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Foaming behaviour of EDTA-treated α-lactalbumin

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Abstract

The foaming properties of partially denatured α-lactalbumin was investigated. The partially denatured state was produced by removing bound Ca^{2+} by treatment with ethylenediaminetetraacetic acid (EDTA) at pH 8.0 and 25°C. Surface tension measurements showed that partially denatured α-lactalbumin unfolds easily at liquid interfaces compared with the native protein. The results of foam volume and stability measurements were consistent with the results of surface tension measurements. In the presence of EDTA a considerable amount of foam was obtained at low concentrations, such as 0.1 mg/ml, and the foam stability was improved. This indicates the importance of the protein structure on the adsorption of molecules at liquid interfaces. The presence of Ca^{2+} also resulted in an increase in the foamability and foam stability of α-lactalbumin compared with native protein, due to the saturation of the surface charges. This shows the binding affinity of protein to Ca^{2+}. The investigation of the effect of Ca^{2+} on the surface behaviour of β-lactoglobulin, another whey protein, also showed an improvement in the foaming properties of protein.

1. Introduction

Adsorption changes the native conformation of proteins. The protein adopts a conformation in between the highly organized native state and the completely unfolded state. It has been stated (Dickinson, 1992) that the adsorbed protein loses its tertiary structure while its secondary structure is retained. For some globular proteins, an intermediate state similar to that of the adsorbed protein, can be produced in several ways, such as by changing pH, by heating, by adding denaturants, or by cleavage of disulfide linkages (Matsumura, Mitsui, Dickinson, & Mori, 1994). This intermediate state is a partially folded conformation characterized by a compact globular configuration having a native-like secondary structure and unfolded tertiary structure. It might be stated that the configuration of an adsorbed protein at a liquid interface is similar to that of a protein which is changed with mild analytical treatments. Being in the intermediate state might affect the functional properties of proteins at the liquid interfaces. The foaming capacity of a protein is directly related to its ability to reduce the interfacial tension which is, in turn, related to the ease of the protein unfolding (Dickinson, 1989). For a globular protein having a rigid structure, the exposure of hydrophobic and hydrophilic groups to their preferred environments takes longer than the unfolding of a partially disordered protein (Kinsella, 1981). It was observed (Kinsella & Whitehead, 1988) that partial denaturation of globular proteins improves the emulsifying and foaming capacities. In addition, the behaviour of the adsorbed protein layers plays an important role in the stability of food foams (Prins, 1988). Therefore, the partially unfolded intermediate state might be used for further improvement of potential foaming properties of proteins.

Many globular proteins have partially unfolded states. α-Lactalbumin has an intermediate state (Kuwajima, Nitta, Yoneyama, & Sugai, 1976). It retains the native-like backbone secondary structure in the partially unfolded form, but the tertiary structure is observed to be very different from the native state. α-Lactalbumin exhibits a greater exposure of hydrophobic residues in the partially unfolded state than in the native state (Bacon, Hemmant, Lambert, Moore, & Wright, 1988). This state of α-lactalbumin can be produced in several ways, such as by changing pH to alkaline pH, by heating to 90°C, by adding denaturants, by cleavage of disulphide linkages or by removal of bound Ca^{2+} (Dickinson & Matsumura, 1994). Determination of the foaming properties of α-lactalbumin in its intermediate

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state may provide an understanding of how the protein structure affects the adsorbed protein layers at the liquid interface.

Foaming properties are usually represented by two parameters: foamability and foam stability. Rapid adsorption and rearrangement determines foamability, whereas flexible, cohesive film formation imparts foam stability. Sparging of gas at a constant flow rate through a sintered glass disc is the most reproducible method for the foam formation in the laboratory and it gives more uniform bubble sizes than other methods (Halling, 1981).

In the present study, we have investigated the relative foaming properties of α-lactalbumin in the native state and in the partially denatured state. Measurements are made of foam volume and stability, as a function of protein concentration. The molten globule state was prepared by removing Ca^{2+} using ethylenediamine–tetraacetic acid. α-Lactalbumin is described as an important Ca-binding protein (de Wit & Klarenbeek, 1984). β-Lactoglobulin, the most abundant whey protein component, is larger than α-lactalbumin, which is more compact in structure with four disulfide bonds. The surface behaviour of β-lactoglobulin is influenced by the presence of ions (Ca^{2+}, Na^{+}) which mask some exposed ionic groups and alter the electric double layer to facilitate chain interactions between β-lactoglobulin polypeptides. The presence of Ca^{2+} also changes the solubility of protein. Ca^{2+} is capable of forming bridges between adjacent peptides, contributing to β-lactoglobulin aggregation (Xiong, Dawson, & Wan, 1993). The present study also investigates the change in foaming ability and foam stability of β-lactoglobulin with Ca^{2+}.

2. Materials and methods

α-Lactalbumin (L-5385), ethylenediaminetetraacetic acid (EDTA) (E-5134) and Trisma-base (Tris[hydroxymethyl]aminomethane) buffer salt, $CaCl_2$, NaCl, Na_2SO_4 were purchased from Sigma Chemicals.

2.1. Foam formation and stability

Experiments were carried out in 10 mM tris–HCl buffer at pH 8. Protein solutions (ionic strength of 25 mM) were prepared at different concentrations (0.01, 0.05, 0.1, 0.2 wt%) by dissolving protein in the buffer including either 5 mM EDTA or 5 mM $CaCl_2$. The samples were foamed in a glass sintered column of 25 cm in length and 9.5 cm in internal diameter. The column has a sintered disc of 40–100 μm located throughout the whole cross-section at the lower end and an extension for nitrogen inlet below sinter. The temperature was kept at 25°C throughout the experiments by means of a water jacket around the column. Nitrogen gas was fed into the column after passing through a copper coil placed in a thermostat tank and a flowmeter. A 25 ml sample solution was sparged at a constant flow rate (0.1 ± 0.05 dm^3/min) for 1 min each time. Immediately after turning off the gas the foam height was read from the calibrated markings on the column and the foam volume was calculated. The time for the collapse of the foam to half of its initial value is measured to express the foam stability.

2.2. Surface tension measurements

A digital tensiometer (model K10ST, Kruss Instruments) was used. The Wilhelmy plate was made of platinum and has a height of 19.9 mm, a thickness of 0.1 mm, and a wetting length of 40 mm. A 10^{-2} wt% α-lactalbumin solution was prepared in buffer and diluted to 10^{-3} wt% for the measurements. The measurements were carried out at 25°C.

3. Results and discussion

Relationships between globular protein structure and the properties of adsorbed layers at air–water interfaces were investigated by following the reduction in the surface tension, and the amount of foam produced and the foam stability. Fig. 1 shows the change in surface tension of α-lactalbumin solutions as a function of time. A rapid decline in tension was observed in the presence of EDTA. Treatment with EDTA removes Ca^{2+} from the structure of protein and may lead to a change in conformation. The rapid change in surface tension may be attributed to increased surface activity as a result of increased flexibility of protein and increased rate of unfolding (Dickinson & Matsumura, 1994). In the

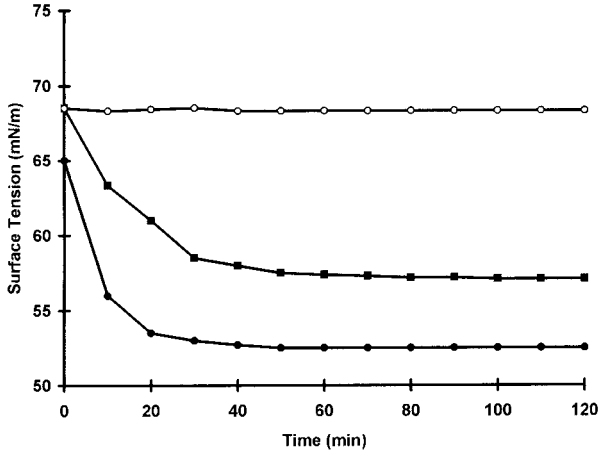

Fig. 1. The change in surface tension with time at air–water interface at pH 8, 25°C; α-lactalbumin solution (10^{-3} wt%) in the presence of EDTA (●), α-lactalbumin solution (10^{-3} wt%) in the absence of EDTA (■), EDTA solution without α-lactalbumin (○). The curves present the average of three replicates within the experimental error of ±1 mN/m.

absence of protein, a reduction in surface tension was not observed from the solution of EDTA alone (Fig. 1). The results of surface tension measurements correlate well with those of foaming experiments. Fig. 2 represents the change in foam volume as a function of concentration for the native protein without any salt and for the protein in the presence of either EDTA or $CaCl_2$. A measurable quantity of foam was obtained from the solution of native protein at a concentration of 0.01 wt%. Foam volume increased with concentration at low concentrations and reached a steady value at 0.1 wt%. The same trend was obtained in the presence of $CaCl_2$ and EDTA. In fact, the steady value of foam volume was reached at a lower concentration (0.05 wt%) in the presence of EDTA and $CaCl_2$. The largest foam volume was obtained with an α-lactalbumin solution containing EDTA. At high concentrations there was not a dramatic difference between the foam volume of partially denatured protein and that of native protein with excess Ca^{2+}. The ability of a protein to form foam strongly correlates with its ability to lower the tension at the air–water interface (Dickinson, 1992). This ability is, in turn, related to the ease of protein unfolding, i.e. the rate of exposure of hydrophobic and hydrophilic groups to their preferred non-aqueous or aqueous environments.

Unfolding occurs more easily for flexible protein molecules as compared to globular protein molecules. It has been revealed (Bacon et al., 1988) that α-lactalbumin, in the partially unfolded intermediate state, exposes more hydrophobic residues than when in the native state. Also, the increase in the relative affinity of α-lactalbumin for the interface has been observed during emulsification after transformation to the partially denatured state (Matsumura et al., 1994). The increase in foam volume in that state might be attributed to the enhanced flexibility and exposure of hydrophobic residues. The effect of the change of state was larger on foaming properties at low concentrations. As the concentration increased, the difference could presumably be compensated for by the presence of a large number of molecules. However, the difference was still quite significant even at high concentration. The foam volume was also improved in the presence of excess Ca^{2+}. α-Lactalbumin has a high Ca^{2+} binding affinity (Owusu, 1992), and it is likely to exist in a more compact, more folded structure in the presence of Ca^{2+}. However, the excess amount of Ca^{2+} in solution saturates the negative charges on protein molecules and reduces the repulsion between them. Therefore, the foaming ability of protein might be improved.

Fig. 3 records the foam stability of α-lactalbumin in the native state with and without excess Ca^{2+} and in the partially denatured state as a function of concentration. The partially denatured form of α-lactalbumin showed a dramatic increase in foam stability compared to the native state with and without Ca^{2+}. The exposure of hydrophobic residues might be expected to give rise to protein–protein interactions and to facilitate the formation of a cohesive film which improves the stability. Foam stability of native α-lactalbumin was found to be better with Ca^{2+} than without Ca^{2+}. This might be interpreted as an increase in the interactions of protein molecules when the repulsion between them is low. This also leads to the formation of a more cohesive film.

The ionic strength (I) of tris–HCl used was 10 mM. However, the ionic strength of protein solution in the

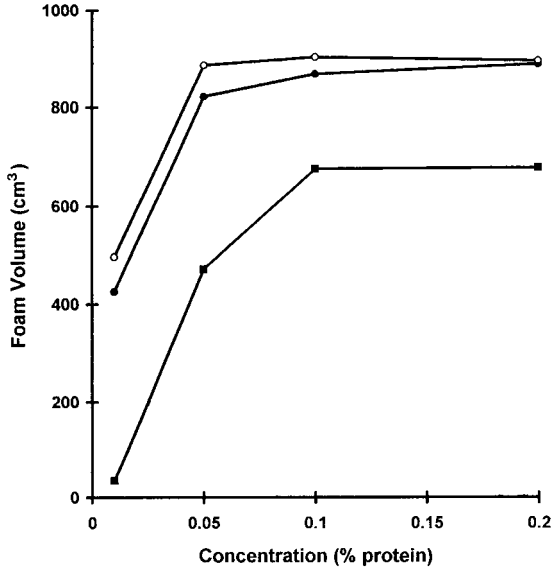

Fig. 2. Foam volume of α-lactalbumin as a function of concentration at 25°C, pH 8, in 10 mM tris–HCl buffer (I=10 mM) (■), in the presence of $CaCl_2$ (I=25 mM) (●), in the presence of EDTA (I=25 mM) (○).

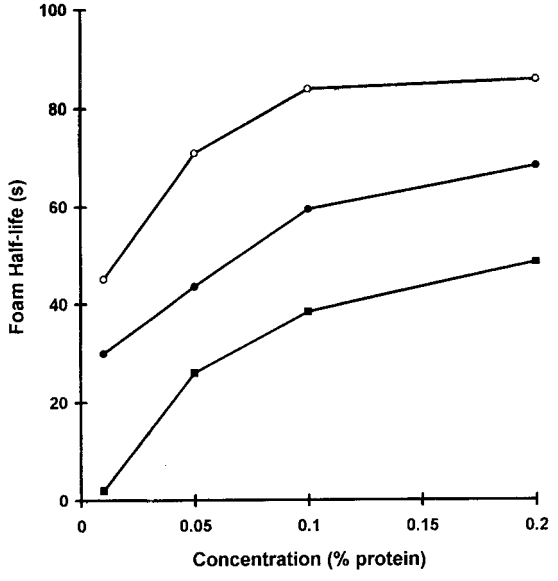

Fig. 3. Half-life of α-lactalbumin foam as a function of concentration at 25°C, pH 8, in 10 mM tris–HCl buffer (I=10 mM) (■), in the presence of $CaCl_2$ (I=25 mM) (●), in the presence of EDTA (I=25 mM) (○).

presence of EDTA and $CaCl_2$ was 25 mM. In order to understand whether it is the Ca^{2+} affecting the foaming properties, or the I is playing a role in determining the foaming properties, the experiments were repeated in the presence of different salts and at different ionic strengths at an α-lactalbumin concentration of 0.1 wt%. The protein solution was made up to 25 and 40 mM by adding NaCl and Na_2SO_4 to the buffer solution ($I=10$ mM). The foam volumes obtained at 10 and 25 mM with NaCl and Na_2SO_4 were the same within experimental error (Table 1). Only at 40 mM with NaCl, was the foam volume slightly higher, but it was still less than the foam volume obtained with $CaCl_2$ at 25 mM. These results show the relative effect of different ions on the foaming properties of α-lactalbumin. Foam stability also showed similar results. Foam half-lives at 25 mM with NaCl, Na_2SO_4, and 40 mM with NaCl were higher than that at 10 mM and lower than that at 25 mM with $CaCl_2$.

Regarding the effect of particular ions, the foaming properties of another whey protein, β-lactoglobulin, were examined in the presence of $CaCl_2$ ($I=25$ mM). Figs. 4 and 5 show the foam volume and the half-life of β-lactoglobulin in the presence and absence of $CaCl_2$. Foaming ability and foam stability both increased dramatically. It has been reported (Zhu & Damodaran, 1994) that calcium ions strongly bind to β-lactoglobulin and the amount of calcium bound is stoichiometrically equivalent to the net charge of the protein. These results not only demonstrate the specific ion effect, but also indicate the importance of net charge of the protein molecules on their functional properties.

At high concentrations, no dramatic changes in foam volume and stability were observed. To be able to follow the changes in foaming properties with concentration, a low concentration (0.01 wt%) was chosen below which the foam volume of native α-lactalbumin was difficult to measure under the experimental conditions. Since foam volume and foam stability of proteins are temperature- and pH-dependent, the conditions (pH 8, 25°C) were kept constant throughout the experiments. α-Lactalbumin carries net negative charge at pH 8. Therefore, it undergoes an ionic interaction with positively charged ions. Proteins are expensive ingredients and difficult to isolate. They are used in a variety of foods to enhance foaming, emulsifying, gelling and water-binding properties. Understanding the protein structure–function relationship responsible for these phenomena (Dickinson, Murray & Stainsby, 1988) and improvement in the functional properties of food proteins have been the aim of food scientists (Ibrahim, Kobayashi, & Kato, 1993; Kinsella & Whitehead, 1987; Li-Chan &

Table 1
Foam volume and foam stability of α-lactalbumin solution in the presence of different salts (I = ionic strength, mM)

	Foam volume ($cm^3 \pm 10$)	Foam half-life ($s \pm 3$)
No added salt ($I=10$)	676	38
EDTA ($I=25$)	904	84
$CaCl_2$ ($I=25$)	868	59
Na_2SO_4 ($I=25$)	699	46
NaCl ($I=25$)	684	44
NaCl ($I=40$)	769	56

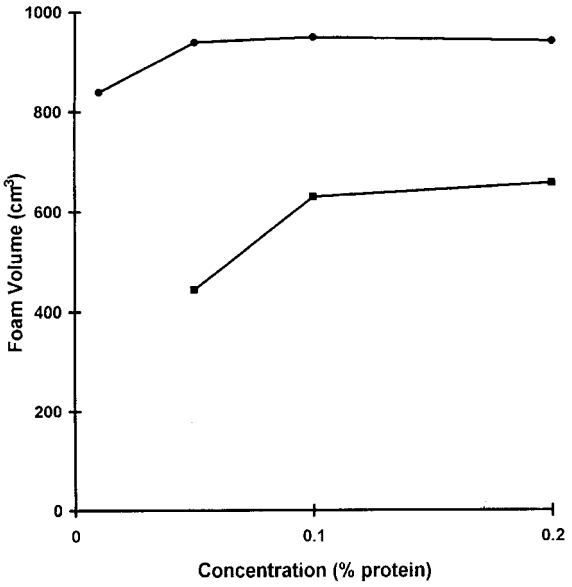

Fig. 4. Foam volume of β-lactoglobulin as a function of concentration at 25°C, pH 8, in tris–HCl buffer in the absence (■) and in the presence (●) of $CaCl_2$.

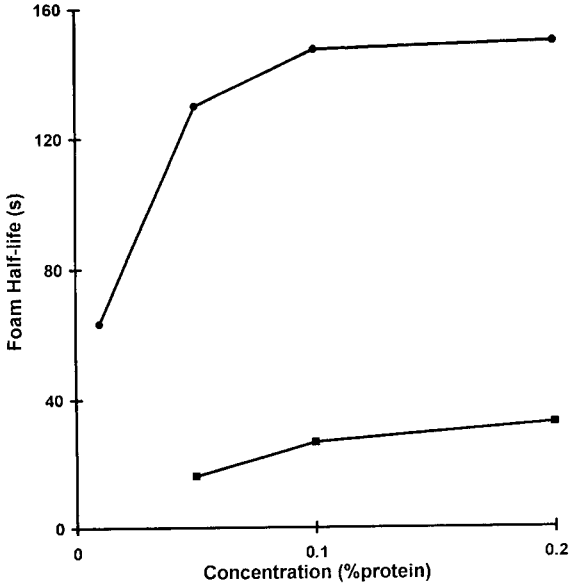

Fig. 5. Half-life of the β-lactoglobulin foam as a function of concentration at 25°C, pH 8, in tris–HCl buffer in the absence (■) and in the presence (●) of $CaCl_2$.

Nakai, 1989). The treatment with EDTA leads to better foaming properties by changing the conformation of α-lactalbumin. The data presented here show the effect of ions on the surface behaviour of α-lactalbumin and the significance of surface charge of the proteins in their functionality in a simple model system. The future work on this subject would be the investigation at neutral and acidic pH and at different concentrations, to apply the results to real food formulations.

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