Interactions of Calcium Binding Proteins, Parvalbumin and α-lactalbumin, with Dipalmitoylphosphatidylcholine Vesicles

E. A. PERMYAKOV, D. I. KREIMER, L. P. KALINICHENKO and V. L. SHNYROV

Institute of Biological Physics, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

Abstract. Interactions of Ca^{2+} binding proteins, pike (*Esox lucius*) parvalbumins pI 4.2 and 5.0, and bovine and human α-lactalbumins, with dipalmitoylphosphatidylcholine vesicles were studied by means of scanning microcalorimetry and intrinsic tyrosine and tryptophan fluorescence methods. The interactions of pike parvalbumins are modulated by Ca^{2+} and Mg^{2+} binding to the protein and induce some changes in the physical properties of both the proteins and liposomes. Liposomes increased thermal stability of Ca^{2+}-loaded parvalbumin and decreased thermal stability of both Mg^{2+}-loaded and metal-free protein. The interaction of parvalbumin with liposomes affects the phase transition from gel to liquid-crystalline state in liposomes. Ca^{2+}-loaded α-lactalbumin interacts with liposomes in its native state while the metal-free protein binds to the liposomes mainly in its thermally denatured state. The results of the microcalorimetric and spectrofluorometric studies are supported by data obtained by means of gel-chromatography on Sepharose 4B. It may be suggested that these metal-modulated interactions of Ca^{2+}-binding proteins with membranes have some functional significance.

Key words: Parvalbumins — α-lactalbumins — Liposomes — Interaction-Ca^{2+} and Mg^{2+} binding — Fluorescence-microcalorimetry

Introduction

Calcium binding proteins play an important role in cell regulation. The regulatory functions of proteins such as calmodulin or troponin C is well known. However, there are other calcium binding proteins with hither to unclear physiological functions. The group of these proteins includes parvalbumins and α-lactalbumins.

Parvalbumins are a family of low molecular mass proteins (11.000—13.000). The parvalbumin molecule consists of six helical regions (A to F)

connected by nonhelical loops. Two loops between helical regions C and D, and E and F each, form two high affinity Ca^{2+}/Mg^{2+}-binding sites (Kretsinger and Nockolds 1973). Their effective calcium binding constants are about 10^5—10^8 l/mol, while their effective magnesium binding constants are about 10^3—10^5 l/mol (Permyakov et al. 1980, 1983). These sites can bind also sodium and potassium ions but with a rather low affinity (effective binding constants ranging between 10 an 100 l/mol) (Permyakov et al. 1983; Permyakov et al. 1984). The binding of cations increases parvalbumin stability to pH-, urea-, or temperature-induced denaturation (Permyakov et al., 1982b, 1983). Parvalbumins interact not only with cations but also with nucleotides ATP and ADP (Permyakov et al. 1982a). The binding of the nucleotides to parvalbumins is metal ions dependent.

It has been proposed that parvalbumins are soluble relaxing factors that accelerate the removal of Ca^{2+} from Ca^{2+} specific regulatory sites of troponin to bring about relaxation (Haiech et al. 1979). On the other hand, the results of computer simulation show that this model cannot work since Mg^{2+} and Ca^{2+} ions do not dissociate fast enough from these sites (Potter et al. 1981). These results are inconsistent with data on computer simulation by other authors who have shown that parvalbumins can serve as soluble relaxation factors (Baylor et al. 1983; Cannell et al. 1984).

Parvalbumins are usually believed to not interact with any cell membranes. To investigate this question, we examined possible interactions of parvalbumins with liposomes formed from dipalmitoylphosphatidylcholine.

α-lactalbumin is one of the two components of the lactose synthetase system which catalyzes the final step in lactose biosynthesis in the lactating mammary gland (Brew et al. 1968). It has been shown that α-lactalbumin is a calcium metalloprotein (Hiraoka et al. 1980). α-lactalbumin posseses one high-affinity binding site for Ca^{2+} ions (effective binding constant approx. 10^8 l/mol) which can bind also Mg^{2+} and Mn^{2+} (Permyakov et al. 1981a, b; Kronman et al. 1981; Murakami et al. 1982; Segawa and Sugai 1983; Permyakov et al. 1985). Attempts to elucidate the role of calcium ions in the functioning of α-lactalbumin as a component of lactose synthetase have not been successful as yet (Kronman et al. 1981; Murakami et al. 1982; Kronman and Bratcher 1984).

It is well known that α-lactalbumin can interact with dimyristoylphosphatidylcholine vesicles (Hanssens et al. 1980; 1983; Herreman et al. 1981; Ameloot et al. 1984; Brown, 1984). However, these interaction have been studied only in the presence of Ca^{2+} ions. On the other hand, the knowledge of effects of Ca^{2+} and Mg^{2+} ions on the interactions of α-lactalbumin with liposomes seems very important.

Here we present results of fluorimetric and microcalorimetric studies of effects of Ca^{2+} and Mg^{2+} ions on interactions of two Ca^{2+}-binding proteins, parvalbumin and α-lactalbumin, with liposomes formed from dipalmitoylphos-

phatidylcholine and used as the simplest model of cell membrane. It has been shown that parvalbumins of pike are capable of metal ions dependent interactions with liposomes, and that the interactions of bovine and human α-lactalbumins with liposomes are modulated by calcium ions.

Materials and Methods

Bovine and human α-lactalbumins were isolated and purified in a preparative sequence similar to that described by Kaplanas and Antanavichius (1975). The purity of the protein preparations was confirmed by sodium dodecyl sulfate gel electrophoresis. Pure preparations of pike parvalbumins pI 4.2 and pI 5.0 were isolated, purified and generously supplied by Dr. V. N. Medvedkin, Institute of Protein Research Academy of Sciences of the USSR, Pushchino. The purity of these preparations was checked electrophoretically, spectrophotometrically and spectrofluorometrically. Protein concentrations were determined spectrophotometrically using molar absorption coefficients $\varepsilon_{257} = = 2700\,M^{-1}cm^{-1}$ for pike parvalbumin pI 4.2 (Bhushana Rao and Gerday 1973) $\varepsilon_{259} = 1810\,M^{-1}cm^{-1}$ for pike parvalbumin pI 5.0 (Closset and Gerday 1975), $\varepsilon_{280} = 28540\,M^{-1}cm^{-1}$ for bovine α-lactalbumin (Kuwajima and Sugai 1978), and $\varepsilon_{280} = 26130\,M^{-1}cm^{-1}$ for human α-lactalbumin (Segawa and Sugai 1983).

All chemicals used were of chemically pure grade. All solutions were prepared with deionized water. Only polyethylene or quartz ware was used in this work.

Dipalmitoylphosphatidylcholine was purchased from Sigma and used without further purification, the purity being verified by thin layer chromatography (Kostetsky and Shchipunov 1983).

Protein and lipid concentrations were 1 mg/ml. The buffer routinely used in this study was 10 mmol/l Hepes, pH 8, 100 mmol/l KCl.

Single bilayer vesicles were prepared by sonification of phospholipid suspended in buffer solution with a UZDN-1 disintegrator for 1 min az 45°C (Hanssens et al. 1980). The lipid concentration was determined from the content of P_i according to the method of Vaskowsky et al. (1975). The protein-lipid mixtures were used in experiments after incubation at room temperature for 1 hour.

Fluorescence measurements were performed with a home built spectrofluorimeter described elsewhere (Permyakov et al. 1977). The fluorescence was taken from the front surface of the cell. The temperature in the thermostatted cell of the instrument was measured by means of a copper-constantan thermocouple with an accuracy of approx. 1 K. The heating rate was approx. $1\,K\,min^{-1}$.

All fluorescence spectra were corrected for the spectral sensitivity of the instrument. For tryptophan fluorescence of α-lactalbumin the position of a chord drawn at the 80% level of the maximal intensity ($\bar{\lambda}$) was taken as the measure of the spectral position. Relative fluorescence quantum yield of α-lactalbumin was evaluated from the area under corrected emission spectra of protein samples. Since neither the position nor the shape of the tyrosine (pike parvalbumin pI 4.2) and phenylalanine (pike parvalbumin pI 5.0) fluorescence spectra do depend on pH, temperature or cation content, tyrosine or phenylalanine fluorescence intensity at a fixed wavelength was used in some measurements as the measure of relative fluorescence quantum yield.

The use of intrinsic protein fluorescence to study thermal denaturation of proteins is limited by the fact that thermal denaturation induces changes in fluorescence developed on the background of a common decrease in fluorescence quantum yield due to activation of collisions of the protein chromophores with neighbouring quenching groups (the thermal quenching process). In order to separate the thermal quenching from the contribution of conformational changes we used the method described in our earlier works (Permyakov et al. 1983b, 1985; Permyakov and Burstein 1984). The method employs plots of reciprocal fluorescence yields (or intensities at a fixed wavelength) against T/η (T is the temperature and η is the solvent viscosity). For proteins within

nondenaturating temperature ranges these plots are straight lines. Fluorescence quantum yield for a given temperature T is

$$q = (1 - \Delta)q_{N,T} + \Delta \cdot q_{H,T}$$

where the subscripts N and H refer to the native and "high" temperature conformers respectively, Δ is the fractional conversion of N to H form, and $q_{N,T}$ and $q_{H,T}$ represent fluorescence yields of the two conformers at temperature T. $q_{N,T}$ and $q_{N,T}$ can be determined from extrapolation of the linear parts of $1\ q$ versus $T\ \eta$ plots to the thermal transition regions. From $q_{N,T}$ and $q_{H,T}$, Δ can be obtained.

Calorimetric measurements were carried out in a DASM-4 (U.S.S.R.) adiabatic microcalorimeter with 0.5 ml cell volume and a heating rate of $1\ K\ min^{-1}$.

The vesicle-protein complexes were separated from the free protein molecules by gel-filtration chromatography on a 27×1.6 cm Sepharose-4B column (Hanssens et al. 1980). One ml of the complex solution was given to the column. Fractions of 0.5 ml were collected for analysis. Parvalbumin and α-lactalbumin in vesicles and free fractions were detected by means of intrinsic protein fluorescence.

Ultraviolet absorption spectra were registered with a Specord UV-VIS spectrophotometer (Karl Zeiss, Jena).

Results

Fig. 1 shows a calorimetric record for pure dipalmitoylphosphatidylcholine liposomes in 10 mmol/l Hepes, pH 8; in the presence of 100 mmol/l KCl. The

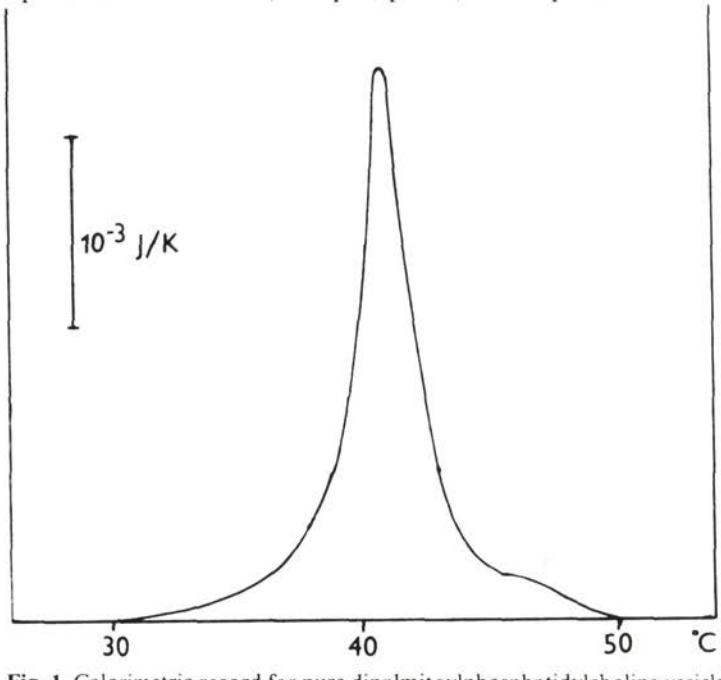

Fig. 1. Calorimetric record for pure dipalmitoylphosphatidylcholine vesicles (1 mg/ml) in 10 mmol/l Hepes, pH 8, in the presence of 100 mmol/l KCl.

narrow peak with the maximum at 41°C is due to phase transition from gel to liquid-crystalline state. Interaction of liposomes with various substances result in some changes in transition (Kursch et al. 1983). In order to increase the sensitivity of the microcalorimetry method to changes in position and shape of the thermogram for liposomes in the presence of proteins, the difference between heat sorption curves for liposome solutions with and without protein was estimated.

Interactions of the proteins with liposomes might change also thermal denaturation curves of the proteins. In the present work thermal denaturation of parvalbumins and α-lactalbumins was monitored by means of microcalorimetry and the intrinsic protein fluorescence method.

Parvalbumins

Fig. 2 shows results of the calorimetric and fluorometric studies of thermal transitions in pike parvalvumin pI 4.2 both in absence and in the presence of dipalmitoylphosphatidylcholine vesicles. The measurements were carried out with metal-free, Mg^{2+}-loaded, and Ca^{2+}-loaded protein. The fluorescence of pike parvalbumin pI 4.2 is completely due to a single tyrosine residue (Permyakov et al., 1983). The fluorometric denaturation curves for Ca^{2+}-loaded protein shown in Fig. 2 were not normalized owing to difficulties with fluorescence measurements at high temperatures (> 90°C). In this case, we only can record the start of thermal transition.

The binding of Mg^{2+} and particularly Ca^{2+} ions results in pronounced stabilization of parvalbumin to thermal denaturation. It is clearly seen that the denaturation curves obtained in absence of liposomes differ from these in their presence. The addition of liposomes to Ca^{2+}-loaded pike parvalbumin shifted both the calorimetric and the fluorometric denaturation curve to higher temperatures (by 2—3°C), while the addition of liposomes to metal-free or Mg^{2+}-loaded protein was followed by similar shifts of the denaturation curves but towards lower temperatures. Moreover, the presence of the protein in the liposome solution induced changes in calorimetric transition in the liposomes which caused an appearance of a calorimetric difference in the temperature region from approx. 35°C to approx. 50°C.

The data obtained seem to suggest the existence of some interactions between pike parvalbumin pI 4.2 and dipalmitoylphosphatidylcholine vesicles. The interactions induce changes in the physical properties of some populations of both parvalbumin and liposomes.

A decrease of the protein concentration at a constant lipid concentration (0.5 mg/ml) resulted in a decrease of the calorimetric difference with half-effect at $(1—2) \times 10^{-2}$ mole protein/mole lipid.

The calorimetric and fluorometric data are supported by the results of

gel-chromatography on Sepharose 4B. Fig. 3*C* shows the results of gel-chromatography of a mixture of pike parvalbumin with dipalmitoylphosphatidylcholine vesicles in the presence of 1 mmol/l $CaCl_2$. It is clear that a fraction of the protein eluted with the vesicles as determined by fluorescence measurements.

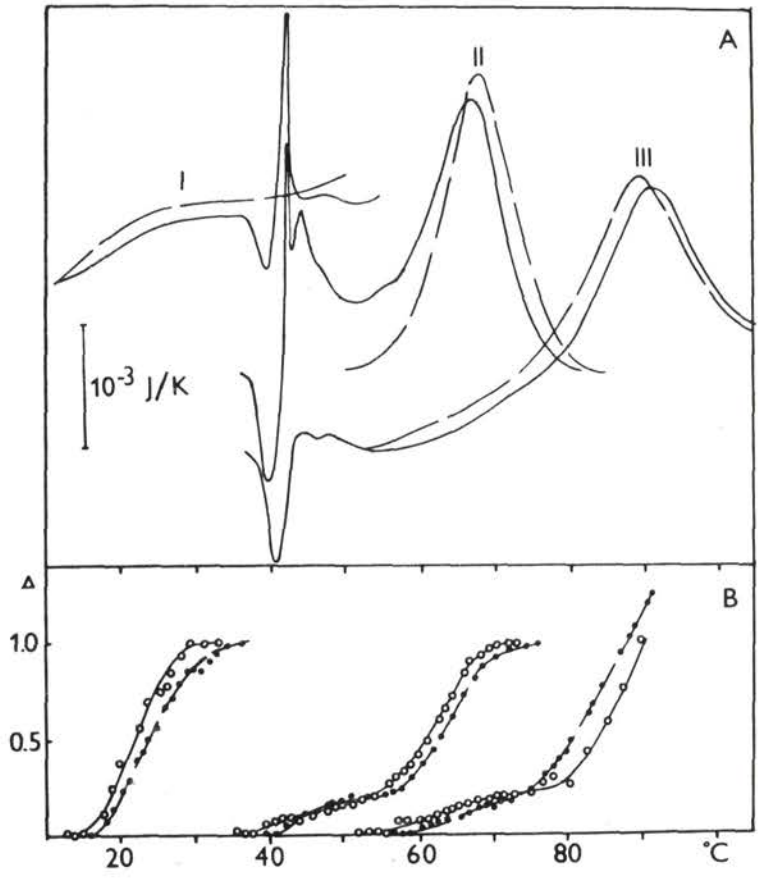

Fig. 2. *A*: calorimetric records for metal free (I) (3 mmol/l EGTA), Mg^{2+}-loaded (II) (3 mol/l EGTA, 8 mmol/l $MgCl_2$) and Ca^{2+}-loaded (III) (1 mmol/l $CaCl_2$) pike parvalbumin pI 4.2 in absence (dotted line) and in the presence (solid line) of dipalmitoylphosphatidylcholine vesicles. The calorimetric measurements with the respective protein in the presence of liposomes were carried out against buffer solution with the same liposome content. *B*: the temperature dependence of the fraction of conversion from native to thermally unfolded state for metal-free (I), Mg^{2+}-loaded (II) and Ca^{2+}-loaded (III) pike parvalbumin pI 4.2 in absence (dotted line) and in the presence (solid line) of liposomes. Curves III were not normalized. 10 mmol/l Hepes, pH 8; 100 mmol/l KCl.

Similar data were obtained for pike parvalbumin pI 5.0 devoid of tryptophan and tyrosine residue. Its fluorescence is due to 9 phenylalanine residues (Permyakov et al. 1983).

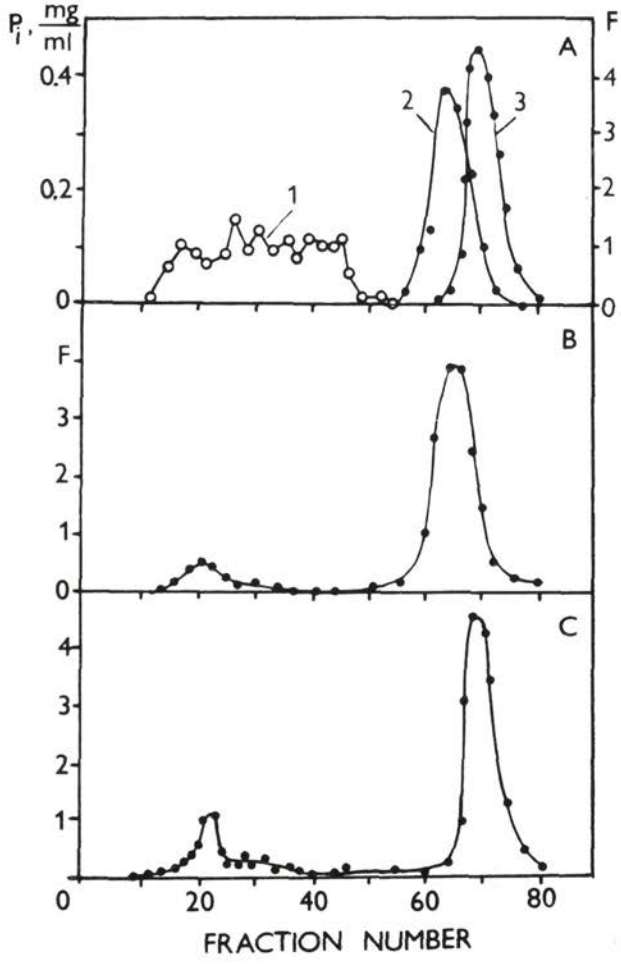

Fig. 3. Gel-chromatography of dipalmitoylphosphatidylcholine vesicles and pike parvalbumin pI 4.2, and bovine α-lactalbumin on Sepharose 4B, 10 mmol/l Hepes, pH 8, 100 mmol/l KCl, 1 mmol/l $CaCl_2$. Fraction volume: 0.5 ml. *A*: 1 — pure dipalmitoylphosphatidylcholine vesicles (1 mg/ml) determined by phosphate analysis; 2 — pure bovine α-lactalbumin (1 mg/ml); 3 — pure pike parvalbumin (1 mg/ml); 2 and 3 were (determined by intrinsic fluorescence. *B*: relative intrinsic fluorescence of a mixture of vesicles (1 mg/ml) with bovine α-lactalbumin (1 mg/ml). *C*: relative intrinsic fluorescence of a mixture of vesicles (1 mg/ml) with pike parvalbumin pI 4.2 (1 mg/ml).

α-lactalbumin

Fluorescence data on thermal denaturation of metal-free and Ca^{2+}-loaded bovine α-lactalbumin in absence and in the presence of dipalmitoylphosphatidylcholine vesicles are shown in Fig. 4. Since α-lactalbumin has a rather low affinity to Mg^{2+} ions, the data obtained in the presence of millimolar concentrations of Mg^{2+} do not differ significantly from those for apo-α-lactalbumin. As in the case of parvalbumins, the binding of Ca^{2+} ions to α-lactalbumin increases the stability of the protein to thermal denaturation. A temperature-induced shift of the tryptophan fluorescence spectrum of the protein towards longer wavelengths corresponds to protein denaturation (Permyakov et al. 1985). The spectral shift is accompanied by an increase in the fluorescence quantum yield.

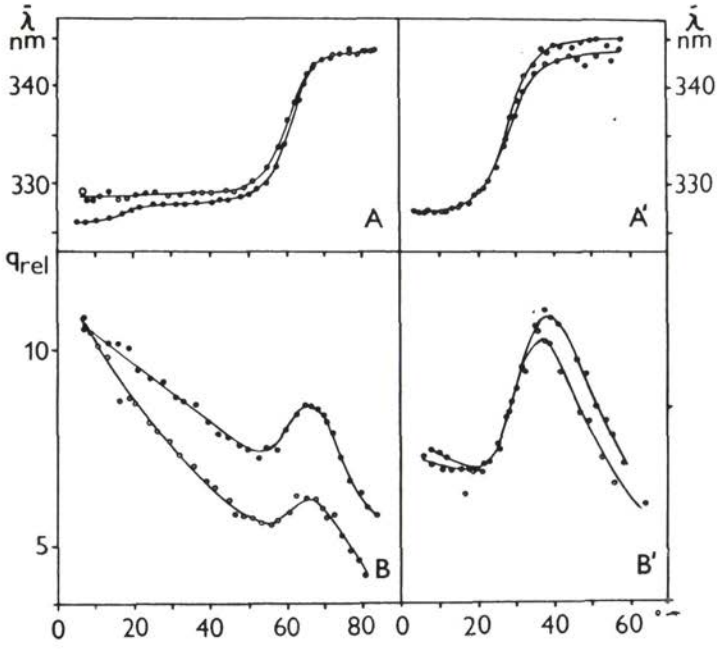

Fig. 4. Thermal denaturation of Ca^{2+}-loaded (*A*, *B*) (1 mmol/l $CaCl_2$) and metal-free (*A'*, *B'*) (1 mmol/l EGTA) bovine α-lactalbumin (1 mg/ml) in absence (●) and in the presence (○) of dipalmitoylphosphatidylcholine vesicles (1 mg/ml); 10 mmol/l Hepes, pH 8; 100 mmol/l KCl. *A*, *A'* — spectrum position; *B*, *B'* — relative fluorescence quantum yield. Excitation wavelength 280.4 nm.

It is well seen from Fig. 4 that the curves obtained in the presence of liposomes differ from these obtained without them. The addition of liposomes to Ca^{2+}-loaded protein at temperatures below the denaturation-induced transition shifts the tryptophan fluorescence spectrum towards longer wavelengths

by 2—3 nm and decreases the fluorescence yield. After thermal transition the difference in spectrum position for the protein with and without liposomes disappears. In contrast, the differences in fluorescence parameters for metal-free α-lactalbumin in absence and in the presence of liposomes appear only after its thermal denaturation. The fluorescence spectrum of the thermally denatured apo-α-lactalbumin in a mixture with liposomes is shifted towards shorter wavelengths by 2 nm as compared with that of pure thermally denatured protein.

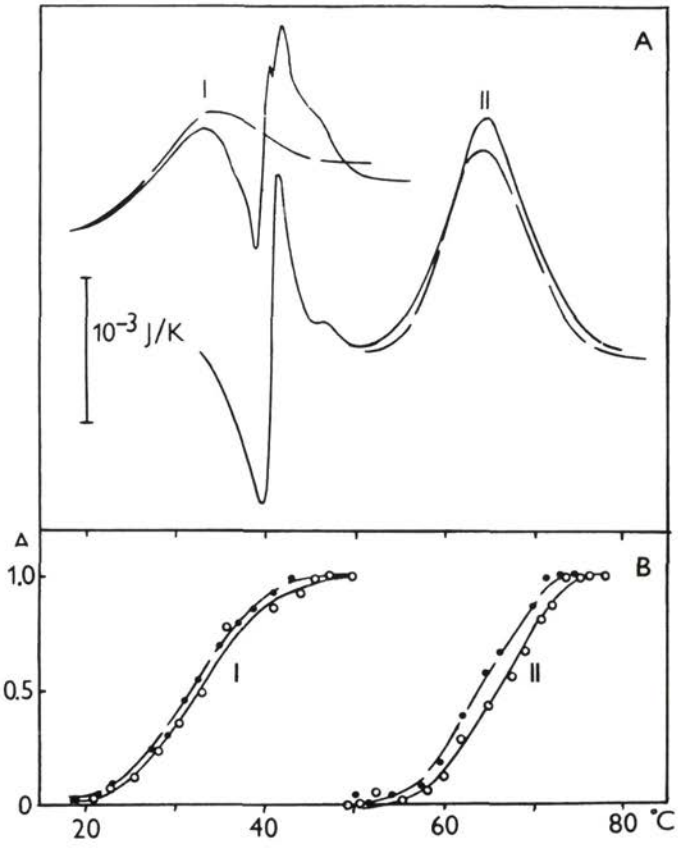

Fig. 5. *A*: calorimetric records for metal-free (I) (1 mmol/l EGTA) and Ca^{2+}-loaded (II) (1 mmol/l $CaCl_2$) bovine α-lactalbumin in absence (dotted line) and in the presence (solid line) of dipalmitoylphosphatidylcholine vesicles. The reference solutions had the same liposome content. *B*: the temperature dependence of the fraction of conversion from native to thermally denatured state for metal-free (I) and Ca^{2+}-loaded (II) bovine α-lactalbumin in absence (dotted line) and in the presence (solid line) of liposomes; 10 mmol/l Hepes, pH 8; 100 mmol/l KCl.

Fig. 5*B* shows the temperature dependence of the fraction of conversion from native to thermally denatured state for apo- and Ca^{2+}-loaded α-lactalbumin in the presence and in absence of liposomes. These data were obtained from the results presented in Fig. 4. Calorimetric records for these preparations are shown in Fig. 5*A*. It is clearly seen from the Figures that the effects of the liposomes are weak with the metal-free protein, and more pronounced with Ca^{2+}-loaded bovine α-lactalbumin.

Similar effects were observed for human α-lactalbumin. The results of gel-chromatography of a mixture of liposomes with Ca^{2+}-loaded bovine α-lactalbumin are shown in Fig. 3. In can be seen that, as with pike parvalbumin, a fraction of α-lactalbumin elutes with the liposomes.

Discussion

The calorimetric and fluorometric data obtained clearly suggest the existence of some interactions between pike parvalbumins and dipalmitoylphosphatidylcholine vesicles. The systems studied thus contained both proteins bound to liposomes and free proteins. Interactions induce changes in physical characteristics of both the proteins and the liposomes, increasing or decreasing their thermal stability. In addition, it should be noted that these interactions are modulated by Ca^{2+} and Mg^{2+} ions. Liposomes increased the thermal stability of Ca^{2+}-saturated parvalvumins and decreased the thermal stability of Mg^{2+}-loaded and metal-free proteins. Liposomes predominantly affect the higher temperature transitions, leaving the lower temperature transitions effectively unchanged (Permyakov et al. 1983). The different metal states of the parvalbumins have different effects on the phase transition in liposomes. The most pronounced effects were observed with Mg^{2+}-loaded protein. These effects were much smaller with Ca^{2+}-loaded parvalbumin. Considering the opposite signs of the effects of liposomes on thermal transition in Ca^{2+} and Mg^{2+}-loaded parvalbumins, it may be suggested that interactions of Ca^{2+}- and Mg^{2+}-loaded proteins with dipalmitoylphosphatidylcholine vesicles are of different nature.

It would be attractive to suppose that similar Ca^{2+}- and Mg^{2+}-modulated interactions of parvalbumins with membranes also occur in vivo. If parvalbumin binds not only to liposomes but also to natural membranes it may be assumed that during the rest stage of a muscle, when parvalbumin is in Mg^{2+}--loaded state, some portion of it is bound to membranes. The release of Ca^{2+} from the sarcoplasmic reticulum must induce dissociation of some of the parvalbumin-membrane complexes since the interaction of Ca^{2+}-loaded protein with membrane is weaker than that of Mg^{2+}-loaded protein. This hypothesis should be tested in future.

Our results concerning interactions of Ca^{2+}-loaded bovine α-lactalbumin with liposomes are in good agreement with data reported by Hanssens et al. (1980). These authors concluded that at neutral pH α-lactalbumin is mainly electrostatically adsorbed to the outer surface of dimyristoylphosphatidylcholine vesicles, while at pH 4 the protein is able to penetrate the vesicles and to interact hydrophobically with the phospholipid fatty acid chains (Herreman et al. 1981). Since the acid form of α-lactalbumin resembles in some aspects its apo-form at neutral pH (Permyakov et al. 1985) it could be suggested that the interactions of both forms with liposomes are similar. The results of our study show that this is not the case. Metal-free α-lactalbumin interacts with dipalmitoylphosphatidylcholine vesicles mainly in the thermally denatured state.

Thus, our data suggest that the calcium-binding proteins, parvalbumins and α-lactalbumins, can interact with dipalmitoylphosphatidylcholine vesicles. The interactions are modulated by binding of Ca^{2+} and Mg^{2+} ions to the proteins. At pH 8 and room temperature, some portion of Ca^{2+}-loaded parvalbumin and α-lactalbumin is in a complex with liposomes. It may be suggested that similar interactions play some role in the functioning of the calcium-binding proteins.

Acknowledgements. We would like to thank Dr. V. N. Medvedkin for supplying the parvalbumin preparations, and Dr. B. I. Medvedev for performing thin layer chromatography of lipids.

References

Ameloot M., Hendrickx H., Herreman W., Pottel H., Van Cauwelaert F., Van der Meer (1984): Effects of orientational order on the decay of the fluorescence anisotropy in membrane suspensions. Experimental verification on unilamellar vesicles and lipid/α-lactalbumin complexes. Biophys. J. **46**, 525—539

Baylor S. M., Chandler W. K., Marshall M. W. (1983): Sarcoplasmic reticulum calcium release in frog skeletal muscle fibers estimated from arsenaso III calcium transients. J. Physiol. (London) **344**, 625—666

Bhushana Rao K. S. P., Gerday C. (1973): Low molecular weight proteins of pike (Esox lucius) white muscles. I. Extraction and purification — polymorphism. Comp. Biochem. Physiol. **44B**, 931—937

Brew K., Vanaman T. C., Hill R. L. (1968): The role of α-lactalbumin and A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. Proc. Nat. Acad. Sci. USA **59**, 491—497

Brown E. M. (1984): Interactions of β-lactoglobulin and α-lactalbumin with lipids: a review. J. Dairy Sci. **67**, 713—722

Cannell M. B., Allen D. G. (1984): Model of calcium movements during activation in the sarcomere of frog skeletal muscle. Biophys. J. **45**, 913—926

Closset J., Gerday C. (1975): Conformational studies on parvalbumins by circular dichroism. Biochim. Biophys. Acta **405**, 228—235

Haiech J., Derancourt J., Pechere J. F., Demaille J. G. (1979): Magnesium and calcium binding to parvalbumins: evidence for differences between parvalbumins and an explanation of their relaxing function. Biochemistry **18**, 2752—2758

Hanssens I., Houthuys C., Herreman W., Van Cauwelaert F. H. (1980): Interaction of α-lactalbumin with dimyristoyphosphatidylcholine vesicles. I. A microcalorimetric and fluorescence study. Biochim. Biophys. Acta **602**, 539—557

Hanssens I., Herreman W., Van Ceunebroeck J. C., Dangreau H., Gielens C., Preaux G., Van Cauwelaert F. (1983): Interaction of α-lactalbumin with dimyristoylphosphatidylcholine vesicles. III. Influence of the temperature and of the lipid-to-protein molar ratio on the complex formation. Biochim. Biophys. Acta **728**, 293—304

Herreman W., Van Tornout P., Van Cauwelaert F. H., Hanssens I. (1981): Interaction of α-lactalbumin with dimyristoylphosphatidylcholine vesicles. II. A fluorescence polarisation study. Biochim. Biophys. Acta **640**, 419—429

Hiraoka Y., Segawa T., Kuwajima K., Sugai S,. Nurai N. (1980): α-lactalbumin: a calcium metalloprotein. Biochem. Biophys. Res. Commun. **95**, 1098—1104

Kaplanas R. I., Antanavichius A. I. (1975): Isolation and purifications of α-lactalbumin, the component of lactose synthase. Biokhimiya **40**, 584—587 (in Russian)

Kostetsky E. Y., Shchipunov Y. A. (1983): Evolution of phospholipid content of marine invertebrates. Zh. Evol. Biokhimii Fiziologii **19**, 11—20 (in Russian)

Kretsinger R. H., Nockolds C. F. (1973): Carp muscle calcium-binding protein. II. Structure determination and general description. J. Biol. Chem. **248**, 3313—3326

Kronman M. J., Bratcher S. C. (1984): Conformational changes induced by zinc and terbium binding to native bovine α-lactalbumin and calcium free α-lactalbumin. J. Biol. Chem. **259**, 10887—10895

Kronman M. J., Sinha S. K., Brew K. (1981): Characteristics of the binding of Ca^{2+} and other divalent metal ions to bovine α-lactalbumin. J. Biol. Chem. **256**, 8582—8587

Kursch B., Lullmann U., Mohr K. (1983): Influence of various cations amphiphilic drugs on the phase-transition temperature of phosphatidylcholine liposomes. Biochem. Pharmacol. **32**, 2589—2594

Kuwajima K., Sugai S. (1978): Equilibrium and kinetics of the thermal unfolding of α-lactalbumin. Biophys. Chem. **8**, 247—254

Murakami K., Andree P. J., Berliner L. J. (1982): Metal ion binding to α-lactalbumin species. Biochemistry **21**, 5488—5494

Permyakov E. A., Burstein E. A. (1984): Some aspects of studies of thermal transitions in proteins by means of their intrinsic fluorescence. Biophys. Chem. **19**, 265—271

Permyakov E. A., Burstein E. A., Sawada Y., Yamazaki I. (1977): Luminiscence of phenylalanine residues in superoxide dismutase from green pea. Biochim. Biophys. Acta **491**, 149—154

Permyakov E. A., Yarmolenko V. V., Emelyanenko V. I., Burstein E. A., Closset J., Gerday C. (1980): Fluorescence studies of the calcium binding to whiting (*Gadus merlangus*) parvalbumin. Eur. J. Biochem. **109**, 307—315

Permyakov E. A., Kalinichenko L. P., Morozova L. A., Yarmolenko V. V., Burstein E. A. (1981a): α-lactalbumin binds magnesium ions: study by means of intrinsic fluorescence technique. Biochem. Biophys. Res. Commun. **102**, 1—7

Permyakov E. A., Yarmolenko V. V., Kalinichenko L. P., Morozova L. A., Burstein E. A. (1981b): Calcium binding to α-lactalbumin: structural rearrangement and association constant evaluation by means of intrinsic protein fluorescence changes. Biochem. Biophys. Res. Commun. **100**, 191—197

Permyakov E. A,. Kalinichenko L. P., Yarmolenko V. V., Burstein E. A., Gerday C. (1982a): Binding of nucleotides to parvalbumins. Biochim. Biophys. Res. Commun. **105**, 1059—1065

Permyakov E. A., Yarmolenko V. V., Burstein E. A., Gerday C. (1982b): Intrinsic fluorescence spectra of tryptophan containing parvalbumin as a function of thermal, pH and urea denaturation. Biophys. Chem. **15**, 19—26

Permyakov E. A., Medvedkin V. N., Kalinichenko L. P., Burstein E. A. (1983): Comparative study of physicochemical properties of two pike (*Esox lucius*) parvalbumins by means of their intrinsic tyrosyl and phenylalanyl fluorescence. Arch. Biochem. Biophys. **221**, 9—20

Permyakov E. A., Kalinichenko L. P., Medvedkin V. N., Burstein E. A., Gerday C. (1984): Sodium and potassium binding to parvalbumins measured by means of intirnsic protein fluorescence. Biochim. Biophys. Acta **749**, 185—191

Permyakov E. A., Morozova L. A., Burstein E. A. (1985): Cation binding effects on the pH, thermal and urea denaturation transitions in α-lactalbumin. Biophys. Chem. **21**, 21—32

Potter J. D., Robertson S. P., Johnson J. D. (1981): Magnesium and the regulation of muscle contraction. Fed. Proc. **40**, 2653—2656

Segawa T., Sugai S. (1983): Interactions of divalent metal ions with bovine, human and goat α-lactalbumin. J. Biochem. **93**, 1321—1328

Vaskovsky V. E., Kostetsky E. Y., Vasendin G. M. (1975): A universal reagent for phospholipid analysis. J. Chromatogr. **114**, 129—141

Final version accepted May 20, 1987