Effect of Acetylation and Succinylation on the Physicochemical Properties of Winged Bean (*Psophocarpus tetragonolobus*) Proteins

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Winged bean flour was acylated to various degrees by acetic and succinic anhydrides. Changes in physicochemical properties such as PAGE, fluorescence spectra, and ultracentrifuge patterns of acylated proteins were measured. Acetylation and succinylation dissociated the proteins of winged bean to lower molecular weight proteins. The extent of dissociation depends upon the degree of acetylation/succinylation. Succinylation dissociates winged bean proteins to a greater extent than acetylation.

INTRODUCTION

Chemical modification, especially acylation, has been shown to improve the functional properties of vegetable proteins. Acetylation and succinylation have been used to improve/modify the functional properties of oilseed and legume proteins (Kinsella and Shetty, 1979). Narayana and Narasinga Rao (1984) have reported that acylation of winged bean proteins increases solubility, water and fat absorption capacity, emulsion capacity, and foaming properties. According to Kinsella (1976) the functional properties are related to their physicochemical properties. In this investigation the effect of acetylation and succinylation of winged bean proteins on their physicochemical properties has been studied.

MATERIALS AND METHODS

Winged bean (*Psophocarpus tetragonolobus*) seeds obtained from the National Botanical Research Institute, Lucknow, India, were dehusked and defatted as described earlier (Narayana and Narasinga Rao, 1982). The defatted flour (60 mesh, British Standard Screen) had 43% crude protein and 0.62% fat. Analytical reagent grade acetic anhydride was obtained from British Drug House, India, and succinic anhydride from Sigma Chemical Co.

Acylation of Winged Bean Flour. Acylation using acetic anhydride and succinic anhydride was performed by the procedure described earlier (Narayana and Narasinga Rao, 1984). The degree of acylation was determined by estimation of "available lysine" in the acylated flours. The procedure of Carpenter (1960) using 1-fluoro-2,4-dinitrobenzene (FDNB) reagent was followed. The degree of acylation is expressed as percent reduction of available lysine in the acylated flours.

The proteins from the acylated flours were extracted with water at pH 9.2. The supernatant protein solution, after centrifugation, was dialyzed extensively against 0.01 M phosphate buffer (pH 7.2). After centrifugation, the supernatant protein solution was appropriately diluted for analysis. Winged bean proteins (WB) acylated to different levels were characterized by polyacrylamide gel electrophoresis (PAGE), analytical centrifugation, and fluorescence spectra by the following procedures.

Polyacrylamide Gel Electrophoresis (PAGE). PAGE was carried out in a Shandon electrophoresis apparatus. Gels (7.5%) in phosphate buffer (0.01 M) of pH 7.2 were used. The same buffer was used as running buffer. Gel tubes of 0.5 × 7.5 cm and 100 μg of protein/gel tube were used, and electrophoresis was carried out at a constant current of 3 mA/tube. The gels were stained in 0.025 Coomassie brilliant blue Rs-260 solution and destained with a mixture of acetic acid 2-propanol/water (75:50:875).

Ultracentrifugation. The ultracentrifuge experiments performed in a Spinco Model E Analytical centrifuge equipped with rotor temperature indicator control (RTIC) and phase plate schlieren optics. By use of 1% dialyzed protein solution the runs were made at 59 780 rpm at room temperature (~28 °C). From the photographs taken at different intervals of centrifugation, $s_{20,w}$ values were calculated by the standard procedure (Schachman, 1959).

Fluorescence Spectra. Fluorescence spectra were recorded in the range 300–400 nm after excitation at 280 nm by using a Perkin-Elmer fluorescence spectrophotometer Model 203. Protein solutions of 0.01 absorbance at 280 nm were used. The fluorescence of the buffer solution was measured and subtracted from that of the protein solution.

RESULTS AND DISCUSSION

The PAGE patterns of untreated and 32, 78, and 90% acetylated winged bean (WB) proteins are shown in Figure 1. The PAGE pattern of winged bean total proteins in phosphate buffer (pH 7.2) shows one major band with low mobility and another band of high mobility along with a few diffused bands (Figure 1). The relative mobilities suggest that the major band may be a high molecular weight protein, while the thin band with higher mobility may be a low molecular weight protein. The ultracentrifuge pattern of untreated winged bean total proteins supplements the PAGE data (Figure 3).

Ultracentrifugation and PAGE studies of Yanagi et al. (1983) have shown that the "6.5S" component was one of the major protein groups and the other component of the "2.5S" peak was a mixture of many proteins. The components with large electrophoretic mobilities were all included in the 2.5S group. Acetylated samples gave patterns that consisted of a band of low mobility and another of high mobility. The intensities of the two bands were nearly the same. Interestingly, the PAGE patterns of all acetylated samples were similar to one another. The observation that the pattern of acetylated samples consisted of a major band with high mobility would suggest that the proteins are possibly dissociated due to acetylation; the intensity of the band of high mobility with the unmodified protein was lower than that of the band of low mobility.

The PAGE patterns of untreated and 54, 77, and 87% succinylated WB proteins are shown in Figure 2. The patterns consisted of several bands of high mobility. As the extent of succinylation increased, the mobilities of the bands increased; the intensity of bands of high mobility also increased. The 87% succinylated sample had a band of high mobility that was not observed in the other two samples.

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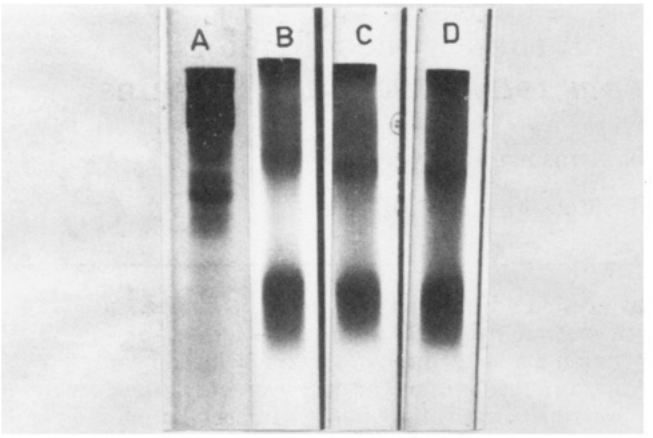

Figure 1. PAGE patterns of untreated and acetylated winged bean proteins (100 μg of protein/gel tube). (A) Untreated; (B) 32% acetylated; (C) 78% acetylated; (D) 90% acetylated.

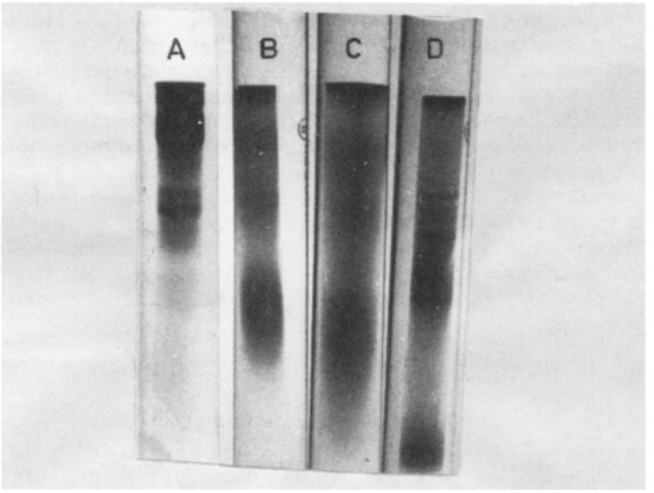

Figure 2. PAGE patterns of untreated and succinylated winged bean proteins (100 μg of protein/gel tube). (A) Untreated; (B) 54% succinylated; (C) 77% succinylated; (D) 87% succinylated.

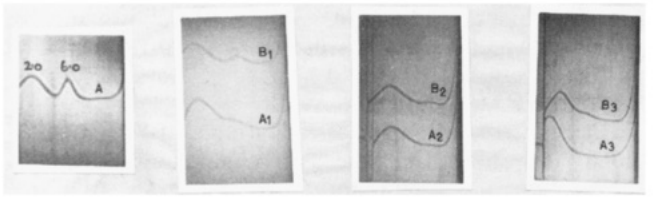

Figure 3. Ultracentrifuge patterns of untreated, acetylated, and succinylated winged bean proteins. (A) Untreated; (A_1) 54% succinylated; (B_1) 32% acetylated; (A_2) 77% succinylated; (B_2) 78% acetylated; (A_3) 87% succinylated; (B_3) 90% acetylated.

Separation in PAGE is based both on the charge and on the size of the protein molecule. Both acetylation and succinylation modify the net charge of the protein. Therefore, it would not be correct to attribute high mobility in PAGE to dissociation of protein. To supplement PAGE data, ultracentrifugation studies were also performed.

The sedimentation velocity patterns of proteins of acetylated/succinylated WB proteins are shown in Figure 3. The pattern of the proteins of untreated WB consisted of two peaks with $s_{20,w}$ values of 2 S and 6 S; their proportions were 70 and 30%, respectively (Figure 3). The 32% acetylated sample gave a pattern wherein the proportion of the 6 S protein had decreased markedly while that of the 2 S protein had increased (Figure 3). With the 78% acetylated sample the proportion of the 6 S protein had decreased even further, and 90% acetylated sample gave a pattern that consisted entirely of the 2 S protein fraction.

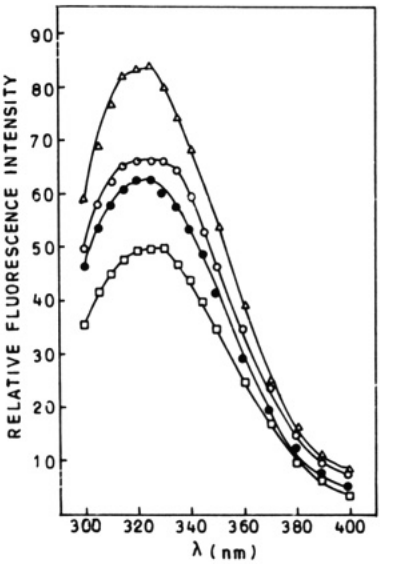

Figure 4. Fluorescence emission spectra of untreated and acetylated winged bean proteins. (□) Untreated; (●) 32% acetylated; (○) 78% acetylated; (△) 90% acetylated.

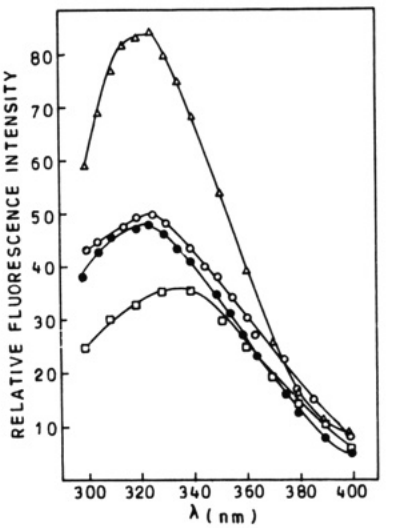

Figure 5. Fluorescence emission spectra of untreated and succinylated winged bean proteins. (□) Untreated; (●) 54% succinylated; (○) 77% succinylated; (△) 87% succinylated.

Similar observations were made with the succinylated samples. In fact, the 77% succinylated sample gave a pattern that consisted almost entirely of 2 S protein. These studies clearly indicate that acetylation and succinylation dissociate WB proteins and the extent of dissociation depends upon the degree of acetylation/succinylation. Further, at the same level of acetylation or succinylation the latter method causes greater dissociation of WB proteins. Thus, the results of PAGE and ultracentrifugation were compatible.

Jayarama Shetty and Narasinga Rao (1978) have reported that succinylation of arachin causes dissociation of the protein and the extent of dissociation depends upon the degree of succinylation. Similar results have been reported with glycinin (Appu Rao and Narasinga Rao, 1979). Acetylation also dissociates arachin (Shyamasundar, 1980).

Narayana and Narasinga Rao (1984) have reported that acetylation or succinylation increases the water absorption capacity of WB flour. This increase could be due to the dissociation of WB proteins on acylation; dissociated proteins would have a greater number of water binding

(hydrophilic) sites. Narayana and Narasinga Rao (1984) have also reported that foam stability of acylated WB flours is low; this could be due to dissociation of proteins.

The fluorescence emission spectra of untreated WB proteins acylated to various degrees are shown in Figures 4 and 5. In the case of acetylated samples there was quenching of fluorescence; it increased with the degree of acetylation. But there was no shift in the emission maximum (Figure 4). However, in the succinylated samples there was both quenching of fluorescence and a red shift in the emission maximum (Figure 5). The 87% succinylated WB proteins had a peak at 340 nm, whereas the unmodified proteins had one at 325 nm. Fluorescence quenching and red shift are indicative of protein denaturation (Brand and Withholt, 1967).

These studies suggest that due to acetylation/succinylation the proteins of winged bean are dissociated to lower molecular weight proteins. The degree of dissociation of WB proteins depends on the level of acetylation/succinylation. Succinic anhydride dissociates the WB proteins to a larger extent than acetic anhydride because of the net charge effect. These effects could be the cause of the altered functional properties of WB proteins, such as increase of nitrogen solubility, water absorption capacity, fat absorption capacity, and emulsion capacity reported earlier (Narayana and Narasinga Rao, 1984).

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