P-19

Extraction behavior of α-lactalbumin using reverse micellar system

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Abstract

This study reports the extraction behavior of α-lactalbumin using bis(2-ethylhexyl) sulfosuccinate sodium (AOT) reverse micelles. Forward extraction of α-lactalbumin in the reverse micellar organic phase from aqueous feed solutions was strongly dependent on the AOT concentration and the complete forward extraction of 0.03 mM α-lactalbumin was successfully achieved at an AOT concentration of ca. 100 mM. A similar dependency of the forward extraction on the AOT concentration was obtained in isooctane, n-hexane, and n-octane systems. In the backward extraction from the micellar organic phase, the recovery of the protein as high as ca. 90% was obtained with pH control and/or salt addition.

INTRODUCTION

α-Lactalbumin is an acidic milk protein with a small molecular weight (M.W. = 14200, pI = 4.5) and has Ca^{2+} binding sites. α-Lactalbumin performs an important function in mammary secretory cells: it is one of the two components of lactose synthase, which catalyzes the final step in lactose biosynthesis in the lactating mammary gland [1]. Moreover it has been found recently that some forms of α-lactalbumin can induce apoptosis in tumor cells [2,3]. Cow and human milk have nutritional differences, because concentration of α-lactalbumin in human milk is higher than cow milk. As a result, cow-milk-based infant formulas are lack in essential amino acids, especially tryptophan [4]. The enrichment with α-lactalbumin in cow milk provides a nutritional improvement of infant formulas. However, increased commercial use of milk proteins is hampered by the difficulty in obtaining purified sources of the individual proteins.

Reverse micelles are spontaneous aggregates of amphiphilic molecule in non-polar media, and capable to solubilize water and hydrophilic proteins. The protein extraction using reverse micellar system has gained much attention because liquid-liquid extraction can be performed, which is especially attractive for use in large-scale, continuous processing [5].

In this study, the extraction behavior of milk protein α-lactalbumin into bis(2-ethylhexyl) sulfosuccinate sodium (AOT) reverse micelles is investigated.

MATERIALS AND METHODS

Chemicals

α-Lactalbumin from bovine milk (M.W. = 14200, pI = 4-5) was purchased from Sigma Chemical Co. (L-5385, purity 85%) (St. Louis, MO). Bis(2-ethylhexyl) sulfosuccinate sodium (AOT) was used as an amphiphilic molecule, which (purity 99.8%) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Organic solvents, isooctane (2,2,4-trimethylpentane), n-octane, and n-hexane were purchased from Wako Pure Chemical Industries (Osaka, Japan). All these chemicals were used without further purification. Phosphate (sodium dihydrogen phosphate–disodium hydrogen phosphate) buffer (0.1 M, pH 6.0) was used as an aqueous phase.

Methods

The forward extraction was carried out by mixing an equal volume (10 mL) of an AOT reverse micellar solution with the initial aqueous phase for 15 min with a magnetic stirrer, then reverse micellar organic phase containing α-lactalbumin was separated by centrifugation. In the backward extraction, the reverse micellar phase containing α-lactalbumin was mixed with a fresh buffer solution for 20 min with a magnetic stirrer. After centrifugation, the recovered aqueous phase could be obtained. The concentrations of α-lactalbumin in the aqueous and organic phases were determined by measuring the UV absorbance at 280 nm using a UV/visible spectrophotometer (Shimadzu UV-2400 PC). The water content of the reverse micellar organic phase was measured by Karl-Fischer titration with Hiranuma AQV-5S. Circular dichroic (CD) spectra were recorded with a Jasco J-820, using 1 mm cells at wavelengths from 200 to 250 nm. The ellipticity is expressed in mean residue ellipticity. All the experiments were carried out at 298 K.

RESULTS AND DISCUSSION

Effect of amphiphile concentration

Figure 1 shows the effect of AOT concentration of the reverse micellar organic phase on the forward extraction of 0.03 mM α-lactalbumin using isooctane system. The forward extraction percentage of α-lactalbumin increased with the AOT concentration and reached a plateau of ca. 95% at an AOT concentration of 100 mM. In the cases of cytochrome *c*, lysozyme, and ribonuclease A, the minimal concentration of AOT required for 100% forward extraction into reverse micelles were reported [6-8]. This critical amphiphile concentration is called 'minimal AOT concentration,' and is a significant operating condition for the practical

design of micellar extraction processes. The AOT concentration of 100 mM obtained in this study corresponds to the minimal AOT concentration for 0.03 mM α-lactalbumin.

Effect of organic solvent species

The effect of organic solvent species on the forward extraction was examined (Figure 2). Isooctane, n-hexane, and n-octane were used as organic solvents. In all the solvent systems, a similar dependency of the forward extraction on the AOT concentration was obtained. The minimal AOT concentrations were different at each solvent system. The minimal AOT concentration obtained in the isooctane system was lowest.

Protein recovery from reverse micellar organic phase

The backward extraction of α-lactalbumin from the reverse micellar organic phase was examined. Figure 3 shows the effect of stripping aqueous phases on the backward extraction of α-lactalbumin. The stripping aqueous phases used were 0.1 M phosphate buffer solution (pH = 8.5), 1.5 M KCl aqueous solution, and 0.1 M phosphate buffer solution containing 1.5 M KCl, respectively. All the tested stripping phases gave the recovery of α-lactalbumin as high as more then 70 %. The phosphate buffer solution containing 1.5 M KCl enabled the most successful protein recovery to be achieved. Thus, the backward extraction of α-lactalbumin was successfully

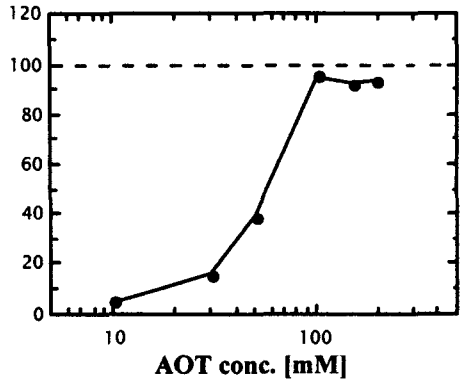

Fig. 1 Forward extraction percentage of α-lactalbumin using AOT / isooctane reverse micellar system at various AOT concentrations. Extraction conditions: α-lactalbumin concentration = 0.03 mM, pH = 6.0.

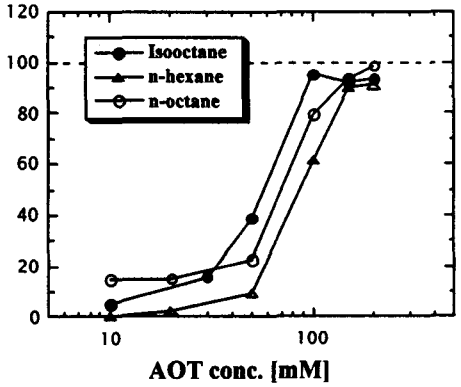

Fig. 2 Effect of solvent species on forward extraction percentage of α-lactalbumin in AOT reverse micellar system at various AOT concentrations. Forward extraction conditions: initial pH = 6.0, initial protein concentration = 0.03 mM.

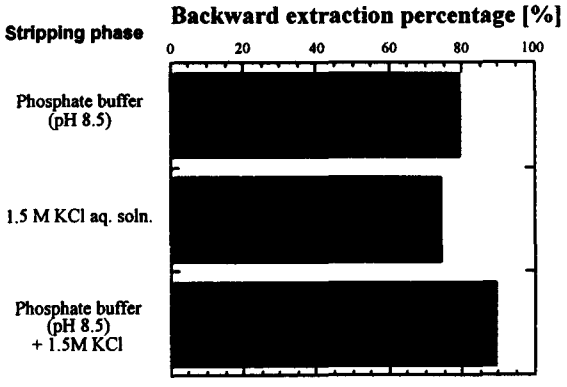

Fig. 3 Backward extraction of α-lactalbumin using various stripping phases from AOT/isooctane reverse micellar organic phase. The stripping phases used were as follows: 0.1 M phosphate buffer (pH = 8.5), 1.5 M KCl aqueous solution, 0.1M phosphate buffer (pH = 8.5) containing 1.5 M KCl.

achieved with the pH control and/or salt addition.

CONCLUSION

The extraction behavior of milk protein α-lactalbumin into AOT reverse micelles was investigated. The forward extraction percentage of 0.03 mM α-lactalbumin increased with the AOT concentration and reached a plateau of ca. 95% at an AOT concentration of 100 mM. A similar dependency of the forward extraction on the AOT concentration was obtained in all the solvent systems. The backward extraction of α-lactalbumin from reverse micellar phase was successfully achieved with pH control and/or salt addition.

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