Influence of Heat Treatment on Antigenicity of Bovine Serum Albumin in Milk and Model Systems

ABSTRACT

Electroimmunodiffusion on a cellulose acetate support medium was used to study the effect of heat treatment on the antigenic activity of bovine serum albumin suspended in water, simulated milk ultrafiltrate (J & K buffer), or saline (.85%) and of bovine serum albumin in milk per se. Heat treatments were at 70, 73, and 75 °C for 0 to 30 min.

The suspending medium influenced the loss of antigenicity of bovine serum albumin upon heat treatment. The loss in increasing order was J & K buffer, saline, and water. In milk, the loss of antigenicity of bovine serum albumin was 70 °C for 30 min, none; 73 °C for 30 min, 24.6%; and 75 °C for 20 min, almost complete with no characteristic cone formation upon electroimmunodiffusion.

INTRODUCTION

Several investigators (6, 7, 8) have studied the effect of heat on the antigenic response of milk proteins by several qualitative procedures because it was postulated that milk proteins upon heat treatment would exhibit reduced antigenicity and, thus, were less likely to elicit a reaction in individuals allergic to milk. Allergenicity determined by in vivo skin test reactions may not be the same as antigenicity or denaturation measured by various in vitro immunological methods.

This study makes application of a recently developed quantitative immunological technique, electroimmunodiffusion (EID), to ascertain the effect of heat on the antigenicity of bovine serum albumin (BSA) in milk and model systems.

MATERIALS AND METHODS

Antiserum

Antibovine serum albumin was obtained from Dexter Biologicals, Dexter, MI, in lyophilized form and was reconstituted with .0125 M phosphate buffer, pH 7.4, before use.

Electroimmunodiffusion

For EID, the technique of Laurell (5) with modifications developed in our and other laboratories was used (1, 9, 10).

Titan III cellulose acetate plates, 78 x 98 mm purchased from Helena Laboratory, Beaumont, TX, were used. The plate was soaked for 10 min in 5% BSA antiserum diluted in .0125 M phosphate buffer, pH 7.4. Excess antiserum was removed by blotting with filter paper. The plate then was stored in a humidity chamber until spotted with the sample containing BSA.

Samples of .4 µl were spotted in duplicate on the plate with a 1-µl Hamilton microsyringe equipped with a Chaney adapter. Standards of BSA, prepared in the appropriate system, were in duplicate at four different concentrations. The spotted plate was held for 10 min in the humidity chamber. It then was electrophoresed in the cold (4 °C) at 5 mA/plate. The chamber contained .1 M phosphate buffer, pH 7.4. After electrophoresis, the plate was washed for 1 h with .0125 M phosphate buffer, pH 7.4, containing .2 M sodium chloride, stained with Nigrosin (.125% in 6% acetic acid), and to remove excess dye, washed with 6% acetic acid and rinsed with distilled water.

Quantitation

Known concentrations of BSA (Sigma, St. Louis, MO) were applied always on the same plate as unknowns. The cone or peak height was measured from the point of sample application to the apex of the cone by a microcalipher. A standard curve was prepared on semilog paper. The log of the concentration of the
standard protein was plotted versus the cone height. The concentration of the unknown was read from the standard curve.

**Heat Treatments**

Two milliliters of raw milk or an equal volume of a suspension containing 1 mg BSA/ml of distilled, deionized water, simulated milk ultrafiltrate (J & K buffer) (3), or .85% saline were placed in a screw-capped test tube. A tube also was prepared to contain 2 ml of the heating menstruum with a thermometer to monitor the temperature. The test tubes were held in a thermostatically controlled ethylene glycol bath at the desired temperature. When the contents had attained the desired temperature and at selected time intervals, a tube was withdrawn, cooled in ice water, and assayed for BSA by the EID procedure. At 75 C, come-up time did not exceed 3 min.

**RESULTS**

The typical cone shaped patterns achieved with the EID procedure for known concentrations of BSA in saline and J & K buffer, and for BSA following heat treatments are in Fig. 1 and 2.

The EID patterns for BSA suspended in .85% saline (Fig. 1) are for experiments at 70 C. The cone height decreased as the heating time increased. The EID patterns were similar for BSA suspended in J & K buffer (Fig. 2). However, the heat effect was less pronounced.

All data were quantified. Selected results of percent loss of antigenicity of BSA in water, saline, and J & K buffer were determined (Table 1).

At 75 C for 10 min the antigenicity of BSA suspended in saline and J & K buffer had decreased 35.7 and 24%. In comparison, BSA

**TABLE 1. Loss of bovine serum albumin antigenicity upon heat treatment in three different menstrua.**

<table>
<thead>
<tr>
<th>Heating menstrua (temperature)</th>
<th>Heating time (min)</th>
<th>Water (70 C)</th>
<th>Saline (75 C)</th>
<th>J &amp; K buffer (75 C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>23.6</td>
<td>.7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50.3</td>
<td>35.7</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>62.9</td>
<td>50.9</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>76.4</td>
<td>61.8</td>
<td>45.3</td>
</tr>
</tbody>
</table>

*Results of three trials in duplicate.*
suspended in water and heated at 70 C for 10 min had lost over 50% of its antigenic activity. When BSA was heated in water at 75 C, no characteristic cone patterns were observed, although there was evidence that an antibody-antigenic reaction had occurred at the point of sample application.

The effect of heat on the antigenicity of BSA of raw milk is shown in Fig. 3. The upper patterns (A) are for heat treatments at 75 C and the lower ones (B) at 73 C. Tabular data are in Table 2. When milk was heated at 70 C, no antigenic activity was lost. At 73 C for 30 min, the loss of BSA antigenicity was 24.6%. At 75 C for 20 and 30 min, typical cone patterns were not formed (Fig. 3A, g and h). However, antigenicity was not lost completely; a reaction was observed at the point of sample application.

TABLE 2. Loss of bovine serum albumin antigenicity in milk upon heat treatment.a

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Heating temperature (73 C)</th>
<th>Heating temperature (75 C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.5</td>
<td>Not determined</td>
</tr>
<tr>
<td>10</td>
<td>13.0</td>
<td>No loss</td>
</tr>
<tr>
<td>20</td>
<td>18.3</td>
<td>Cones not formed</td>
</tr>
<tr>
<td>30</td>
<td>24.6</td>
<td>Cones not formed</td>
</tr>
</tbody>
</table>

aResults of two trials in duplicate.

DISCUSSION

The heat effects on the antigenic response of BSA were greater in water than in saline or milk ultrafiltrate. The presence of ions in the latter two systems may have decreased the degree of change in antigenicity by preventing protein-protein interactions and subsequent coagulation. In water, heat treatment had the greatest effect, and coagulation was at 70 C. This was probably due to the fact that BSA is held in suspension primarily by hydration. In the intact milk system, the loss of antigenic activity by BSA was greater than in J & K buffer or saline. Because milk is a complex system, heat-induced interactions are likely to occur between the various components, which could contribute to increased heat sensitivity and coagulation.

The degree of loss of antigenicity may not be the same as the degree of denaturation. Sapersteint and Anderson (8) using the precipitin test and a passive cutaneous anaphylaxis test demonstrated that -lactalbumin, -lactoglobulin, and casein retained their antigenic properties in most commercial milk formulae. These findings led them to the supposition that heat denaturation is not synonymous with loss of antigenicity.

REFERENCES


