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[Received October 5, 1984]

Modification of Surface Charges of Soy Protein by Phospholipids

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ABSTRACT

Lecithin is used to prevent soy protein isolates from clumping in food processing. A PenKem Inc. System 3000 Electrokinetic Analyzer was used to investigate how the phospholipid modified the surface charge of soy protein. Electrophoretic mobility-pH curves showed that a commercial soy lecithin lowered the isoelectric point (pI) and increased the electrical mobility of soy protein more than did a pure phosphatidylcholine. The modification of the surface charge of the protein was a function of the phospholipid added. Lecithinated soy isolate was more negatively charged and thus more dispersible in water than the nonlecithinated soy control.

INTRODUCTION

Protein phospholipid interactions related to food problems have been studied and reviewed extensively (1-7). However, few studies have been made on how phospholipids change colloidal properties of proteins. Because proteins form colloids in water and the surface charge plays an important role in dispersion (8), it was of interest to investigate how phospholipids modify the surface charges of soy protein and how these modifications affect colloidal properties.

In dilute aqueous suspensions the electrophoretic mobility is proportional to the zeta potential which, in turn, is proportional to the surface charge (8-11). Therefore, we have measured electrophoretic mobility in order to characterize the surface charge of the particles of soy isolate, phospholipid and phospholipid/soy protein complex.

MATERIALS AND METHODS

L-alpha-phosphatidylcholine was from Sigma Chemical Co. (St. Louis, Missouri), and Centrolene A (a food grade, hydroxylated soybean lecithin) was from Central Soya Co. (Ft. Wayne, Indiana). Soy protein isolate obtained from Kraft, Inc. (Glenview, Illinois) had the following composition: nitrogen, 13.3%; fat, 2.7%; moisture, 6%; and ash, 2.8%. Lecithinated soy isolate was prepared by spraying 0.2% (w/w) liquified lecithin onto the soy protein while mixing the powder in a ribbon blender.

L-alpha-phosphatidylcholine and Centrolene A were added into distilled water in small portions with stirring to form 1 mg/ml dispersions. These phospholipid dispersions were then mixed with various concentrations of soy protein solution to obtain dispersions with different phospholipid/protein ratios. The dispersions of phospholipids, soy protein and phospholipid/soy protein were adjusted to various pH values with either 0.1 M HCl or NaOH. In order to obtain the mean mobility values for the electrophoretic mobility vs. pH plots (Figs. 1, 5, and 6) all protein/phospholipid mixtures were stirred for 30 min or until a single peak was obtained. To capture the presence of multiple peaks (Fig. 2), mobility measurements were taken approximately 1 min after mixing.

Because electrophoretic mobility was unaffected by the concentration of the colloid dispersion used in this study (0.4-1.0 mg/ml), the overall concentration of protein and phospholipids was not adjusted to the same final value.

Electrophoretic mobilities of phospholipids, soy protein and phospholipid-soy protein dispersions were measured at various pH values with a PenKem System 3000 automated electrokinetic analyzer at 25 C. The procedure described in the instruction manual was followed. One mobility unit is equal to 1.0×10^{-8} meters/sec/volt/meter.

Particle size analysis of 1% (w/v) dispersions of protein in distilled water, pH 6.3 and 25 C, were measured in an Electrozone/Celloscope manufactured by Particle Data, Inc., Elmhurst, Illinois.

RESULTS AND DISCUSSION

The electrophoretic mobility-pH curves of food grade lecithin and soy isolate in Figure 1 show that lecithin has a higher negatively charged surface than soy protein. As a result, two groups of peaks appear immediately after the phospholipid

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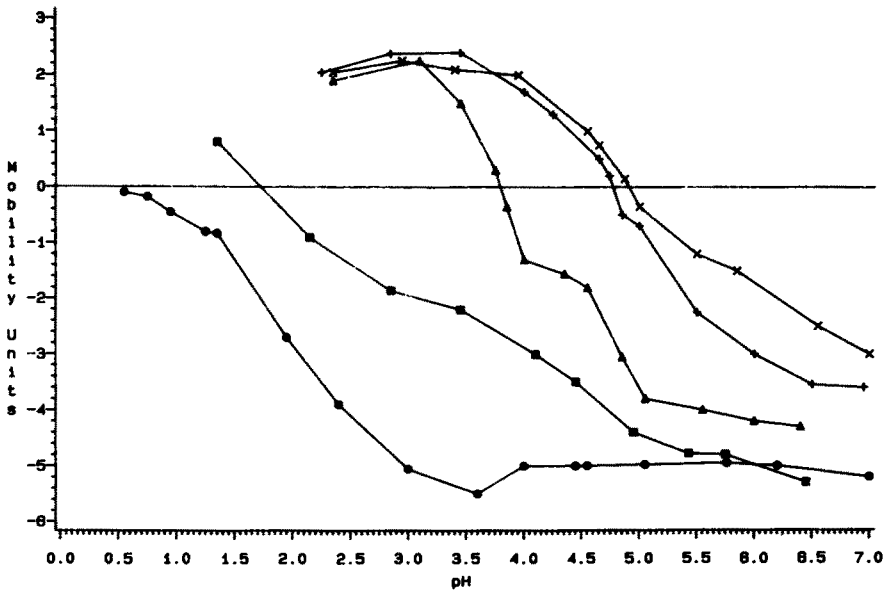

FIG. 1. Electrophoretic mobility-pH curves of (a) 0.4 mg/ml soy isolate (×); (b) 0.5 mg/ml soy isolate and 0.2 mg/ml lecithin (+); (c) 0.5 mg/ml soy isolate and 0.5 mg/ml lecithin (▲); (d) 0.167 mg/ml soy isolate and 0.67 mg/ml lecithin (■), and (e) 0.4 mg/ml lecithin (●). All dispersions were in distilled H_2O. L, lecithin (food grade); S, soy isolate.

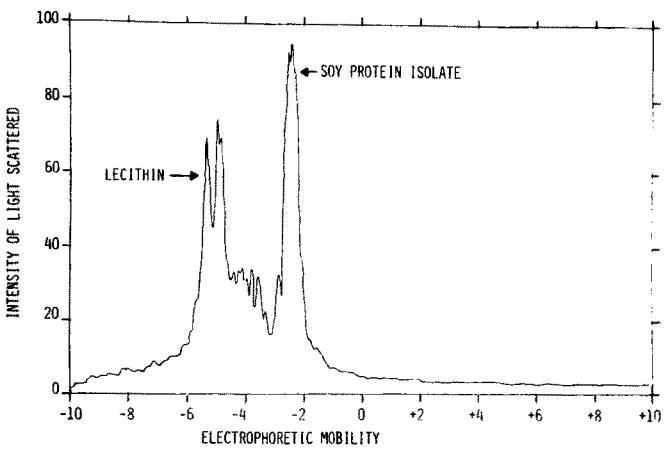

FIG. 2. Electrophoretic mobility histogram of 0.7 mg/ml lecithin/soy isolate (2:5) mixture at pH 6.5 and 25 C. The peaks between −4 and −6 mobility units correspond to lecithin, while the peak centered at −2.4 mobility units corresponds to soy isolate.

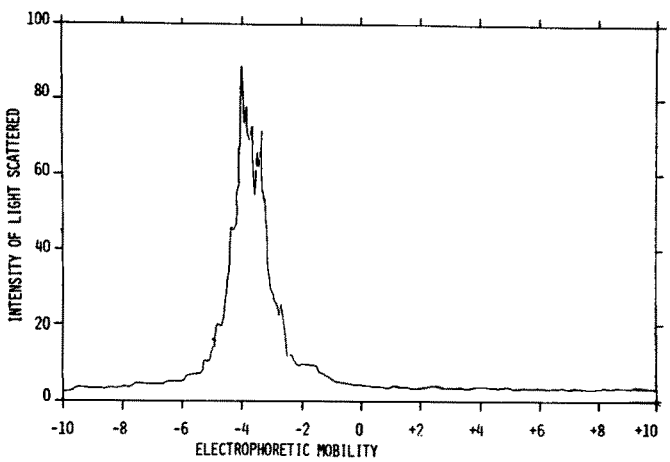

FIG. 3. Electrophoretic mobility histogram of 1 mg/ml lecithin/soy isolate (1:1) complex at pH 5.1 and 25 C.

and protein are mixed and stirred (this requires sampling within one min as described in Materials and Methods). The electrophoretic mobility distribution of the phospholipid/protein (2:5) mixture at pH 6.5 is shown in Figure 2. The peaks between -4 and -6 mobility units and the peak centered at -2.4 mobility units correspond to lecithin and the soy protein, respectively, because the mean electrophoretic mobility of lecithin and that of the soy protein alone at pH 6.5 are -5.1 and -2.4 units, respectively, as shown in Figure 1. In contrast, when a lecithin/soy protein (1:1) mixture was adjusted to pH 5.1 and stirred for 30 min at 25 C, only a single peak centered at -3.8 units was obtained as shown in Figure 3. This single peak corresponds to neither the phospholipid nor the soy protein, but to a phospholipid/protein complex. This type of analysis has been used to identify whether or not the phospholipid and the protein form a complex when they are mixed and adjusted to various pH values.

Figure 1 also shows that the isoelectric point of soy isolate decreases as a function of lecithin added. The lecithination effect on the isoelectric point of the soy protein is also shown in Figure 4. The charged surface of the soy protein is modified to become more negative when it is complexed with lecithin. As a result, the plot of isoelectric point versus lecithin/soy protein ratio, as shown in Figure 4, can be used to monitor the degree of lecithination of the protein.

Above the isoelectric point of the protein (Fig. 1), the electrophoretic mobility of soy protein increases as a lecithin concentration increases. Figure 5 shows this lecithination effect on the mobility of the soy protein. The data in Figure 5 further demonstrate that lecithin makes the surface of soy protein colloid progressively more negatively charged as the lecithin concentration increases.

Below the isoelectric point the net surface charge of soy protein can be converted from positive to negative by the addition of lecithin (Fig. 1). For example, in the 4:1 lecithin/soy isolate mixture, the soy protein remains negatively charged and does not become neutral until pH 1.7, which is 3.2 pH units lower than the isoelectric point of the protein in the absence of lecithin.

Phosphatidylcholine has a higher isoelectric point and gives a lower electrophoretic mobility than the food grade lecithin (Figs. 1 and 6).

This indicates that phosphatidylcholine has a less negatively charged particle surface than food grade lecithin particles. The reason for this is that the food grade lecithin is composed of phosphatidylinositol and phosphatidylethanolamine in addition to phosphatidylcholine. Consequently, the soy protein becomes more negatively charged in the presence of the food grade lecithin than in the presence of pure phosphatidylcholine. The negativity difference is especially apparent when the phospholipid/protein ratio in both complexes is 4:1, as shown in Figures 1 and 6. The electrophoretic mobility-pH curves in these figures show that the isoelectric point of the food grade lecithin/soy isolate (4.1) complex is 2 pH units lower than that of the phosphatidylcholine/soy isolate (4:1) complex. It should be noted that the three electrophoretic mobility-pH curves of phosphatidylcholine/soy isolate mixtures at ratios of 2:5, 1:1 and 4:1 can nearly be superimposed (Fig. 6). Two parts of phosphatidylcholine seem to be adequate to saturate five parts of soy isolate; therefore, any further addition of lecithin has no effect on the surface charge of the particles.

Lecithination of soy isolate with 0.2% lecithin increased electrophoretic mobility by 12% compared to the soy isolate control, as shown in Table I. This increase of negative electrophoretic mobility due to lecithination agrees closely with that by mixing the two dispersions of lecithin and soy isolate, as shown in Figure 5. Although the negativity of the charged surface of the lecithinated soy isolate colloid is only 12% greater than that of the soy isolate control, this small increase of negativity was adequate to reduce the particle size of the

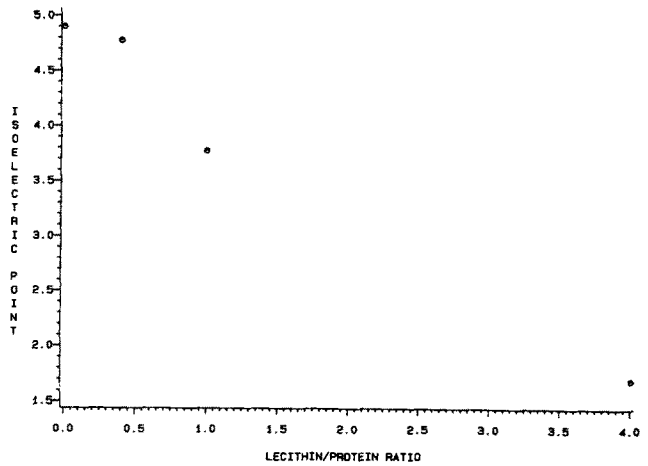

FIG. 4. Effect of lecithination on the isoelectric point of soy isolate. The lecithin/soy ratios were taken from the curves in Figure 1 where the mobility was zero. A protein is at its isoelectric point (pI) when electrokinetic mobility = 0 because this is the point where the number of cationic and anionic charges are equal to each other, resulting in a net charge of zero.

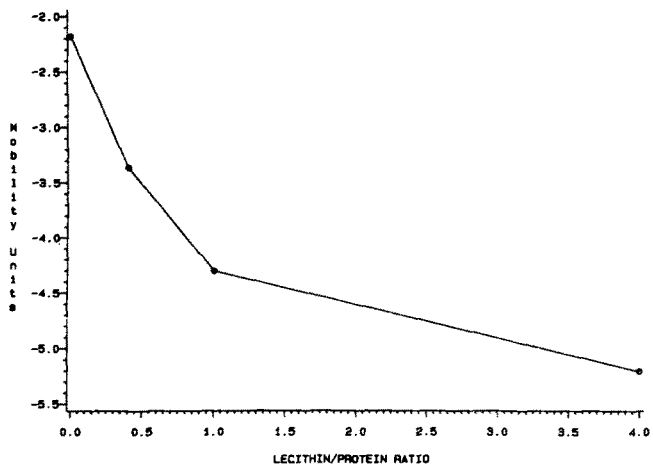

FIG. 5. Effect of lecithination on the electrophoretic mobility of soy isolate at pH 6.3 and 25 C. The data points correspond to the electrophoretic mobilities of soy isolate in the absence and in the presence of various concentrations of lecithin, as shown in Figure 1.

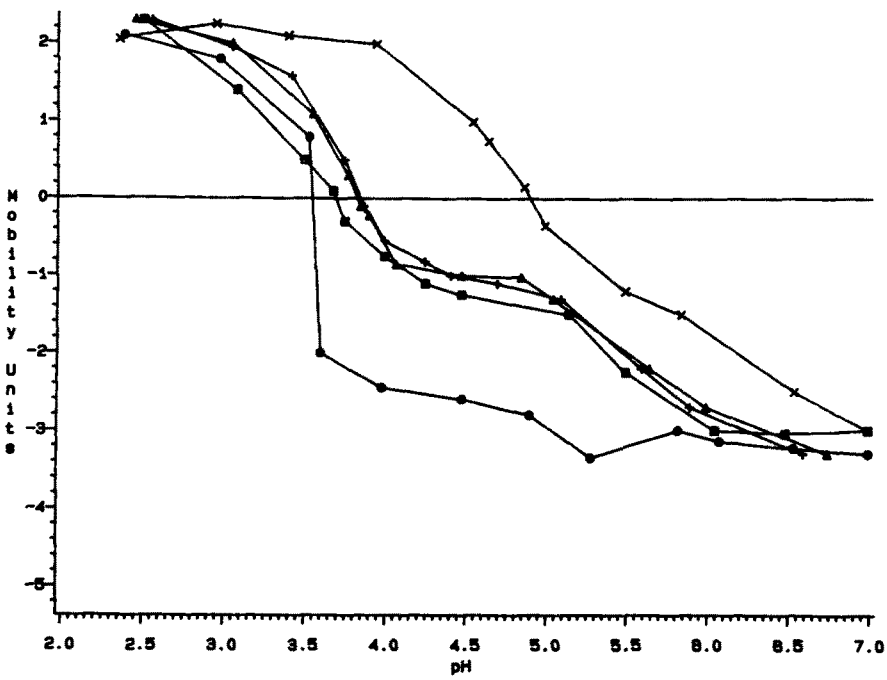

FIG. 6. Electrophoretic mobility – pH curves of (a) 0.4 mg/ml soy isolate (×); (b) 0.5 mg/ml soy isolate and 0.2 mg/ml phosphatidylcholine (+); (c) 0.5 mg/ml soy isolate and 0.5 mg/ml phosphatidylcholine (▲); (d) 0.167 mg/ml soy isolate and 0.67 mg/ml phosphatidylcholine (■), and (e) 0.4 mg/ml phosphatidylcholine (●). All dispersions were in distilled H_2O.

TABLE I

Electrophoretic Mobility of Protein Colloids

Protein dispersion[a]	Electrophoretic mobility units[b]
Soy isolate	2.20 ± 0.05
Lecithinated soy	2.46 ± 0.05

[a]Conditions: 1 mg/ml, pH 6.3, 60 micromhos/cm and 25 C.
[b]The number of measurements for each sample was 10.

TABLE II

Particle Sizes of Protein Colloids

Protein dispersion[a]	Mean diameter of particles (microns)
Soy isolate	15.4
Lecithinated soy	8.1

[a]Conditions: 1 mg/ml, pH 6.3, 60 micromhos/cm and 25 C.

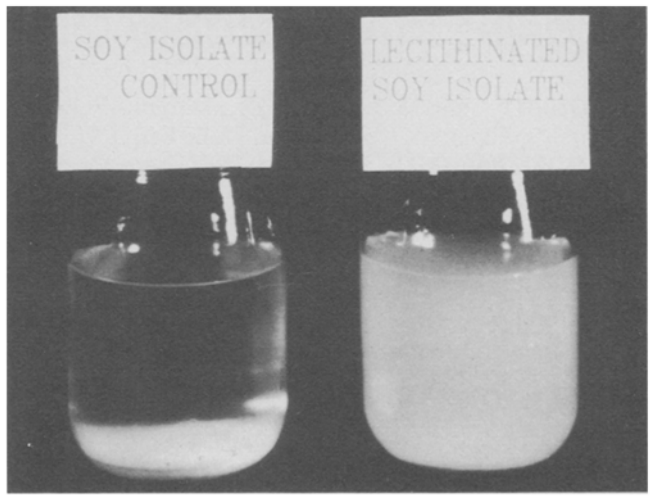

FIG. 7. A 1 mg/ml dispersion of soy isolate and lecithinated soy isolate, which were adjusted to pH 6.3, allowed to stand at 5 C for 18 hr, and then brought to 22 C for photographing.

protein colloid by 47% (Table II). The lecithination stabilized the protein colloids so that the lecithinated soy protein remained dispersed while the nonlecithinated soy isolate agglomerated and precipitated (Fig. 7).

Electrokinetic analysis measures the charge of the molecular components composing the colloid surface. The surface is formed from complexation between the protein and phospholipid molecules. Lecithination increased the negativity of the surface of the protein and caused greater charge repulsion between particles than did the untreated control. As a result, when the lecithinated protein was suspended in water the smaller and more highly charged particles dispersed readily.

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[Received January 28, 1985]