Comparison of calregulins from vertebrate livers

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Calregulins were purified from bovine, rabbit and chicken liver, and their structural properties were compared. Significant differences between the three calregulins include a lower M_r for chicken calregulin (57000) than for rabbit and bovine calregulin (63000), and the glycosylation of only bovine calregulin. Amino acid composition and peptide maps of the three calregulins were very similar. No major differences were detected in the Ca^{2+} -binding properties of the three proteins. Zn^{2+} -induced changes in calregulin conformation and hydrophobicity monitored by intrinsic protein fluorescence and the hydrophobic fluorescent probe 8-anilino-1-naphthalenesulphonate were very similar, suggesting that the Zn^{2+} -dependent increase in the hydrophobicity of bovine, rabbit and chicken calregulin was conserved. These studies more fully define what is a calregulin, demonstrate that calregulin is a relatively invariant constituent of vertebrate liver, and indicate that calregulin structure has been highly conserved in bovine, chicken and rabbit liver.

INTRODUCTION

Previous studies in our laboratory (Waisman *et al.*, 1985) have documented the presence of a M_r -63000 Ca^{2+} -binding protein, called calregulin (Khanna & Waisman, 1986; Khanna *et al.*, 1987), in the 100000 *g* supernatant of a variety of bovine tissues. That this protein might be an important intracellular receptor for second-messenger Ca^{2+} has been suggested by the high concentration of calregulin in bovine tissues (Khanna & Waisman, 1986) and by the high affinity of binding of Ca^{2+} under physiological conditions (Khanna *et al.*, 1986). In our previous report (Khanna *et al.*, 1986) the presence of distinct and specific ligand-binding sites on bovine calregulin for Ca^{2+} and Zn^{2+} were documented. Measurement of Ca^{2+} - and Zn^{2+} -induced changes in tryptophan fluorescence suggested that both Ca^{2+} and Zn^{2+} induced distinct conformational changes in bovine calregulin. Studies with the fluorescent hydrophobic probe ANS suggested that Zn^{2+} , but not Ca^{2+} , induced an increase in the hydrophobicity of bovine calregulin (Khanna *et al.*, 1986). This result presented the possibility that mechanistically calregulin might act in a manner similar to calmodulin, whose Ca^{2+} -dependent increase in hydrophobicity has been shown to be a necessary step in the activation of many enzymes by this protein (LaPorte *et al.*, 1980).

As part of a systematic study of the structure and evolution of calregulin, we have isolated calregulin from bovine, chicken and rabbit livers, using the same isolation protocol for each species. We report here a comparison of the physico-chemical and structural properties of the liver calregulins from these species. These studies more fully define what constitutes a calregulin and demonstrate that the physicochemical and structural properties of calregulin from a single tissue have been highly conserved during vertebrate evolution.

EXPERIMENTAL

Materials

All chemicals were reagent grade, unless specified otherwise. Deionized water was used throughout. Chelex

100 was obtained from Bio-Rad. $^{45}\text{CaCl}_2$ (20 mCi/mg of Ca^{2+}) was purchased from Amersham Corp. *Staphylococcus aureus* V8 proteinase was obtained from Pierce Chemical Co. Hepes, Mops and ANS were obtained from Sigma.

Purification of calregulin

Calregulins from bovine, chicken and rabbit livers were purified by the procedure of Waisman *et al.* (1985).

Ca^{2+} binding of purified calregulins

This was determined by equilibrium dialysis. Calregulin was first dialysed overnight against 1000 vol. of a solution containing 150 mM-KCl, 10 mM-Mops (pH 7.1), 3 mM-MgCl₂, 1.0 mM-dithiothreitol and 0.1 mM-EGTA, to remove bound Ca^{2+} from the proteins. The dialysed proteins were then used for equilibrium dialysis as follows: a 0.5 ml portion of protein was dialysed with shaking for 48 h at 4 °C against 100 ml of the same solution, containing various amounts of CaCl_2 and $^{45}\text{Ca}^{2+}$ (5 μCi) to achieve the desired free Ca^{2+} concentration. The solutions outside and inside the dialysis tubing were removed, the A_{278} was determined, and the protein concentration calculated from the absorption coefficient. Samples of these solutions were subjected to liquid-scintillation spectrometry, and the total Ca^{2+} concentration was calculated from the contaminating Ca^{2+} (1.0 μM) determined by atomic absorption, plus the amount of Ca^{2+} added. The association constants for metal and H^+ binding of EGTA were based on values measured by Fabiato (1981).

Amino acid analysis

Calregulins were dialysed overnight against two changes of 1000 vol. of 0.04 M- NaHCO_3 , and portions of the protein solution were transferred to hydrolysis tubes containing 0.1% phenol and 0.02% 2-mercapto-ethanol. Norleucine (10 nmol) was used as an internal standard to correct for losses of protein. Hydrolysis was carried out at 110 °C for 24, 48 and 72 h. The calculated values were based on mean values from eight determinations. Performic acid oxidation was performed by the method of Hirs (1967). Tryptophan determinations

were performed by the method of Simpson *et al.* (1976). Recoveries of tryptophan were typically 90–95%.

Protein sequencing

Calregulin from chicken and rabbit livers was sequenced on an Applied Biosystems 470A gas-phase protein sequencer by using methanolic-HCl conversion and reagents from Applied Biosystems. Biobrene (30 μ l; Applied Biosystems' Polybrene) was precycled for three cycles (2×0.2 nfil degradation cycles plus 1×0.2 nvac degradation cycle) before each run (0.2 nfil and 0.2 nvac are standard programme cycles supplied by Applied Biosystems for Polybrene pre-conditioning and protein degradation respectively). The amino acid phenylthiohydantoin from the sequencer were identified by h.p.l.c. by using a Varian 401 data system, a Waters WISP 710B autosampler and a Waters 440 two-channel u.v. detector (254 and 313 nm). A Beckman Ultrasphere ODS column (5 μ m; 0.46 cm $\times$ 25 cm) with a Whatman Col: Pell ODS guard column (0.21 cm $\times$ 5.0 cm) was eluted by the 25 min sodium acetate (pH 5.2, 15–50 cm)/acetonitrile programme at 40 $^{\circ}$ C, at a flow rate of 1.0 ml/min. All amino acid phenylthiohydantoin except the lysine/phenylalanine pair were well resolved, and that of pyridylethyl-cysteine was eluted in front of that of methionine. Norleucine phenylthiohydantoin was used as an internal standard in each sequencer vial.

Peptide mapping

Peptide mapping by limited proteolysis was performed by the method of Cleveland *et al.* (1977). Purified protein was dissolved at approx. 0.5 mg/ml in sample buffer, containing 0.1 M-Tris/HCl, pH 6.8, 0.5% SDS, 10% (v/v) glycerol and 0.001% Bromophenol Blue. The samples were then heated to 100 $^{\circ}$ C for 2 min. Proteolytic digestions were carried out at 37 $^{\circ}$ C for 30 min by addition of *Staph. aureus* V8 protease (10 μ g/ml). After addition of 2-mercaptoethanol and SDS to final concentrations of 10% and 2% (v/v) respectively, proteolysis was stopped by boiling the samples for 2 min. About 20–30 μ l (10–20 μ g) of each sample were loaded into a sample well of the 15%-acrylamide gel, which was run in the normal manner, and stained with Coomassie Blue.

Fluorescence measurements

These were performed in a Perkin-Elmer MPF 4 spectrofluorimeter in water-jacketted cuvette holders at 25 $^{\circ}$ C operated in the normal mode. All fluorescence measurements were done with protein solutions in 10 mM-Mops/3 mM-MgCl₂/150 mM-KCl, pH 7.1. Zn²⁺ titrations were performed by sequentially adding small portions of ZnCl₂ from a stock solution to protein solutions and to a blank solution containing no protein and allowing the sample to equilibrate after each addition, for 10 min. Titration measurements were made at an excitation wavelength of 286 nm and an emission wavelength of 345 nm. The binding of the hydrophobic fluorescent probe ANS was monitored by its fluorescence enhancement. Again, the ANS was added from concentrated stock solution (10 mM in water) to cuvettes containing calregulin or buffer only, and the fluorescence of the solution was measured at the emission maximum, 480 nm, after excitation at 360 nm. The effects of Ca²⁺ and Zn²⁺ on the binding of ANS to calregulin were evaluated by performing titrations and analysing spectra

Fig. 1. Polyacrylamide-gel electrophoresis of purified cal-regulins

Panel (a) shows SDS/polyacrylamide-gel electrophoresis of the purified bovine (B), rabbit (C) and chicken calregulin (D); panel (b) shows the non-denaturing polyacrylamide-gel electrophoresis of bovine (B'), chicken (C') and rabbit calregulin (D'). Positions of M_r markers are indicated on the left.

in the presence and absence of metal ion. All titration data were corrected for the fluorescence of the blank and for dilution.

Gel electrophoresis

Electrophoresis in gels containing SDS was performed in a slab-gel apparatus, by using the system described by Laemmli (1970). For analysis of peptides generated by proteolysis, 15% (w/v) acrylamide gels [75:2 (w/w) acrylamide/bisacrylamide] were routinely used. Non-denaturing gel electrophoresis was performed by the Laemmli (1970) method, except that SDS was omitted from the system and unboiled samples were used for analysis. Gels were stained in a solution containing (final concentrations) 0.1% Coomassie Blue, 50% (v/v) methanol and 10% (v/v) acetic acid, and destained by diffusion in 5% methanol/10% acetic acid.

Determination of total carbohydrate

Protein solutions were filtered through a 0.45 μ m-pore-size Millipore filter. Total carbohydrate content of bovine, chicken and rabbit calregulins was measured by the method of Dubois *et al.* (1956). In brief, 0.5 ml of each protein sample (1 mg/ml) was mixed with 0.5 ml of 0.5% phenol. After thorough mixing, the colour was developed by the rapid addition of 2.5 ml of conc. H₂SO₄. The samples were incubated at 50 $^{\circ}$ C for 15 min and, after cooling, their A_{495} was measured. Glucose (100 μ g/ml) was used as a standard.

Fluorescein isothiocyanate-linked lectins (concanavalin A, wheat-germ, soya-bean, lentil, asparagus, pea, ricin I and gorse seed) were used as probes to evaluate the glycosylated nature of the purified calregulins. The purified calregulins dotted on a nitrocellulose sheet were incubated at room temperature for 1 h with 50 mM-Hepes (pH 7.6) containing 3% (w/v) bovine serum albumin, 0.15 M-NaCl and 0.1% Triton X-100. The sheets were

Fig. 2. Ca^{2+} binding by calregulins

The experiment was carried out by equilibrium dialysis as described in the Experimental section. The medium used contained 150 mM-KCl, 10 mM-Mops (pH 7.1), 3 mM- MgCl_2 , 1 mM-dithiothreitol and 0.1 mM-EGTA. Scatchard plots of data: V , mol of Ca^{2+} bound/mol of calregulin; C , free $[\text{Ca}^{2+}]$ (μM). (■) Bovine, (▲) chicken and (●) rabbit calregulin.

washed with the above filter without albumin, and were incubated separately with various lectins for 1 h at room temperature. After extensive washing with the same buffer (without albumin), the sheets were evaluated under u.v. light.

Protein determination

Protein concentrations were determined by the method of Bradford (1976). Calregulin concentration was estimated by its absorption coefficient.

RESULTS

Purification of calregulins

Calregulins from bovine, chicken and rabbit livers were purified to electrophoretic homogeneity by the previously published procedure (Waisman *et al.*, 1985). Fig. 1(a) shows SDS/polyacrylamide-gel electrophoresis of the purified calregulins. Bovine and rabbit calregulins (lanes B and C) have M_r 63000, whereas chicken calregulin has M_r 57000 (lane D). Since the extensive use of proteolytic inhibitors has failed to allow the observation of a higher- M_r form of chicken calregulin, it is unlikely that the M_r -57000 protein is a proteolytic fragment of a higher- M_r form of the protein. Fig. 1(b) shows non-denaturing polyacrylamide-gel electrophoresis of bovine, chicken and rabbit calregulins: the three calregulins migrate similarly under these conditions, indicating a similar mass-to-charge ratio of these proteins.

Table 1. Amino acid composition (mol %) of liver calregulins

Amino acid	Bovine	Rabbit	Chicken
Aspartic acid	16.5	15.7	15.7
Threonine	3.7	3.7	3.9
Serine	3.7	3.7	3.9
Glutamic acid	17.7	16.8	17.7
Proline	6.4	6.0	6.7
Glycine	6.8	6.4	6.9
Alanine	4.6	4.8	4.1
Half-cystine	0.5	0.7	1.0
Valine	4.2	4.9	4.5
Methionine	0.9	0.7	1.0
Isoleucine	4.4	4.2	3.9
Leucine	4.4	4.8	4.1
Tyrosine	3.5	3.3	3.0
Phenylalanine	4.6	4.4	4.9
Histidine	2.0	1.6	1.8
Lysine	9.5	10.1	10.0
Arginine	2.4	3.1	3.9
Tryptophan	4.4	5.1	3.3
No. of residues	547	547	492

Ca^{2+} -binding properties of calregulin

Fig. 2 presents Scatchard (1949) plots for Ca^{2+} binding to bovine, chicken and rabbit calregulins. Analysis of the data reveals that, in the presence of 3.0 mM- MgCl_2 and 150 mM-KCl, all the calregulins bind 1.0 mol of Ca^{2+} /mol of protein, with apparent K_d values of 0.05 μM (bovine) and 0.03 μM (chicken and rabbit).

Ca^{2+} -binding studies were also performed in the absence of MgCl_2 (results not shown), but no significant differences were observed between the values for binding of Ca^{2+} to any calregulin in the presence or absence of MgCl_2 . These results indicate that the Ca^{2+} -binding properties of calregulin are phylogenetically conserved in these three species.

Amino acid composition and sequence of calregulins

The amino acid compositions of bovine, chicken and rabbit calregulins are shown in Table 1; they are very similar. All the proteins contain very similar amounts of acidic, basic and aromatic residues. However, the mol % of tryptophan appears to differ significantly in bovine (4.4%), chicken (3.3%) and rabbit (5.1%) calregulins. The high contents of tryptophan account for the unusually high A_{280} . The absorption coefficients ($\epsilon_{280}^{1\%}$) of bovine, chicken and rabbit calregulins are 17.8, 17.8 and 18.8 respectively. The pI values of bovine (4.15), rabbit (4.15) and chicken (4.20) calregulins indicate their acidic nature.

The *N*-terminal amino acid sequence of the first 15 amino acids of chicken and rabbit calregulins is shown in Fig. 3. The bovine calregulin could not be sequenced, owing to its blocked *N*-terminus. The amino acids at positions 2, 6–8 and 11–15 are identical in chicken and rabbit calregulins. Moreover, positions 5 and 9 show a conserved substitution of amino acids. Such a close homology in the amino acid sequence of chicken and rabbit calregulins is consistent with its highly conserved phylogenetic nature.

Fig. 3. N-Terminal amino acid sequences of chicken and rabbit calregulins

Boxes with continuous lines indicate homology, and those with broken lines indicate conservative substitutions.

Carbohydrate analysis of calregulins

Analysis of the total carbohydrate content of bovine, rabbit and chicken calregulins was estimated by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956). Bovine calregulin contained 16 μg of carbohydrate/mg of protein. In contrast, rabbit and chicken calregulin did not contain any detectable sugar residues. Lectins were also used as a probe to identify the sugar residues of bovine calregulin (see the Experimental section). Of seven lectins tested, only concanavalin A bound to bovine calregulin. Rabbit and chicken calregulins failed to interact with any of the lectins tested. We have shown that about 90% of bovine calregulin is glycosylated and binds to a concanavalin A-agarose affinity column (Khanna *et al.*, 1986). However, a small population of bovine calregulin (0.5–1.0%) is unglycosylated, and can be resolved from the glycosylated calregulin by concanavalin A-agarose affinity chromatography. Concanavalin-A-

binding proteins are reported to be asparagine-linked proteins rich in α -D-mannopyranosyl, α -D-glycopyranosyl and sterically related residues.

Peptide mapping

Similar patterns were obtained when proteolytic digests of bovine, chicken and rabbit calregulins were analysed by SDS/15%-polyacrylamide-gel electrophoresis. Fig. 4 shows the V8-proteinase-cleaved pattern of bovine (lanes B and C), chicken (lane D) and rabbit (lane E) calregulins. Lanes B and C compare with the peptide pattern of the glycosylated population of bovine calregulin (lane B) and that of the unglycosylated population (lane C). The peptide pattern of unglycosylated bovine calregulin (lane C) is very similar to that of chicken (lane D) and rabbit calregulin (lane E). In all three species there is one major band of M_r about 18000, which seems to be more resistant to V8 protease. This band also seems to be preserved in the glycosylated population of bovine calregulin, but it has a lower mobility, possibly owing to the presence of sugar residues.

Fig. 4. Peptide maps of calregulins

Bovine, chicken and rabbit calregulins, each at a concentration of 0.5 mg/ml in sample buffer (see the Experimental section), were incubated at 100 °C for 2 min. After cooling, proteolytic digestions were carried out at 37 °C for 30 min by addition of V8 protease (10 μg /ml). About 20–30 μl of each sample was subjected to SDS/15%-polyacrylamide-gel electrophoresis. Lanes: A, M_r ($\times 10^{-3}$) markers; B, glycosylated bovine calregulin; C, non-glycosylated bovine calregulin; D, chicken calregulin; E, rabbit calregulin.

Increased intrinsic fluorescence of calregulins on binding Zn^{2+}

Fig. 5 shows the intrinsic and Zn^{2+} -induced fluorescence-emission spectra of bovine, chicken and rabbit calregulins. When excited at 286 nm (where predominantly tryptophan absorbs), the tyrosine contribution to the emission spectra (emission maximum 303 nm) was negligible, and the emission maximum at 334 nm was typical of tryptophan. Addition of Zn^{2+} (0.5 mM) causes a dose-dependent increase of about 2-fold in intrinsic fluorescence intensity of all three calregulins, with a red-shift of about 11 nm in λ_{max} of bovine and chicken calregulin and 10 nm for rabbit calregulin. With similar protein concentrations, chicken and rabbit calregulins (0.1 mg/ml) exhibit 3-fold higher intrinsic tryptophan emission fluorescence as compared to bovine calregulin (Figs. 5a, 5b and 5c). Addition of 1 mM-EDTA to the protein solution containing 0.5 mM- Zn^{2+} reversed the Zn^{2+} -induced increase in fluorescence intensity and the wavelength shift of the emission spectrum of all three calregulins. Titration curves of the Zn^{2+} -induced shift in intrinsic λ_{max} of bovine, chicken and rabbit calregulins are shown in Fig. 5(d). Approx. 8–20 μM - Zn^{2+} was required to produce 50% of the maximal effect of the Zn^{2+} -induced shift in λ_{max} of bovine, chicken and rabbit calregulins. The shift in λ_{max} of intrinsic protein fluorescence of all three calregulins was complete at about 100 μM - Zn^{2+} .

Zn^{2+} -induced enhancement of ANS fluorescence

ANS is an anionic amphiphile used as a probe for hydrophobic regions of proteins (Fairclough & Canter, 1978). Fig. 6 shows the Zn^{2+} -induced enhancement of ANS fluorescence by chicken (a), rabbit (b) and bovine

Fig. 5. Increased intrinsic fluorescence of calregulins on binding Zn^{2+}

Emission spectra are shown of calregulins (a, chicken; b, rabbit; c, bovine calregulin) in 10 mM-Mops (pH 7.1)/3 mM- $MgCl_2$ /150 mM-KCl at excitation wavelength of 286 nm. The protein solutions contained no Zn^{2+} (a), 0.5 mM- Zn^{2+} (b) or 0.5 mM- Zn^{2+} + 1 mM-EDTA (c). Panel (d) shows the titration curve of the Zn^{2+} -induced shift in intrinsic λ_{max} of bovine (■), chicken (▲) and rabbit (●) calregulins.

(c) calregulins. Chicken calregulin blue-shifted the λ_{max} of ANS from 520 nm to 485 nm (Fig. 6a). Addition of Zn^{2+} sequentially blue-shifted the λ_{max} of the ANS-protein complex from 485 to 465 nm and produced about a 3-4-fold enhancement of ANS fluorescence intensity. Rabbit calregulin produced a shift in ANS emission maximum from 520 to 490 nm (Fig. 6b). Again, the sequential addition of Zn^{2+} blue-shifted the λ_{max} of ANS-protein complex from 490 to 470 nm, with 3-4-fold enhancement of ANS fluorescence intensity (Fig. 6b). Bovine calregulin blue-shifted the ANS emission spectra from 520 to 500 nm (Fig. 6c). Sequential addition of 0.5 mM- Zn^{2+} produced a shift in λ_{max} of ANS-protein complex from 500 to about 480 nm, with a 2-3-fold enhancement of fluorescence intensity. The Zn^{2+} -induced effect on the ANS emission spectrum was reversible by EDTA. Fig. 6(d) shows the titration curve of Zn^{2+} -induced shift in λ_{max} of

ANS-protein complex. About 16-32 μM - Zn^{2+} was required to produce 50% of the maximal effect of the Zn^{2+} -induced shift in λ_{max} of ANS-protein complex. Rabbit calregulin required about 110 μM - Zn^{2+} to produce a similar effect in the Zn^{2+} -induced shift in λ_{max} of ANS-protein complex.

DISCUSSION

Previous studies from our laboratory have documented the presence of a M_r -63000 Ca^{2+} -binding protein in bovine liver (Waisman *et al.*, 1985). This protein, called calregulin (Khanna & Waisman, 1986; Khanna *et al.*, 1986, 1987), was quantified by radioimmunoassay in a variety of bovine tissues (Khanna & Waisman, 1986), and it was shown that calregulin exists in particularly high amounts in pancreas (540 $\mu g/g$ of tissue), liver (375 $\mu g/g$ of tissue) and testis (256 $\mu g/g$ of tissue). The

Fig. 6. Effect of calregulin and Zn^{2+} on the fluorescence emission spectra of ANS

Emission spectra are shown of ANS ($25 \mu M$) in 10 mM-Mops (pH 7.1)/150 mM-KCl/3 mM-MgCl₂ at excitation wavelength of 360 nm, with calregulin: (a) chicken; (b) rabbit; (c) bovine. Panel (d) shows the titration curve of the Zn^{2+} -induced shift in ANS λ_{max} by bovine (■), chicken (▲) and rabbit (●) calregulin. The protein solutions contained no Zn^{2+} (a), 0.5 mM- Zn^{2+} (b), or 0.5 mM- Zn^{2+} + 1 mM-EDTA (c). Trace d shows the emission spectrum of ANS; 10 μM -ANS was used with bovine calregulin.

only bovine tissue shown not to contain calregulin was the erythrocyte. Considering the presence of large amounts of calregulin in bovine tissues and the affinity and specificity of Ca^{2+} binding ($K_d = 0.05 \mu M$), we suggested that the calregulin might be an important intracellular receptor for Ca^{2+} (Waisman *et al.*, 1985; Khanna *et al.*, 1986).

In a previous report (Khanna *et al.*, 1986), we have examined the effects of metal binding on the conformation of bovine calregulin. It was shown that bovine calregulin binds both Ca^{2+} and Zn^{2+} at distinct and specific sites. Ca^{2+} binding to calregulin resulted in the movement of tryptophan away from the solvent, whereas Zn^{2+} binding caused a movement of tryptophan into the solvent and exposure of a hydrophobic domain. Although the cytosolic free concentration of Zn^{2+} is

unknown, the total cytosolic concentration of Zn^{2+} has been estimated at about 200 μM (Thiers & Vallee, 1957), and the cytosolic free Ca^{2+} concentration is known to be about 50 nM (Snowdowne & Borle, 1984). It is therefore reasonable to propose that under physiological conditions calregulin binds both Ca^{2+} and Zn^{2+} .

In order to investigate whether or not calregulin was restricted to bovine tissues, we attempted the purification of calregulin from rabbit and chicken liver. Initially we were unsuccessful in this by our rapid purification procedure (Khanna *et al.*, 1987). However, using our older purification procedure (Waisman *et al.*, 1985) we purified calregulin from rabbit and chicken liver. Our rapid purification procedure for calregulin (Khanna *et al.*, 1987) failed because rabbit and chicken calregulins, unlike bovine calregulin, are unglycosylated proteins and

therefore do not bind to the concanavalin A-agarose column.

In the present paper we have compared the amino acid composition and peptide maps of bovine, rabbit and chicken calregulin and shown that these proteins are very similar. Chicken calregulin (M_r 57000) is significantly smaller than bovine and rabbit calregulin (M_r 63000). The major difference between the three calregulins is the presence of sugar residues on bovine calregulin and the absence of these sugar residues from rabbit and chicken calregulin. It was therefore important to establish whether or not the glycosylation of bovine calregulin affected its metal-binding properties.

A comparison of the Ca^{2+} - and Zn^{2+} -binding properties of the calregulins suggests that the selective glycosylation of bovine calregulin does not affect the response of the protein to these metal ions. All three calregulins bind Ca^{2+} with about the same affinity, and all three calregulins show a Zn^{2+} -dependent conformational change. The latter involves an increased hydrophobicity of the protein, as illustrated by the Zn^{2+} -dependent increase in ANS fluorescence and red-shift in the ANS fluorescence-emission maximum. Zn^{2+} binding also results in a large increase in intrinsic protein fluorescence and a 11 nm red-shift in emission maximum. Although the intrinsic protein fluorescence of bovine calregulin appears to be lower than that of chicken and rabbit calregulin, the magnitude of the Zn^{2+} -induced change in λ_{max} appears identical.

These studies indicate that the physical, chemical and metal-ion-binding properties of bovine, rabbit and chicken calregulin are conserved, and that the selective glycosylation of bovine calregulin does not result in dramatic changes in the metal-binding properties of the protein. Although speculative, it is reasonable to propose that the Zn^{2+} -dependent increase in the

hydrophobicity of the three calregulins represents a general mechanism by which vertebrate calregulin may interact with other proteins in a similar manner to the Ca^{2+} -dependent increase in the hydrophobicity of calmodulin.

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