by heating of the protein molecules. The former usually arises due to electrostatic repulsion and the attractive force originates from the hydrophobic interaction (Schmidt, 1981; Gossett et al., 1984). It is expected that the structure of heat induced protein gel can be controlled by adjusting either surface net charge or hydrophobicity. So, it is possible to design the structure of heat induced protein gel as drug carriers which have a pH-sensitive property, and this means that we can prepare the drug carriers having different structures (pH-sensitivities) with protein. To do this, it is necessary to control the interaction forces between protein molecules. Between attractive and repulsive forces, the latter can be readily controlled by controlling the surface net charge of protein molecules and the easiest way to control the surface net charge is by changing the pH of protein solutions.

In the present study, we prepared thermally denatured albumin gel having different physical state under known pH conditions. Heat induced albumin characterized by Fourier transform infrared (FTIR) analysis. Swelling ratio of the thermally denatured albumin gel was also examined under specified pH conditions.

2. Experimental

2.1. Preparation of thermally denatured albumin

Egg albumin (Kanto chemical, Japan, first grade) was used as received. Given amounts of albumin were dissolved in phosphate buffer solutions having given pH values. The concentration of albumin solution was 80 g/l. The ionic strength of buffer solution was adjusted to 0.2. Albumin solution was taken to measure absorbance at 600 nm using UV-visible spectroscopy (Shimadzu UV 160A, Japan), and then transferred to cylindrical mold to be heated in a water bath at 80°C for 1 h. After heating, the mold was cooled in chilled water for 90 min and the thermally denatured albumin was cut to obtain a tablet shape matrix having regular thickness. The heat induced albumin was dried in desiccator until the weight of the matrix reaches a constant value at room temperature.

2.2. FTIR analysis

FTIR (ATI Mattson, Genesis series FTIR) analysis of thermally denatured albumin was performed to observe the structure changes of thermally denatured albumin matrix.

2.3. Swelling experiment

Predetermined amounts of dried albumin matrix were placed in phosphate buffer solution (ionic strength = 0.2). Temperature was maintained on 37.5 ± 0.5°C in an incubator (SI-900 shaking incubator, Jeio tech., Korea). After 6 h, the albumin matrix in its equilibrium swollen state was weighed. The swelling ratios of albumin matrices were determined from the weight change before and after swelling.

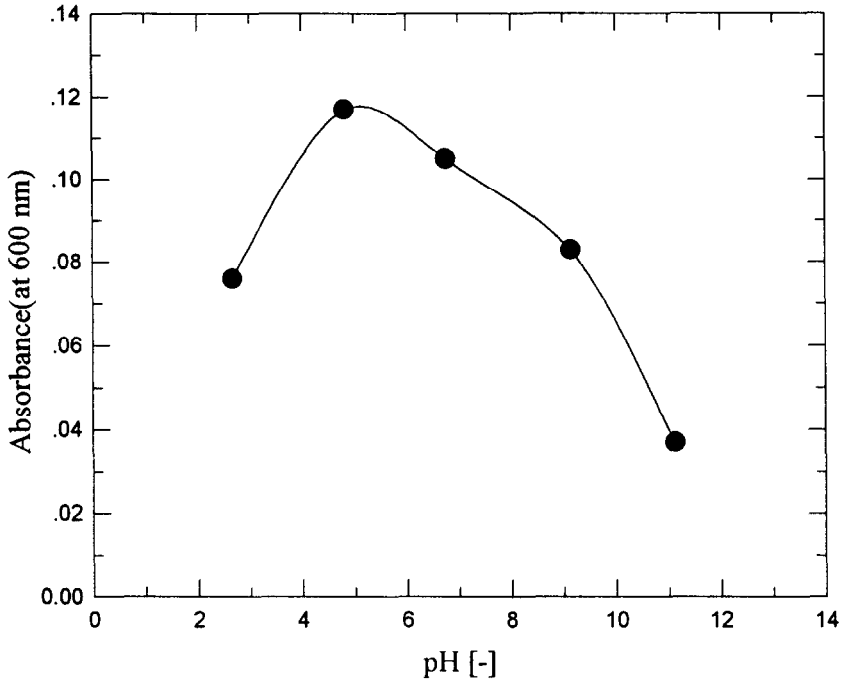

Fig. 1. Absorbance of albumin solution at different pH.

3. Results and discussion

3.1. Preparation of thermally denatured albumin

The absorbance of albumin solution showed the highest value in the vicinity of iso-electric point (pI) of albumin and decreased at the pH away from pI (Fig. 1). The high value of absorbance indicates that albumin molecules in solution are in aggregated state. The concentration of albumin in solutions was constant, and attraction among albumin molecules was equal in each solution, but there was repulsion among albumin molecules due to the surface charge of albumin molecules at different pH. It may be concluded that the difference in absorbance reflected the difference of the repulsive force among albumin molecules. In the vicinity of the pI, surface net charge of the albumin molecule has a minimum value and it means low electrostatic repulsive force among albumin molecules. On the other hand, surface net charge of albumin molecule increases as the pH value increases or decreases from pI and high electrostatic repulsive force results in low absorbance value.

3.2. FTIR analysis

The frequency position and peak intensity ratio of amide I and amide II band is listed in Table 1. The peak intensity ratio is the ratio of the band's intensity to CH_2 deformation vibration (1450 cm^{-1}) by the baseline method (Goringstein et al., 1995).

As shown in Table 1, the peak positions of both amide I and amide II bands are at a minimum when the pH value is near pI. In the case of amide I band, the wave number decrease leads to the increased number of hydrogen bonds in which the carbonyl oxygen is involved as a consequence of the presence of the NH_2 groups (Torreggiani et al., 1997). In addition, the shift in the frequency position of the amide II band to the lower region reflects the increased number of hydrogen bonds (Ong et al., 1994; Muller et al., 1996). The peak intensities of both amide I and amide II bands

Table 1
Band position of amide I and amide II and the intensity ratio (R_I, R_{II}) to the 1450 cm^{-1} band

pH Condition at denaturation stage	Amide I		Amide II	
	Band position (cm^{-1})	R_I	Band position (cm^{-1})	R_{II}
2.7	1652.7	0.37414	1533.1	0.9152
4.8	1644.9	0.39007	1527.3	0.8978
6.8	1648.8	0.29803	1529.2	0.8999
11.1	1658.7	0.24534	1540.8	0.9149

decreased as the pH value differed from pI. From these results, it could be concluded that the sample that denatured at the pH value near pI was more crosslinked than the other samples.

3.3. Swelling ratio

Fig. 2 shows the equilibrium swelling ratio of heat induced albumin denatured at pH 11.13. The equilibrium swelling ratio was at a minimum at pI and increased as the pH of swelling medium either increased or decreased. The net charge of the albumin molecule is at a minimum at pI, which means low electrostatic repulsion between chains in thermally denatured albumin, as mentioned above. Low electrostatic repulsive force resulted in low swelling ratio. However, as the pH differs from pI the net charge of albumin molecule increases (positive below pI, negative above pI). High swelling ratio can be attributed to the increased amount of net charge (either positive or negative). It could be concluded that the charge distribution in thermally denatured albumin affected swelling ratio.

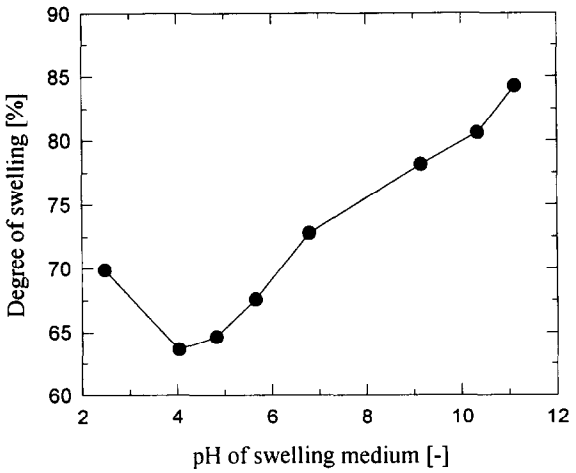

Fig. 2. Effect of pH on the equilibrium swelling ratio of albumin. (Denaturation pH: 11.1.)

First order regression of equilibrium swelling ratios of heat induced albumin denatured at the known pH conditions (pH 2.67, 4.82, 6.75 and 11.13) are illustrated in Fig. 3. The pH range of swelling medium was adjusted to the right side of pI. pH sensitive swelling was observed for all heat induced albumin, but the slope was different for each thermally denatured albumin sample. Fig. 4 showed the slope of first regression lines in Fig. 3 according to the pH of albumin solution before denaturation. The albumin sample denatured at pH 11.13 showed the highest sensitivity among the samples. The sensitivity decreased as denaturation pH decreased. The mechanism of pH sensitive swelling is expected to be governed by ionization of carboxyl groups in the experimental pH region. High equilibrium swelling ratio was attributed to the electrostatic repulsive force originating from the negative charge of ionized carboxyl groups. So, different pH sensitivity means a different amount of ionizable and/or ionized carboxyl groups.

As discussed earlier, heat induced albumin denatured at pI has a more crosslinked structure. More crosslinked structure means more amounts of carboxyl and amino groups participated in the crosslinking reaction resulting in the loss of their functionality. That is to say, there remains more carboxyl and amino groups in the gel that denatured at high pH condition than in the others. However, there are much more carboxyl groups

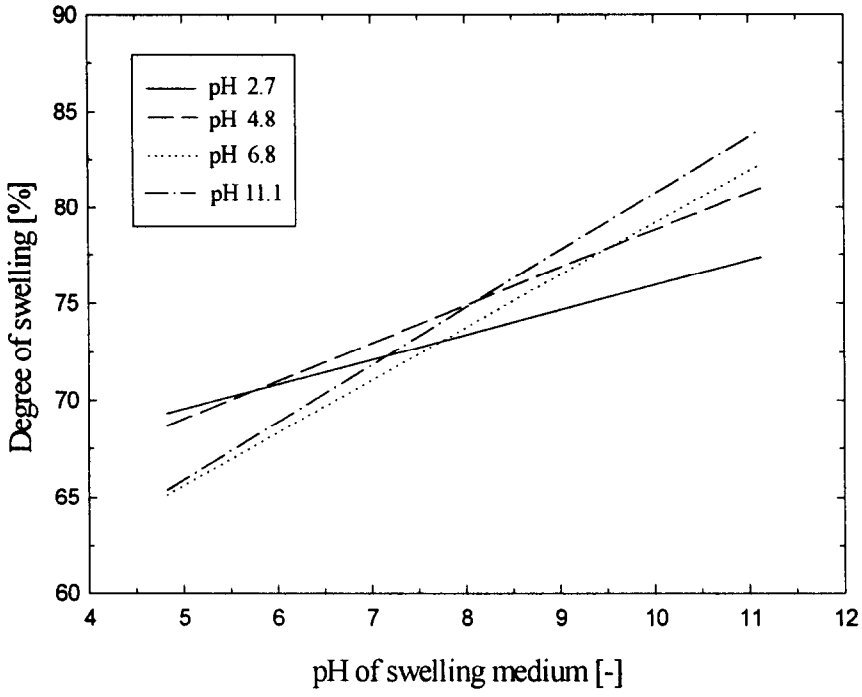

Fig. 3. The degree of swelling as a function of pH of the swelling medium.

than amino groups in native albumin molecules. A large portion of amino groups would participate in self crosslinking reactions regardless of the pH of albumin solution at the preparation stage and could thereby lose their functionality. Although the remaining amount of amino groups might be different for each sample, the contribution of amino groups to the pH sensitive mechanism could be ignored compared to that of carboxyl groups, because there are much more carboxyl groups than amino groups in heat induced albumin gel. From this aspect, the ionization of carboxyl groups is the major factor that gives the pH sensitive swelling property to the thermally denatured albumin matrix.

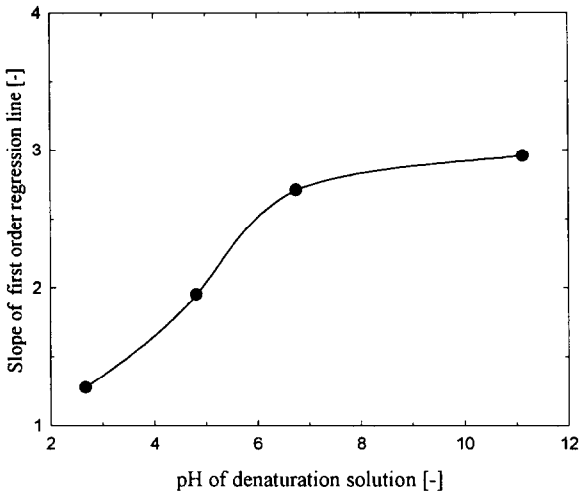

Fig. 4. Variation of pH sensitivity according to the pH of denaturation solution.

In general, there are three types of drug delivery systems associated with biodegradable matrices: diffusion controlled; swelling controlled; and chemically controlled. The degree of swelling is one of the most important factors affecting the drug release characteristics in drug delivery systems (Linhardt, 1989). Since the drug release is dependent on the swelling of matrix, stimuli sensitive swelling of matrix means that the matrix has the ability to release drugs in response to changes in environmental variables such as temperature, pH, ionic strength, etc. In the case of pH sensitive

drug delivery systems, many studies discussing the relationship between the swelling ratio of the vehicle and drug release characteristics were reported (Park et al., 1998; Peppas and Klier, 1991).

4. Conclusions

As the pH of albumin solution changes, the charge distribution of albumin molecule in solution is altered. The structure of thermally denatured albumin depends on the pH of the albumin solution, i.e. the charge distribution of albumin molecules in solution. The structure of heat induced albumin affects the equilibrium swelling ratio and pH sensitivity in swelling. The pH of albumin solution affects pH sensitive swelling characteristics of thermally denatured albumin gel thereby heat induced protein gel matrices having different pH sensitivities could be prepared. Until pI, the equilibrium swelling ratio of heat induced albumin decreased as the pH value of swelling medium rose. However, the swelling ratio increased as the pH of swelling medium increased, on the right side of the isoelectric point. At the isoelectric point, the swelling ratio was minimal. Gel, which was made from albumin solution whose pH was adjusted to a high value, was found to have a high pH sensitivity in swelling.

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