Recovery of Whey Proteins with Sodium Hexametaphosphate

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Abstract

Interactions between proteins and anionic polyelectrolytes may be utilized to recover the protein of milk whey. Depending on the polyelectrolyte used, the protein content of the precipitates can be increased by gel filtration or by ion exchange.

Under optimum conditions (pH 3), over 90% of the protein of whey was precipitated by sodium hexametaphosphate, provided the whey was decationized previously. On dry weight basis, the precipitates contained 70 to 85% protein, 10 to 20% sodium hexametaphosphate, and 10 to 15% lactose. Only a negligible amount of the precipitant remained in the supernatant.

The protein content of the protein-hexametaphosphate complex was increased to 88 to 90% by either gel filtration or ion exchange. Ion exchange removed mainly the phosphate whereas gel filtration removed most of the lactose and part of the phosphate. Small amounts of residual sodium hexametaphosphate could be removed from the upgraded complex by neutralizing the solution with calcium hydroxide and centrifuging. The solubility curve of the product showed that the protein would not be denatured by this process.

Gel filtration of the complex on Biogel P-10 at pH 8 to 10 yielded two protein fractions and the mineral-lactose fraction. The first fraction contained the immunoglobulins and blood serum albumin whereas the second fraction contained α-lactalbumin and β-lactoglobulin.

Introduction

Recent studies on whey utilization have been concerned with the separation of proteins from lactose, minerals, and minor constituents of whey. However, only limited attention has been given to methods based on the complexing of proteins by polyelectrolytes. Smith et al. (16) used anionic hydrocolloids and detergents for precipitating the protein of soybean whey and reported nearly complete protein recovery. Cluskey et al. (3) reported the formation of insoluble complexes between casein and carboxymethylcellulose. Hidalgo and Hansen (9, 10) showed in model systems that individual whey proteins form insoluble complexes with anionic hydrocolloids. They developed a procedure (7) for recovering over 90% of the protein of whey with carboxymethylcellulose and suggested that this procedure can be used on industrial scale. On a dry basis the product contained approximately 65% protein, 30% carboxymethylcellulose, and 5% lactose and minerals. Later, they showed that it was possible to fractionate the whey protein into at least three fractions by selective precipitation with the hydrocolloid under varying conditions of pH and temperature (11). Spinelli and Koury (17) precipitated the protein from diluted solutions of sarcoplasmic fish proteins with sodium trimetaphosphate, sodium hexametaphosphate, and sodium tetrametaphosphate. Protein recovery was nearly complete at pH 3.5 to 4.0. Recovery of whey protein by precipitation with phosphates was first studied by Gordon (5) and later by Hartman and Swanson (8). Jones et al. (12) found that virtually all the protein in commercial acid whey is precipitated by ferricophosphate at pH 3.2 to 4.0 whereas Becker et al. (2) and Wingerd (20) studied the recovery of this protein by a combination of precipitation with phosphates and column chromatography.

This report describes our studies on the recovery of the protein of whey by precipitation with sodium hexametaphosphate (HMP) and the upgrading of the resulting protein concentrates by gel filtration and ion exchange.

Materials and Methods

Acid and rennet whey were prepared from mixed, pasteurized skim milk. For the acid whey, 10% HCl was added to the skim milk at 35 C until the pH of 4.6 was reached. For the rennet whey, skim milk was inoculated with .1% commercial rennet and incubated for

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Analytical grade HMP utilized (sodium hexametaphosphate flake, linear type, product 30147, British Drug Houses) would correspond to a sodium phosphate glass whose Na₂O/P₂O₅ mole ratio falls in the range of 1.10 to 1.15 (18). The solutions of this chemical were prepared by dissolving 10 g at 60 C under rapid agitation, cooling to room temperature, adjusting the pH to 3 with HCl, and completing the volume to 100 ml.

Whey protein was precipitated by adding a predetermined amount of HMP solution to whey at pH 3 at 20 C. Agitation was kept to a minimum to prevent the disaggregation of the precipitate particles. After 30 min the precipitate was separated by centrifugation at 5,000 g for 15 min, washed, and redisolved or washed, freeze-dried, and stored. The precipitate was dissolved in distilled water and neutralized to pH 6 to 7 with 1N sodium hydroxide.

Gel filtration was in 75 x 2.5 cm glass columns with either Biogel P-6 or P-10. Ion exchange was in 35 x 20 cm glass columns with either Amberlite IR-120 (for removing the cations) or Dowex-2 (for removing the HMP). The columns were prepared in the usual manner and activated with 10% HCl and 15% NaOH, respectively (14). The experiments were at 20 C, and the eluate was monitored by absorbance (280 nm) and by electrical conductivity. Data were recorded with an LKB apparatus.

Phosphorus and nitrogen were determined by the methods of Fiske and Subbarow (4) and Kjeldahl (1), respectively. Protein nitrogen (insoluble in 12.5% trichloroacetic acid) was calculated by subtracting the nonprotein nitrogen (soluble in 12.5% trichloroacetic acid) from the total nitrogen. The protein concentration was calculated by multiplying the protein nitrogen by 6.4 (6). Cations were determined by atomic absorption as described by Rebmann and Höth (15). Lactose was determined by the anthrone method (13). Electrophoresis was on polyacrylamide gel containing sodium dodecyl sulfate as described by Weber and Osborn (19). The mineral content was determined by ashing at 650 C.

Results and Discussion

Precipitation of whey protein by HMP. Preliminary experiments showed that maximum protein precipitation occurred at pH 3. Thus, this pH was selected for subsequent experiments. Protein recovery increased with the amount of HMP added up to 80% by weight of the total protein (Fig. 1). Further addition of HMP did not increase yield. Acid whey required approximately 100 mg HMP/100 ml whereas rennet whey required only 70 mg/100 ml.

After removing the protein-HMP complex, we found 60 to 70% of the added HMP in the supernatant. Therefore, additional precipitation experiments were to determine the effect of experimental conditions on the protein-recovery yield and on the residual phosphate in the supernatant. Large differences in protein recovery occurred between different samples of whey, and it became evident that variations in the mineral composition of whey had a strong influence on the protein-HMP interaction. To study this, whey was concentrated to a total solids content of 20% and decationized by elution through a column of Amberlite IR-120. The decationized whey then was rediluted to its original volume. Table 1 shows the Ca, Mg, Na, and K content of the whey before and after ion exchange.

Increasing amounts of calcium chloride were added to aliquots of the decationized whey, and the maximum amount of protein precipitated by HMP was determined for each case. Fig. 2 shows the relationship between the cal-

Table 1. Cationic composition of whey before and after ion exchange on Amberlite IR-120.*

<table>
<thead>
<tr>
<th>Cations</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ion exchange</td>
<td>5,000</td>
<td>482</td>
<td>1,625</td>
<td>6,250</td>
</tr>
<tr>
<td>After ion exchange</td>
<td>&lt;.1</td>
<td>&lt;.1</td>
<td>1.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Concentrated whey; total solids = 23.6%.

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Calcium added and protein recovery. In the absence of this cation, over 92% of the protein was recovered in the precipitate. The recovery decreased sharply as the amount of calcium added was increased. Table 2 shows the protein and phosphate content of a typical sample of decationized whey, before and after protein precipitation with 65 mg HMP/100 ml whey. Over 95% of the added phosphate was precipitated with the protein, and the ratio of protein to phosphate in the product was approximately 9 to 1. Below a HMP of 65 mg/100 ml whey, the amount of protein recovered in the precipitate decreased rapidly whereas an increase in yield was only slight when higher amounts of HMP were added. Presumably, residual cations in the demineralized whey prevented 100% protein recovery.

After centrifugation and washing, the precipitate contained from 50 to 70% dry matter.

**Table 2.** Protein recovered from acid whey by 65 mg hexametaphosphate (HMP)/100 ml of decationized whey (pH 3).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Protein (mg/100 ml)</th>
<th>Phosphate (PO₄) (mg/20 ml sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before addition of HMP: Decationized whey</td>
<td>593.5</td>
<td>212</td>
</tr>
<tr>
<td>After addition of HMP: Sediment</td>
<td>557.6</td>
<td>62</td>
</tr>
<tr>
<td>Supernatant</td>
<td>35.9</td>
<td>215</td>
</tr>
<tr>
<td>Recovery (sediment)</td>
<td>94</td>
<td>95.4</td>
</tr>
</tbody>
</table>

This precipitate was readily soluble upon dilution and neutralization. Atomization of the solution containing 5 to 8% protein yielded a soluble powder. Polyacrylamide-gel electrophoretic pattern of the solubilized protein is in Fig. 3.

Removal of the HMP from the complex. Experiments were performed to upgrade the protein-HMP complex by removing the HMP by either ion exchange or gel filtration. Ion exchange was performed by eluting a 15% solution of the complex at pH 6 to 7 through a column of Dowex-2. Over 92% of the protein was recovered in the eluate (Table 3) whereas over 83% of the HMP and 42% of the lactose were removed. The protein content of the complex increased by approximately 15% and the phosphate content decreased from 16 to 3.5% (Table 4).

Gel filtration experiments were on Biogel P-6 and P-10 in the pH range from 5 to 10. Best
protein upgrading was on Biogel P-6 at pH 6 (Fig. 4). The protein of 20 ml sample was recovered in 150 ml eluate whereas the phosphate was recovered in the next 230 ml. Thus, because of the extensive dilution, gel filtration would be less adequate than ion exchange for the upgrading. Over 97% of the protein was recovered in Fraction 1 together with 11.6% of the lactose (Table 5). However, only about half of the HMP was removed. More complete removal of HMP from the product resulted in important protein losses. The protein content of the product was increased from approximately 70 to 88% by gel filtration (Table 6). However, the upgraded product contained 9.9% HMP. Thus, gel filtration was less efficient than ion exchange for demineralizing the complex, the upgrading mainly being due to lactose removal. It may be possible to obtain a protein concentrate with over 90 to 95% protein by combining ion exchange with molecular sieve. Re-elution of the protein solution through a second gel filtration column did not increase further the protein concentration of the protein.

Gel filtration on Biogel P-10 yielded similar results to those on Biogel P-6. However, the elution rate at pH 6 was approximately 2.5 times lower than with the P-6 column. The protein fraction was composed of a turbid eluate (zone a) and of a clear solution (zone b). At pH 8 these two zones separated more clearly and the elution rate was higher, as shown in Fig. 5.

**Table 5.** Protein, hexametaphosphate (HMP), and lactose content of the protein concentrate before and after gel filtration on Biogel P-6 at pH 6.

<table>
<thead>
<tr>
<th></th>
<th>Protein (mg/20 ml sample)</th>
<th>HMP (NaPO3)</th>
<th>Lactose</th>
<th>Total (mg/20 ml sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before gel filtration</strong></td>
<td>1,700</td>
<td>419</td>
<td>285</td>
<td>2,404</td>
</tr>
<tr>
<td><strong>After gel filtration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>1,663</td>
<td>188</td>
<td>33</td>
<td>1,884</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>...</td>
<td>260</td>
<td>253</td>
<td>513</td>
</tr>
<tr>
<td><strong>Total recovery</strong></td>
<td>1,663</td>
<td>448</td>
<td>286</td>
<td>2,397</td>
</tr>
</tbody>
</table>
TABLE 6. Dry weight composition of the protein-hexametaphosphate complex before and after gel filtration on Bioget P-6 at pH 6.

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>NaPO₅</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before gel filtration</td>
<td>70.7</td>
<td>17.4</td>
<td>11.9</td>
</tr>
<tr>
<td>After gel filtration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>88.3</td>
<td>9.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>...</td>
<td>50.7</td>
<td>49.3</td>
</tr>
</tbody>
</table>

may be improved by precipitating the residual HMP with calcium hydroxide. The soluble calcium-HMP formed can be separated by centrifugation. However, we found that treatment with calcium hydroxide may cause important protein losses if the protein-HMP complex is not upgraded previously by gel chromatography or ion exchange.

The protein of whey can be recovered by precipitation with HMP. However, for complete and efficient recovery, the whey must be demineralized. This operation increases the cost of the recovery procedure. The protein-HMP complex can be upgraded subsequently to contain over 90% protein. The upgraded product shows most of the properties of other undenatured whey protein concentrates.

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References

