Mechanism for Stabilization of Fish Actomyosin by Sodium Lactate

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The mechanism for stabilization of actomyosin (AM) by sodium lactate (SL) was studied by comparing physical properties (viscosity, density, surface tension, and water activity) of SL and sucrose solutions. Relatively high intrinsic viscosity [η] and Huggins factor (k') values showed SL is an effective water structure-maker. Thermal aggregation studies showed that AM stability increased up to 15% SL; higher concentrations of SL destabilized the AM. Surface tension and water activity measurements indicated that the transition from stabilization to destabilization and from increasing to decreasing surface tension coincided with a change in molecular species in the SL solutions. The decrease in surface tension (and AM stability) with increasing SL concentration above the optimum is presented to be due to formation of the lactate dimer lactoyl lactate. This molecule is amphiphilic and would be expected to preferentially absorb at an interface (with for example air or protein) and thus lower surface tension.

Keywords: *Fish; protein; aggregation; amphiphilic; surface tension; water activity.*

INTRODUCTION

In a previous study, we showed that sodium lactate (SL) from one supplier stabilized tilapia actomyosin (AM) against freeze−thaw and heat denaturation (MacDonald and Lanier, 1994). The extent of stabilization was dependent on concentration, with an optimum concentration of about 6% for freeze−thaw protection and 20−25% for protection from heat denaturation. This was in contrast to sucrose with which stabilization increased monotonically with concentration.

Oddly, SL of similar purity from a second supplier destabilized AM at low concentrations and was less effective at higher concentrations. Lithium lactate was not an effective cryoprotectant and generally destabilized AM with increasing concentration (MacDonald and Lanier, 1994). The reason for the variation in stabilizing ability of SL and destabilization by lithium lactate is not known.

The mechanism by which sucrose stabilizes proteins from heat denaturation in aqueous systems has been investigated in detail by Timasheff and others (Back et al., 1979; Lee and Timasheff, 1981; Arakawa and Timasheff, 1982). There appears to be a general mechanism for protein stabilizing solvent systems (Timasheff and Arakawa, 1989) that is mediated through changes in solvent properties or alteration of water structure. Agents that stabilize proteins in solution can also be used for stabilization of proteins in the frozen state (Carpenter and Crowe, 1988). Therefore, the mechanism is most probably the same as that described for proteins in solution by Lee and Timasheff (1981).

Using kinetic experiments, Lee and Timasheff (1981) found that the apparent activation energy of the unfolding process for a number of proteins was increased by the addition of sucrose. Furthermore, protein−solvent interaction studies indicated that sucrose was preferentially excluded from the protein domain, thereby increasing the free energy of the system. Thermodynamically this leads to protein stabilization since the unfolded state of the protein is accompanied by an increase in protein−solvent surface contact area, and hence unfolding becomes thermodynamically less favorable in the presence of sucrose. The exclusion of sucrose from the protein domain appears to be related to a higher cohesive force for the sucrose−water system since all of the experimental observations could be correlated with the effect of sucrose on the surface tension of water.

On the other hand, the ability of a salt to stabilize proteins in solution is a balance between its ability to increase the solution surface tension and its binding to anionic sites and dipolar peptide bonds of the protein. Increasing surface tension tends to stabilize intramolecular hydrophobic interactions, while binding of ions may stabilize or destabilize depending on the specific interactions between the protein and the ions (Arakawa and Timasheff, 1984; Arakawa et al., 1990). The addition of carboxylic acids to a solution presents a potential complication due to their charged state because electrostatic interactions are possible with the charged sites on proteins.

Other efforts to relate the ability of solutes to structure water have concentrated on intrinsic viscosity and the Huggins factor (k') (Mathlouthi et al., 1989; Serghat et al., 1992). These bulk properties of a solution depend on the size and stereochemistry of the solute, and empirical studies have shown a dependence on distribution between OH^- and COO^- groups and the number of these groups (Gekko and Morikawa, 1981; Matsuura et al., 1984; Ooizumi et al., 1984). The ability of sugars to stabilize proteins against heat denaturation has also been related to the number of equatorial OH groups on a molecule (Uedeira and Uedeira, 1980; Ooizumi et al., 1981; Miura et al., 1991).

The objective of this study was to investigate the mechanism for stabilization of AM by SL. The approach taken was to compare the physical properties of solutions of SL and sucrose with the performance of the solute as a stabilizer of AM.

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MATERIALS AND METHODS

Materials. Sodium lactate (60%) of USP grade was obtained from Purac America, Inc. (Lincolnshire, IL). High-purity sucrose was purchased from Pfanstiehl Laboratory Inc. (Waukegan, IL). All other chemicals were of reagent grade or purer.

AM Preparation. For each set of experiments actomyosin was extracted from freshly sacrificed tilapia hybrid (*Tilapia nilotica* × *Tilapia aurea*) by blending 7.5 g of flesh in 75 mL of chilled 0.6 M KCl (pH 7.0) using a Sorvall Omnimixer (DuPont Instruments, Newton, CT). Excessive heating during extraction was avoided by placing the blender cup in ice and blending for 20 s followed by a 20 s interval for a total extraction time of 4 min. The extract was centrifuged at 5000*g* for 30 min (4 °C). Actomyosin was precipitated by diluting with 3 volumes of chilled distilled water and collected by centrifuging at 5000*g* for 20 min (4 °C). The AM was then dissolved by gentle stirring of an equal volume of chilled 1.2 M KCl (pH 7.0) for 30 min (0 °C). The AM solution was centrifuged (5000*g*, 20 min, 4 °C) for a final time to remove insoluble protein. The supernatant was used in the following experiments.

Protein Assay. Protein concentrations were measured according to the biuret method (Gornall et al., 1949) using bovine serum albumin as standard.

Thermal Aggregation. Protein−protein interaction was monitored by measuring optical density changes attributable to light scattering. Actomyosin was adjusted to 0.5 mg/mL protein concentration in deaerated assay buffer (0.6 M KCl, 10 mM phosphate buffer, pH 6.5) with various concentrations of SL. Protein−protein interaction was induced by heating the AM solution at 0.5 °C/min from 25 to 90 °C in a spectrophotometer (Spectronic 1001, Bausch and Lomb, Rochester, NY) equipped with a jacketed cuvette holder. The temperature at the center of the cuvette was controlled using a circulating water bath (EX-100 DD, Neslab Instrument Inc., Portsmouth, NH) and programmer (MTP-5, Neslab Instrument). Preliminary experiments were done to ensure the temperature profile was reproducible so that thermocouples were not used to monitor cuvette temperature when turbidity data was gathered. To prevent evaporation during the experiment, the solution was covered with a layer of paraffin. Turbidity of solutions was recorded every 60 s by measuring the optical density at 320 nm. The differential change in optical density as a function of temperature (dOD_{320}/dT) was calculated to determine apparent transition temperatures (T_r) of protein−protein association.

Measurement of Physical Properties of Solutions. Solutions of SL and sucrose were deaerated by stirring under vacuum for 20 min prior to measurement of physical properties.

Viscosity, Intrinsic Viscosity, and Huggins Factor. Dynamic viscosity of solutions of SL and sucrose were measured at 25 °C using a concentric cylinder viscometer (Bohlin Rheologi, Edison, NJ). To test for Newtonian flow at each solute concentration, shear rates were varied from 4.634 to 4.634 × 10^2 s^{-1}. Intrinsic viscosity [η] was calculated from the initial slope of the relative viscosity (η/η_0) versus concentration (c), where η is the solution dynamic viscosity (Pa·s) at a shear rate of 46.1 s^{-1}, η_0 is the solvent viscosity, and concentration is in g/cm^3 solute. The Huggins factor, k', was derived from the relation of Huggins (1942)

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c + \ldots \quad (1)$$

where specific viscosity, $\eta_{sp} = (\eta - \eta_0)/\eta_0$.

Surface Tension. Solution surface tension was measured using a CSC-Du Nouy Interfacial Tensiometer (Model 70545, CSC Scientific Co. Inc., Fairfax, VA). Freshly degassed solution was prewarmed in stoppered test tubes and then emptied into a clean disposable Petri dish for measurement. Temperature was controlled by placing the Petri dish in a specially constructed water-jacketed holder. Five measurements were taken over 20 min; the standard deviation was typically less than 2% of the average. Solution density was measured by weighing 10 mL from a volumetric pipette. Apparent surface tension measurements were corrected for solution density using the correction factor of Zuidema and Waters (1941).

To test the hypothesis that SL stabilizes mainly by increasing the surface tension of the solution, we measured the surface tension of SL solutions at the corresponding transition temperature (T_r) predicted from the protein thermal denaturation data.

Water Activity. Water activity (a_w) of solutions was measured at 25 °C using a thermocouple psychrometer (Model SC-10A and NT-3 nanovoltmeter, Decagon Devices Inc., Pullman, WA). Output was calibrated against solutions of NaCl, KCl, and $MgCl_2$. Distilled water was taken as the reference solution for $a_w = 1.000$.

We compared our water activity data to values calculated from the freezing point depression data of Dietz et al. (1941) using the following semiemperical equation from Chen (1987):

$$a_w = \frac{1}{1 + 0.0097\Delta T + C\delta T^2} \quad (2)$$

ΔT is the freezing point depression, (°C), and $C = 5 \times 10^{-5}$ $°C^{-2}$.

Data Analyses. Regression analyses were performed using JMP II (SAS Institute, Cary, NC) statistical package to reveal the effect of solute concentration on changes in apparent transition temperatures and solution properties. One-way analysis of variance and Dunnett's method (Dunnett, 1955) for comparisons with a control were performed for comparisons among means.

RESULTS AND DISCUSSION

Thermal Aggregation. Since our earlier study used Ca^{2+}ATPase deactivation as an indicator of denaturation of AM proteins, a further method, that of turbidity changes with thermal denaturation, was used in this study to verify earlier results. As proteins are heated they unfold, and exposed residues are able to associate via noncovalent and covalent bonds to form polymers and so scatter more light. We are therefore able to use the amount of light scattered/transmitted to follow intermolecular interactions using spectrophotometry (Clark and Ross-Murphy, 1987).

The change in optical density with increasing temperature as a function of SL concentration is given in Figure 1a. A first-derivative plot of these data is shown in Figure 1b. Heating of control AM (no added SL) resulted in transitions at 39.5 and 44.5 °C. Each transition peak may represent the aggregation of partially (unfolded domains) or completely unfolded protein molecules and may involve one or more types of proteins. The initial T_r is close to the 39.0 and 39.6 °C transition temperatures reported for tilapia myosin by Wicker et al. (1986) as detected by ANS fluorescence (pH 6.5) and by Park and Lanier (1989) from DSC measurements, respectively.

As in our previous study, addition of SL stabilized the AM (MacDonald and Lanier, 1994) and resulted in an increase in temperature for the initial apparent transition and loss of the second. The increase in T_r as a function of SL concentration is shown in Figure 2. A linear relationship ($R^2 = 0.973$, $p < 0.001$) exists up to 15% SL, followed by a transition region between 15 and ~20% SL, after which there is no further increase in T_r. At 20% SL, proteins were destabilized such that aggregation was detected prior to heating. These results are slightly lower than those of the previous heat denaturation study in which AM was stabilized up to about 25% but destabilized at higher concentrations of SL (MacDonald and Lanier, 1994). The lower optimum

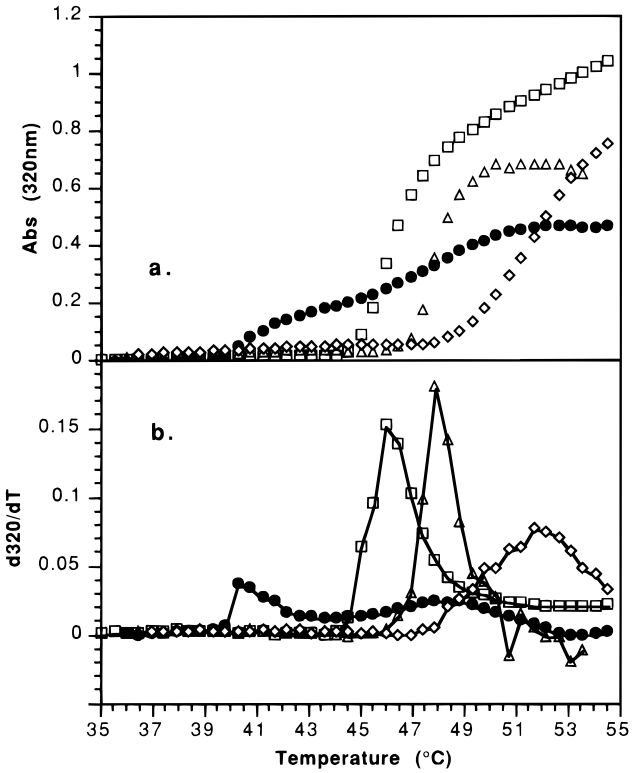

Figure 1. (a) Thermal transitions of protein−protein association for tilapia actomyosin (0.5 mg/mL, pH 6.5) heated at 0.5 °C/min with various concentrations of sodium lactate: ●, 0%; □, 6%, △, 10%; ◇, 15%. (b) First-derivative plot of the data. dOD_{320}/dT is the differential change in optical density. Representative data for each concentration are shown in this figure.

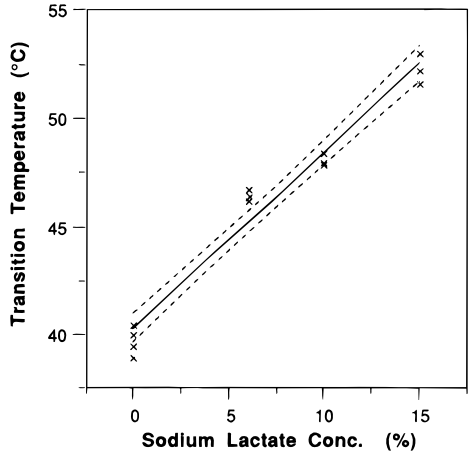

Figure 2. Thermal transition of tilapia actomyosin (0.5 mg/mL, pH 6.5) as a function of sodium lactate concentration. Dashed lines are the 95% confidence interval for the model: $T_r = 40.3\ °C + 0.81[SL](\%)$, $R^2 = 0.973$ ($p < 0.001$).

SL concentration observed in this study could be due to the lower pH used (6.5) compared to pH 7.0 and 7.5 used in the earlier study, since fish AM is more heat labile at lower pH (Hashimoto and Arai, 1985). These results also compare well to our previous freeze−thaw results obtained when the optimum concentration for stabilization was 6% (w/v) SL (MacDonald and Lanier, 1994).

The slope of the relationship between transition temperature and concentration can be used as an indicator of stabilizer effectiveness. In this case, the slope of 0.81 (SE = 0.04) °C per percent SL is nearly double the value found for lactitol (0.41 °C per percent

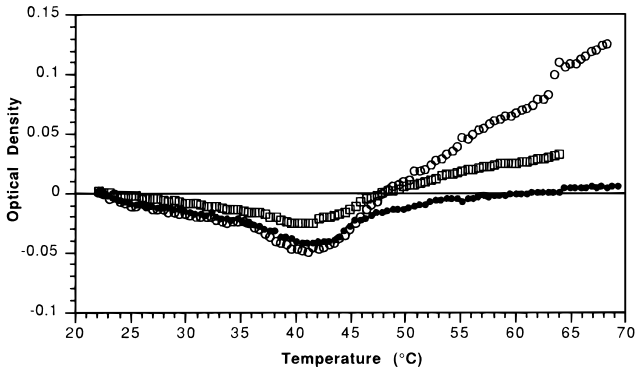

Figure 3. Change in optical density of tilapia actomyosin (0.5 mg/mL, pH 6.5. 0.6 M KCl) heated at 0.5 °C/min with sucrose and sorbitol added: ●, 10% sucrose (320 nm); ○, 10% sucrose (280 nm); □, 5% sorbitol (320 nm). Note: the optical density scale is different from that in Figure 1a.

lactitol) added to cod surimi (Sych et al., 1991) and is also higher than those reported by Back et al. (1979) for sucrose- and sorbitol-stabilized ovalbumin, which were about 0.2 and 0.3 °C per percent, respectively. Hence, these data support our earlier study (MacDonald and Lanier, 1994) that showed, on a percent concentration basis, SL to be a better stabilizer of AM than sucrose.

Aggregation was suppressed with the addition of 10% sucrose, as it was when 5% sorbitol was added (Figure 3). The experiment using sucrose was repeated at 280 nm to improve sensitivity and indicated that unfolding of the AM proteins was taking place, as shown by an increase in absorbance at temperatures above 41 °C, but aggregation was greatly inhibited compared to when SL was used. For example, for the 10% sucrose sample, at 55 °C, the optical density was only 0.04 (280 nm) or −0.005 (320 nm) compared to 0.65 (320 nm) when 10% SL was added. The reason for the difference between denaturation and aggregation of AM proteins in SL and sucrose is not known. According to Hamai and Konno (1990) for fish myosin, the concentration at which turbidity starts to increase with thermal denaturation is species specific. In their study, tilapia S-1, the most stable myosin tested, did not form aggregates. Perhaps, the ability of sucrose to reduce aggregation of proteins, in addition to its ability to stabilize the protein to denaturation, is an important property related to its use in frozen stored fish mince (i.e., surimi) and its ability to inhibit heat-induced gelation of fish proteins (MacDonald et al., 1996).

Physical Properties of Sodium Lactate and Sucrose Solutions. *Viscosity and Density*. The dynamic viscosity of SL solutions greater than 30% increased at a greater rate than sucrose solutions at the same concentration (Figure 4).

Viscometric constants $[\eta]$ and k' may be used as indicators of the extent and stability of solute hydration. Intrinsic viscosity is dependent on the shape and specific volume and includes solvent that is hydrodynamically associated with the molecule. The intrinsic viscosity of SL was more than double that of sucrose (Table 1), which is greater than would be predicted on the basis of a comparison of the molecular weight of SL (112.1) to that of sucrose (342.3). The Huggins factor, k', which is an interaction factor accounting for the mobility of water around the solute, is quite close to that for sucrose (Table 1). These high values for $[\eta]$ and k' could be attributed to the solvation of lactate's carboxyl and hydroxyl groups and suggest that, for its molecular

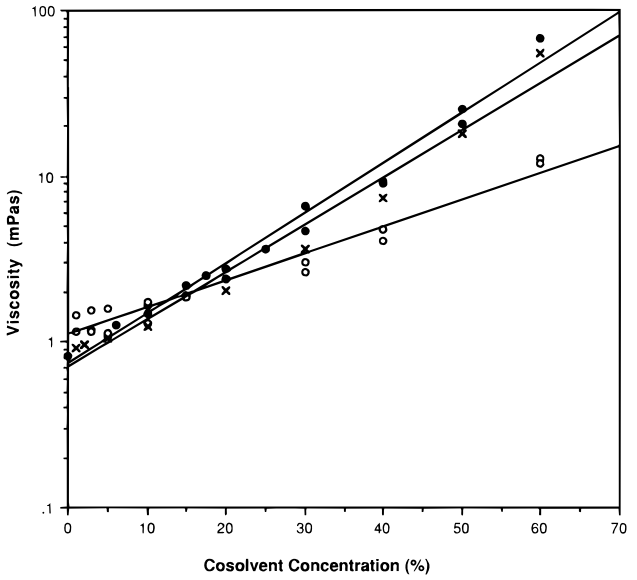

Figure 4. Viscosity of sodium lactate and sucrose solutions at 25 °C as a function of concentration: ●, sodium lactate; ×, sodium lactate data from Dietz et al. (1941); ○, sucrose.

Table 1. Comparison of Intrinsic Viscosity and Huggins Factor for Sodium Lactate and Sucrose

	$[\eta]$ (dL/g)	k'
sodium lactate (USP)	0.056	0.81
sucrose	0.0219	0.94
sucrose[a]	0.0248	0.95
sucrose[b]	0.0203	1.19

[a] From Serghat et al. (1992). [b] From Mathlouthi et al. (1989).

weight, lactate is a relatively effective water structure maker. It is not surprising, therefore, that in our previous study (MacDonald and Lanier, 1994) SL was shown to be an effective stabilizer of AM.

An important restriction concerning the usefulness of the viscometric constants $[\eta]$ and k' for prediction of a solute's effectiveness as a protein stabilizer is that they are based on measurements of the bulk properties of dilute solutions, and hence extrapolation to higher solute concentrations may not be valid. In this instance, viscosity measurements did not predict the destabilization of AM at higher concentrations of SL.

Density was linear with concentration (Figure 5). The results are similar to those of Dietz et al. (1941).

Surface Tension. Surface tensions of SL solutions increased linearly ($p < 0.001$) up to about 15% and decreased above 20% (Figure 6). These data are in contrast to those of Dietz et al. (1941), who found that surface tension decreased to a minimum at 50% and then increased again at 60%. However, it was difficult for them to obtain reproducible results. Importantly, the concentrations of SL that correspond to stabilization and destablization closely correlate with the concentrations at which surface tensions were increasing or decreasing, respectively, in the present study.

In comparison, the surface tension of sucrose solutions increased with increasing concentration, which also correlates with our previous freezing−thawing and heat denaturation studies showing a similar relationship between stability and concentration (Figure 6). The surface tension of sucrose solutions was also lower than the corresponding SL solutions up to 20% SL.

It is possible to estimate the concentration of solute that is present at the interface compared to the bulk concentration by approximating the increasing and

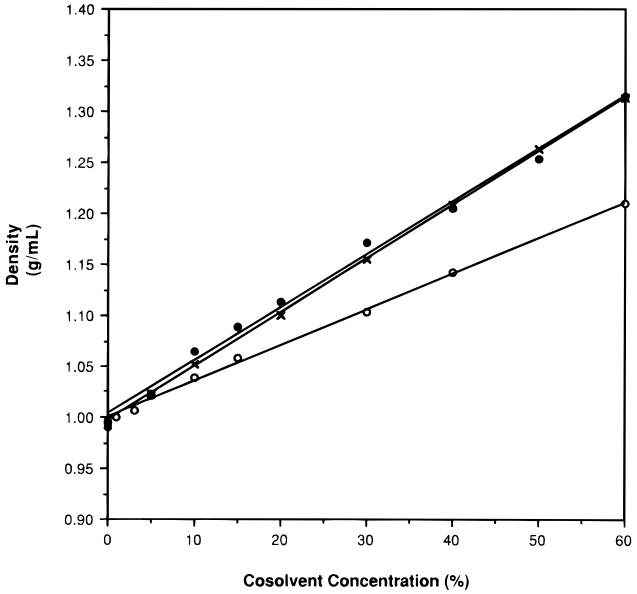

Figure 5. Density of sodium lactate and sucrose solutions at 25 °C as a function of concentration: ●, sodium lactate; ×, sodium lactate data from Dietz et al. (1941); ○, sucrose.

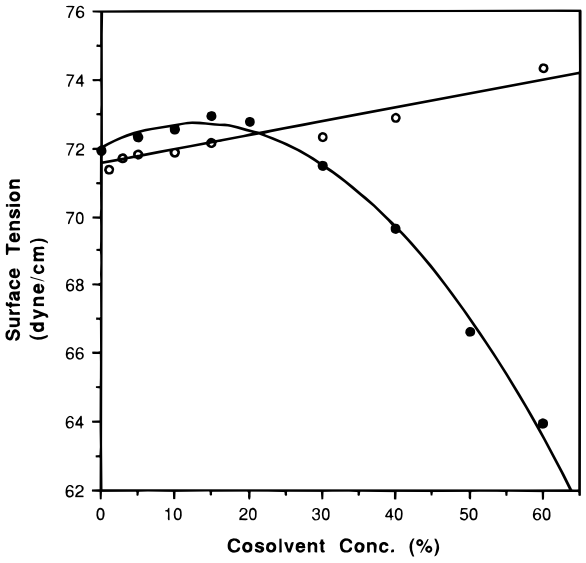

Figure 6. Surface tension of sodium lactate and sucrose solutions at 25 °C as a function of concentration: ●, sodium lactate, $y = 71.8 + 0.096[SL]\ (\%) - 0.00375[SL]^2$; $R^2 = 0.9864$ ($p < 0.001$); ○, sucrose, $y = 71.3 + 0.043[\text{sucrose}]\ (\%)$; $R^2 = 0.9031$ ($p < 0.001$).

decreasing portions of the surface tension curve with linear models and using the Gibbs adsorption isotherm (Tinoco et al., 1985).

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d \ln c} \qquad (3)$$

Γ is the adsorption (excess concentration) of solute at surface (mol m^{-2}), γ is the surface tension (N m^{-1}), R is the gas constant (=8.314 J deg^{-1} mol^{-1}), and c is the concentration of solute in bulk solution.

We estimated that from 0 to 17.5% the concentration of SL at the interface was -2.0×10^{-7} mol m^{-2}. The negative value shows that solute was excluded from the interface compared to the bulk concentration. Using the same procedure, adsorption of solute at the interface for the decreasing surface tension portion of the graph (25−60% SL) was estimated to be 2.5×10^{-6} mol m^{-2}, which

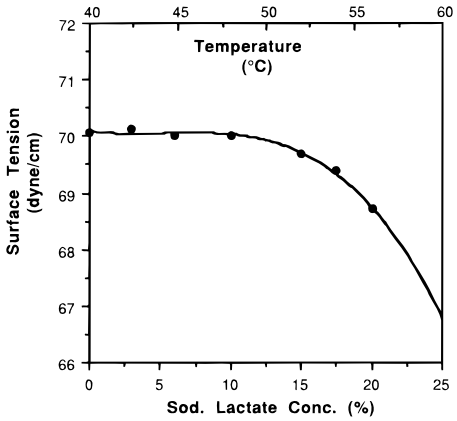

Figure 7. Surface tension of sodium lactate solutions at various temperatures. The combinations of SL concentration and temperature were determined using the linear relationship between thermal transition temperature and sodium lactate concentration from the aggregation data in Figure 2. The equation was extrapolated to 20% SL as confirmation of a decrease in surface tension at this concentration and temperature (56.5 °C) compared to combinations of lower SL concentrations and temperatures.

compares to values of $0.2-4.0 \times 10^{-6}$ mol m^{-2} for typical detergents (Rosen, 1978).

Preferential Exclusion Mechanism Tested. To test the hypothesis that SL stabilizes the protein mainly by increasing the surface tension of the solvent, we measured the surface tension of solutions of different SL concentrations at the corresponding T_r from data in Figure 2. The resultant plot of surface tension versus concentration and temperature shows that, up to about 15%, the surface tension is, within experimental error ($p < 0.05$), close to constant (Figure 7). At higher SL concentrations and temperature, in the region where previously AM proteins were shown to be destabilized, surface tension was lower ($p < 0.05$). Our results verify, experimentally, the results of Lee and Timasheff (1981), who calculated and reported a similar relationship between the surface tension at the transition temperature and sucrose concentration for denaturation of three proteins. The increase in AM T_r in the presence of SL therefore appears to be closely related to the need for lowering the surface tension at the protein−solvent interface to a level at which the free energy change provided by the protein expansion is sufficient to overcome the pressure of the solvent that counteracts the process. It appears, therefore, that increasing surface tension does play an important part in the mechanism by which SL stabilizes AM.

Water Activity. To better understand the basis for an increasing and then a decreasing surface tension with increasing SL concentration (Figure 6), we measured the water activity (a_w) of solutions. In dilute solutions a_w is a colligative property and is only dependent on number of molecules of solvent (Noggle, 1985).

The a_w of SL solutions was characterized by a linear relationship from 0 to 20% and a transition at about 25%; thereafter, a_w continued to decrease but at a greater rate and in a nonlinear manner (Figure 8). Our results compared closely to water activity values calculated from freezing point depression data of Dietz et al. (1941). These results suggested that some interaction between SL molecules was occurring at concentrations above 20% and that possibly a new molecular species was being formed with a greater ability to affect the a_w of the solution. Interestingly, the transition from

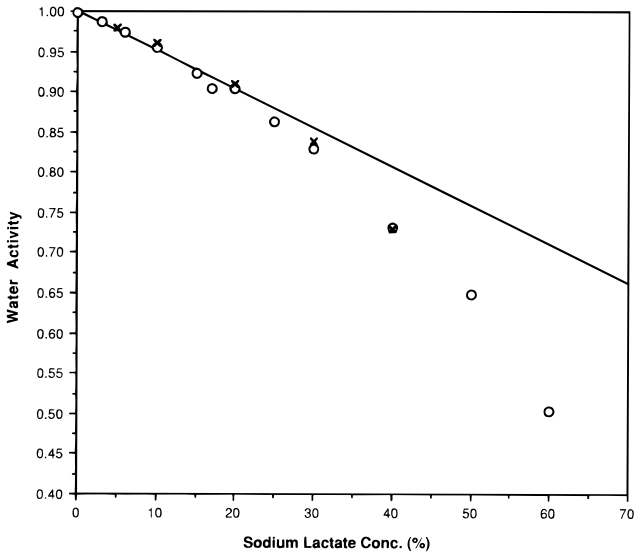

Figure 8. Water activity of sodium lactate solutions as a function of concentration. Crosses represent data calculated from freezing point depression data of Dietz et al. (1941) using eq 2. The linear model shown represents data from 0 to 20% SL, where $a_w = 1.000 - 0.005$[sodium lactate(%)]; $R^2 = 0.994$.

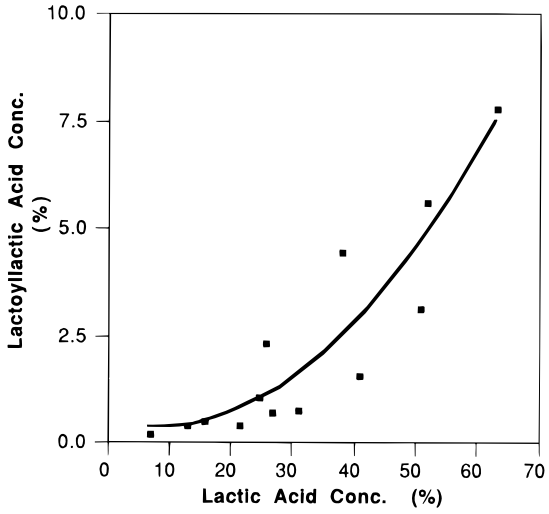

Figure 9. Concentration of the dimer lactoyl lactate as predicted from lactic acid concentration. Data are from Holten et al. (1971).

ideal to nonideal solution coincided with the transition from a positive surface tension increment to a negative surface tension increment.

Proposed Mechanism for Cryoprotection by SL. Holten et al. (1971) demonstrated that lactic acid forms polymers at higher concentrations by interesterification, with the dimer lactoyl lactate being the main polymer formed between 25 and 60% lactic acid. We plotted lactoyl lactate concentration, from their published data, against SL concentration (Figure 9). The resulting model was used to predict lactoyl lactate concentration, which was then plotted against a_w (Figure 10). From this relationship we note that there is a linear relationship between a_w and lactoyl lactate (predicted) for concentrations from 30 to 60% SL ($p < 0.001$). No such relationship was observed below 30% SL.

Inspection of the chemical structure of lactoyl lactate (Figure 11) shows that it is amphiphilic, exhibiting one side that is more hydrophobic and the other more hydrophilic. Hence, lactoyl lactate would be expected to preferentially absorb at an air−water interface and

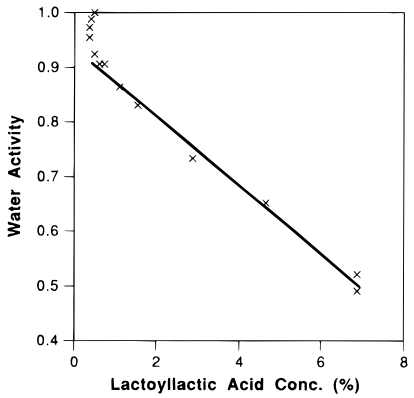

Figure 10. Relationship between water activity and the lactoyl lactate concentration predicted from the data in Figure 9.

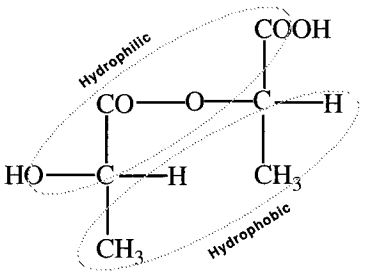

Figure 11. Structure of L-lactoyl-L-lactic acid ester.

lower surface tension. This would then explain the relationship between surface tension and SL concentration, since as the concentration of lactoyl lactate increased with SL concentration, surface tension would be expected to decrease.

The reaction velocity for formation and hydrolysis of the lactoyl lactate ester is slow at room temperature (Holten et al., 1971), which would perhaps explain the difference between our surface tension results and those of Dietz et al. (1941). If solutions had not attained equilibrium prior to testing, this may also explain their reported difficulty in obtaining reproducible results. Further variation may arise from the complex mixture of the four possible combinations of lactate isomers, D, L and (+), (−), that can make up lactoyl lactate. This hypothesis would help to explain the lower cryoprotective effectiveness of SL from the second supplier (brand B) reported in our previous study (MacDonald and Lanier, 1994), since the pH of their concentrate was low (6.19) and the concentration of lactoyl lactate would thus be expected to be higher than that of SL from brand A (Purac), which had a pH of 7.42.

Current theory divides stabilizers into two categories based on their preferential hydration patterns (Timasheff and Arakawa, 1989; Arakawa et al., 1990). The first category includes sugars, amino acids, glycerol, and certain salts such as NaCl, $MgSO_4$, Na_2SO_4, and possibly $(NH_4)_2SO_4$ in which the preferential hydration is almost totally independent of the solvent pH and cosolvent concentration. These cosolvents always act as protein stabilizers, and the role of the protein is restricted to presenting a surface. The principal mechanism for these stabilizers is the strong increase in surface free energy (surface tension) of water upon their addition. It is the increase in the protein−solvent contact surface and, hence, in the surface free energy of the unfolding that shifts the equilibrium toward the more compact native state of the protein.

For the second category of stabilizers, the preferential hydration is strongly dependent on either pH or concentration, or both, and their effect cannot be predicted from their preferential interactions with proteins in the native state. It is the chemical nature of the protein surface that determines the interactions (repulsive or attractive). This category includes $MgCl_2$, poly(ethylene glycol), and 2-methyl-2,4-pentanediol.

In which stabilizer category does SL belong? We believe in the first, since it has a large surface tension increment. At higher concentrations a surface tension decrease, with consequent protein destabilization, can be correlated to the production of the dimer, lactoyl lactate, and not to lactate itself. If we are correct, it may be possible to improve the effectiveness of commercial SL preparations by controlling the content of lactoyl lactate in the concentrate. This could be achieved most easily by increasing the concentrate pH, thereby displacing the chemical equilibrium toward the lactate monomer.

Conclusions. In this study we have reaffirmed that SL is an effective stabilizer of AM and that its effectiveness is strongly correlated with its ability to increase the solution surface tension. In turn, the surface tension of SL solutions appears to be directly dependent on the concentration of the dimer lactoyl lactate that is formed with increasing SL concentration.

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