A Research Note

Relationship of Somatic Cell Count and Total Sulfhydryls in Milk

DANIEL R. SAMPLES, SUSAN L. DILL, RONALD L. RICHTER* and CHARLES W. DILL

Department of Animal Science, Texas A&M University, College Station, Texas 77843

(Received for publication July 15, 1985)

ABSTRACT

Individual milk samples from 32 cows were analyzed to determine the relationship between somatic cell concentration and total sulfhydryl concentration (cysteine plus reduced cystine). A significant relationship was detected between somatic cell count, which ranged from $1.7 \times 10^4$ to $1.0 \times 10^7$ cells/ml, and total sulfhydryls per gram of milk protein. The regression equation, total sulfhydryls/g of milk protein $= 31.96 + 7.99 \times \log_{10}$ somatic cell count, with $r^2 = 0.19$, was calculated. The mean total sulfhydryl concentration was 73.1 $\mu$mol/g of protein. The minimal effect of somatic cell concentration on total sulfhydryl concentration indicates that somatic cell concentration should have little influence on chemical parameters of milk protein determined by sulfhydryl analysis when proper experimental controls are used.

Changes in milk protein concentrations associated with mastitis are well-documented (1,2,6,12,14). During periods of high somatic cell counts, total casein decreases and total whey protein increases. Concentrations of $\beta$-lactoglobulin and $\alpha$-lactalbumin decrease, but serum albumin and immunoglobulins increase causing a net increase in whey protein concentration (2,6,12). Similarly, $\alpha$-casein and $k$-casein concentrations are increased slightly, but a lower concentration of $\beta$-casein results in a net loss of total casein (1,2,12). These altered protein distributions could have a significant effect on analytical procedures, such as dye-binding capacity (7) and sulfhydryl analysis, that are based on a specific property of a protein.

Sulfhydryl analysis has been used to evaluate the effect of heat treatment on milk protein (5,11) to determine the effect of milk storage on the oxidation state of sulfur-containing amino acids (11), and as a possible method to detect the adulteration of nonfat dry milk with whey or whey protein concentrate (10).

Cysteine and cystine residues of milk are concentrated in whey proteins. Approximately 81% of total sulfhydryls (cysteine plus reduced cystine, $\mu$mol/g of milk protein) are distributed among the four principle whey proteins, i.e., $\alpha$-lactalbumin, $\beta$-lactoglobulin, serum albumin and immunoglobulins (4). The concentration of sulfhydryl groups in whey proteins which increase in milk with increased concentrations of somatic cells might have a negative impact on the interpretation of milk parameters based on sulfhydryl group analysis.

MATERIALS AND METHODS

Milk samples

Thirty-two milk samples were collected from individual Holstein and Jersey cows of the Texas A&M University dairy herd. Cow selection was based on somatic cell count information from the Texas Dairy Herd Improvement Association to provide a wide range of somatic cell counts.

Somatic cell counts

Somatic cell counts (SCC) were determined by the direct microscopic field count method (9) using a microscopic factor of 430,000. Averages of triplicate counts for each sample were used for statistical analysis.

Total protein

Milk samples were centrifuged ($2000 \times g$, 10 min) to obtain skim milk for nitrogen analyses. Total nitrogen (TN) and non-protein nitrogen (NPN) were determined by Rowland fractionation (13) using a microkjeldahl-microdiffusion procedure (8). Percent protein was calculated: Protein = (TN - NPN) x 6.38.

Total sulfhydryls

Total sulfhydryl concentration in 100-$\mu$l samples of skim milk was determined in duplicate using Ellman’s reagent as described by Beveridge et al. (3). Total sulfhydryl concentration as cysteine plus half-cystine was expressed as $\mu$mol (-S-) /g of protein.

RESULTS AND DISCUSSION

Somatic cell counts ranged from 17,000 to 10,000,000/ml. The mean count was 1,100,000/ml and the median
value was 140,000/ml. Results of total sulfhydryl analysis are shown in Table 1. The mean total sulfhydryl concentration was in agreement with free sulfhydryl plus half-cystine values compiled by Patrick and Swaisgood (11), which ranged from 70.3 to 72.0 μmol (-S-) g protein for bulk milk samples. Both the mean and range of experimental values were consistent with theoretical calculations of the mean (11) and range of total sulfhydryl groups calculated from the range of protein concentrations as presented by Brunner (4).

<table>
<thead>
<tr>
<th>TABLE 1. Total sulfhydryls (μmol) per gram of protein in skim milk.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Experimental</td>
</tr>
<tr>
<td>Theoretical</td>
</tr>
</tbody>
</table>

a(11; cysteine plus half-cystine).
bCalculated from ranges of individual milk proteins published in reference 4.

Somatic cell counts were transformed to log<sub>10</sub> counts for regression analysis. The regression equation, (S) = 31.96 + 7.99 (log<sub>10</sub> SCC) with r² = 0.19, was calculated for regression of total sulfhydryls per gram of protein on SCC. The stand error of β (7.99) was ±2.98, with the slope statistically significant at P = 0.01. An increase in SCC is associated with an increase in the concentration of total sulfhydryls per gram of protein. This is probably associated with a change in the distribution of milk proteins which has been associated with increased levels of somatic cells (1,2,6,12). However, the SCC accounted for only a small percentage (r² = 0.19) of the variation associated with the total sulfhydryl concentration in milk. Researchers concerned with quantitating the sulfhydryl content of milk proteins should be aware that total sulfhydryls expressed as cysteine plus half-cystine residues is positively associated with SCC. The logarithmic relationship implies a greater effect at a low rather than high SCC. Total sulfhydryl values calculated using the regression equation increased less than 1.0 μmol/g of protein with each increase in SCC of 100,000/ml for somatic cell concentrations from 300,000 to 1,000,000/ml.

Values obtained in this study were determined for individual cow's samples. The standard deviation of the mean for these samples reflects individual cow variation and would be lower for bulk samples. The consistency of mean values for total sulfhydryls per gram of protein for samples in this study with previously reported values, in conjunction with the minimal effect of increased SCC on total sulfhydryls for individual samples, suggests that the SCC of milk should not limit chemical analysis of milk protein from bulk milk samples that are based on the determination of sulfhydryl groups if proper experimental controls are employed.

ACKNOWLEDGMENTS

Published as T.A. 19679 of the Texas Agricultural Experiment Station.

REFERENCES