

Conformational changes induced by binding of bivalent cations to oncomodulin, a parvalbumin-like tumour protein

John P. MACMANUS, Arthur G. SZABO and Ross E. WILLIAMS

Division of Biological Sciences, National Research Council, Montreal Road, Ottawa, Ont. K1A 0R6, Canada

(Received 25 November 1983/Accepted 10 February 1984)

When Mg^{2+} was added to rat oncomodulin, a parvalbumin-like tumour protein, changes in the c.d. spectrum and tyrosine fluorescence intensity were observed. The addition of Ca^{2+} resulted in even greater changes in these spectra. The fluorescence excitation spectra of apo- and Mg-oncomodulin were superimposable, whereas that of Ca-oncomodulin was markedly different. The u.v.-absorption spectrum of the Ca^{2+} form also showed major differences from those of the other two forms. These observations indicate that Ca^{2+} induced a significant and specific conformational change in the protein that was not observed on binding Mg^{2+} . In contrast, the conformational change induced by either Mg^{2+} or Ca^{2+} was identical in the homologous rat parvalbumin. This Ca^{2+} -specific conformational change may be the basis for oncomodulin's Ca^{2+} -dependent protein/protein interaction.

The superfamily of bivalent-cation-binding proteins includes calmodulin, troponin C and the parvalbumins (Barker *et al.*, 1979; Kretsinger, 1980). Oncomodulin is a protein originally isolated from rat hepatomas (MacManus, 1979, 1980; Criss & Kakiuchi, 1982). It is also present in other rodent and human tumours (MacManus *et al.*, 1982; MacManus & Whitfield, 1983). The amino acid sequence of rat oncomodulin showed it to be homologous to the parvalbumins, but a comparison with rat muscle parvalbumin indicated the tumour protein to be more like a β -parvalbumin than a muscle α -parvalbumin (MacManus *et al.*, 1983*a,b*). Besides the primary structural similarities to the Ca^{2+} -binding superfamily, oncomodulin also exhibited calmodulin-like properties by its ability to stimulate some calmodulin-dependent enzymes (MacManus, 1981; MacManus & Whitfield, 1983). In addition, oncomodulin also mimicked calmodulin in stimulating DNA synthesis in Ca^{2+} -deprived cells (Boynton *et al.*, 1982). None of these properties was shared by rat parvalbumin.

The root of the manner in which calmodulin functions as a modulator is the change in conformation it undergoes when Ca^{2+} binds (Klee & Vanaman, 1982). This conformational change brings a hydrophobic area to the surface, which allows protein-protein interaction (LaPorte *et al.*, 1980; Tanaka & Hidaka, 1980; Klee & Vanaman, 1982). Although parvalbumins also undergo conformational change, the change is neither Ca^{2+} -

specific nor adequate for the parvalbumins to function as modulators (Demaille, 1982; Wnuk *et al.*, 1982).

The primary structures of calmodulin, troponin C and parvalbumin contain no information to explain their differential modulator action. The explanation has been presumed to lie in the tertiary structures of these proteins (Demaille, 1982; Klee & Vanaman, 1982). Likewise there is nothing in the primary structure of rat oncomodulin or of rat parvalbumin to explain the differential ability of the tumour protein to mimic some calmodulin actions (MacManus *et al.*, 1983*a,b*). Therefore a study of the conformational changes in rat oncomodulin induced by the binding of metal ions was undertaken, and these were compared with similar changes in rat parvalbumin.

Materials and methods

Rat oncomodulin was purified from Morris hepatoma 5123tc (MacManus, 1980), and rat parvalbumin from skeletal muscle, by using as a basis the procedure of Haiech *et al.* (1979). Both proteins were shown to be homogeneous by amino acid analysis (MacManus *et al.* 1983*a,b*).

The proteins were stripped of metals by precipitation with trichloroacetic acid (Haiech *et al.*, 1981). The apo-oncomodulin had a residual metal content of 0.18 mol of Ca/mol of protein, and no detectable Mg, and the apo-parvalbumin had

0.15 mol of Ca and 0.11 mol of Mg/mol of protein, when measured by atomic absorption spectroscopy on a Pye–Unicam SP.191 instrument. The oncomodulin precipitated with acid was fully renaturable to the native state, since there was no difference in the various spectra of the renatured or untreated proteins.

All buffers were passed through a column of Chelex 100 cation-exchange resin (Bio-Rad Laboratories, Mississauga, Ont., Canada) before use. This treatment decreased the contamination to $3\ \mu\text{M}\text{-Ca}^{2+}$ and $1\ \mu\text{M}\text{-Mg}^{2+}$.

The stock metal salt solutions were made from ultrapure salts (Alfa Division, Ventron Corp., Danvers, MA, U.S.A.). The stock magnesium salt was 0.5M-magnesium acetate in the appropriate buffer and had a $12\ \mu\text{M}\text{-Ca}^{2+}$ contamination. The stock calcium salt solution was 0.5M- CaCO_3 (solubilized with HCl; Aristar BDH Chemicals, Toronto, Ont., Canada) in the appropriate buffer, and had a $40\ \mu\text{M}\text{-Mg}^{2+}$ contamination. When diluted during use, these cross-contaminations were below the 1–3 μM background metal-ion contaminations in the buffers used. Samples were always handled in plasticware.

U.v.-absorption spectra were measured on a Cary 219 spectrophotometer. U.v. difference spectra were obtained by adding equal volumes of a solution of $400\ \mu\text{M}\text{-apo-oncomodulin}$ in 150mM-KCl/0.25mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.2, to cuvettes in the reference and sample beams. After the baseline was balanced, small portions of solutions of the metal ions were added to the sample cuvette and equal volumes of buffer were added to the reference cuvette, and the spectra were recorded.

The c.d. spectra were taken on a calibrated (Gillen & Williams, 1975) Cary–Varian model 61 spectropolarimeter with quartz cells (0.05cm or 1.0cm pathlength) thermostatically maintained at $30 \pm 0.5^\circ\text{C}$. Solutions containing $350\ \mu\text{M}\text{-apo-oncomodulin}$ or $330\ \mu\text{M}\text{-apo-parvalbumin}$ (250–300nm region), or $32\ \mu\text{M}\text{-apo-oncomodulin}$ or $33\ \mu\text{M}\text{-apo-parvalbumin}$ (190–250nm region), were prepared in 10mM-Tris/HCl buffer, pH 8.0. Metal-ion titration experiments were performed by addition of $1\ \mu\text{l}$ portions, to a total of $40\ \mu\text{l}$, of stock metal salt solutions to 1ml samples of apo-protein. The estimates of secondary structure were obtained by a method based on the application of a constrained statistical regularization procedure (Provencher & Glockner, 1981; Provencher, 1982a,b), contained in a CONTIN program obtained from Dr. S. W. Provencher (E.M.B.O., Heidelberg, W. Germany).

The fluorescence spectra were obtained with a Perkin–Elmer MPF 44A spectrofluorimeter equipped with a DCSU 2 corrected spectra unit.

The fluorimeter was interfaced with an analog-to-digital converter and a Commodore PET computer. The spectra were corrected for contributions of the blank, and integrated for quantum-yield measurements, or normalized for comparison and plotting. The fluorescence ($\lambda_{\text{exc.}} = 280\text{nm}$) of a solution of $40\ \mu\text{M}\text{-apo-oncomodulin}$ dissolved in 150mM-KCl/10mM-sodium cacodylate buffer, pH 7.0, was measured in 1cm quartz cuvettes at 20°C (absorbance readings at 280nm approx. 0.1). The excitation and emission bandpasses were 2nm and 3nm respectively.

Results

U.v. spectroscopy

The difference absorption spectra between apo-oncomodulin and Ca- or Mg-oncomodulin are shown in Fig. 1. They clearly show that Ca^{2+} induced significant changes in the oncomodulin spectrum. On the other hand there was very little change when Mg^{2+} was added to the oncomodulin sample. This contrast between the effects of Ca^{2+} and Mg^{2+} on the u.v.-absorption spectrum of oncomodulin was further investigated by c.d. spectroscopy, as it suggested that these metal ions induced different conformational changes in the protein.

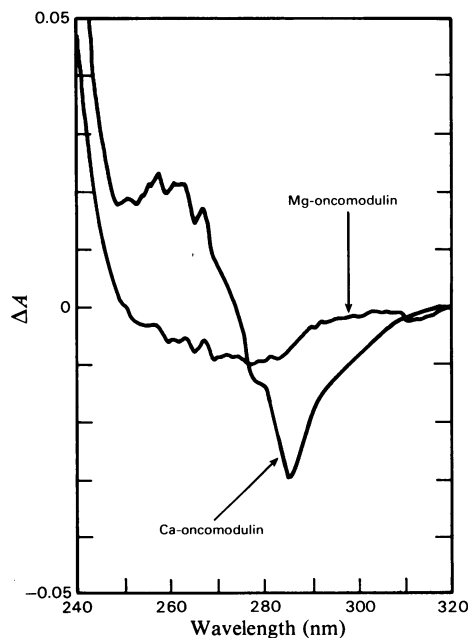


Fig. 1. U.v. difference spectra of Mg-oncomodulin and Ca-oncomodulin when compared with apo-oncomodulin. Oncomodulin was $300\ \mu\text{M}$, and Ca^{2+} or Mg^{2+} were added to a final concentration of 5mM.

C.d. spectroscopy in the 250–300 nm region

Since oncomodulin contains no tryptophan and only one cysteine residue per molecule, the c.d. spectrum in the 250–300 nm region may be assigned to phenylalanine and tyrosine. A multi-band set assignable to the oncomodulin phenylalanine chromophores was present, and was composed of bands centred at 268–262 nm, with a shoulder at 253 nm (Fig. 2*b*). A broad band due to tyrosine was centred at 280 nm. This oncomodulin spectrum was very similar to the c.d. spectrum of calmodulin (Klee & Vanaman, 1982). Mg^{2+} addition caused intensity changes in the bands attributed to phenylalanine, but no change in the intensity of the tyrosine band was recorded. Ca^{2+} addition intensified the spectrum in the phenylalanine region. In contrast with Mg-oncomodulin, the tyrosine region of Ca-oncomodulin exhibited a major loss of c.d. intensity, suggesting either the involvement of tyrosine in forming a complex with Ca^{2+} or a strong perturbation of the tyrosine residue on Ca^{2+} binding.

The binding of Mg^{2+} or Ca^{2+} to rat parvalbumin also caused an increase in intensity, but only in the

phenylalanine region (Fig. 3*b*), since this parvalbumin is devoid of tyrosine (Berchtold *et al.*, 1982; MacManus *et al.*, 1983*b*). With this parvalbumin, both Mg^{2+} and Ca^{2+} produced the same intensity change in apo-parvalbumin, with no Ca^{2+} -specific alteration visible as with oncomodulin.

C.d. spectroscopy in the 190–250 nm region

Oncomodulin had a two-band c.d. spectrum indicative of helical content (Fig. 2*a*). Addition of Mg^{2+} caused an increase in the band intensities, with a further increase caused by Ca^{2+} . The same spectrum was obtained whether the Ca^{2+} was added to apo-oncomodulin or to Mg-oncomodulin.

In contrast, both the Mg^{2+} - and Ca^{2+} -saturated forms of parvalbumin had equal increases in intensity. Mg^{2+} caused only a partial conversion of the apo-oncomodulin into its Ca^{2+} -saturated form (Fig. 2*a*), whereas it caused full conversion in the case of parvalbumin (Fig. 3*a*). Mg-oncomodulin also had an altered ratio of the band intensities at 208 and 222 nm ($[\theta]_{208}/[\theta]_{222} = 1.31$ versus 1.00 for the apo- or Ca-oncomodulin). Parvalbumin had intensity ratios of 1.33 for apo-protein, but only

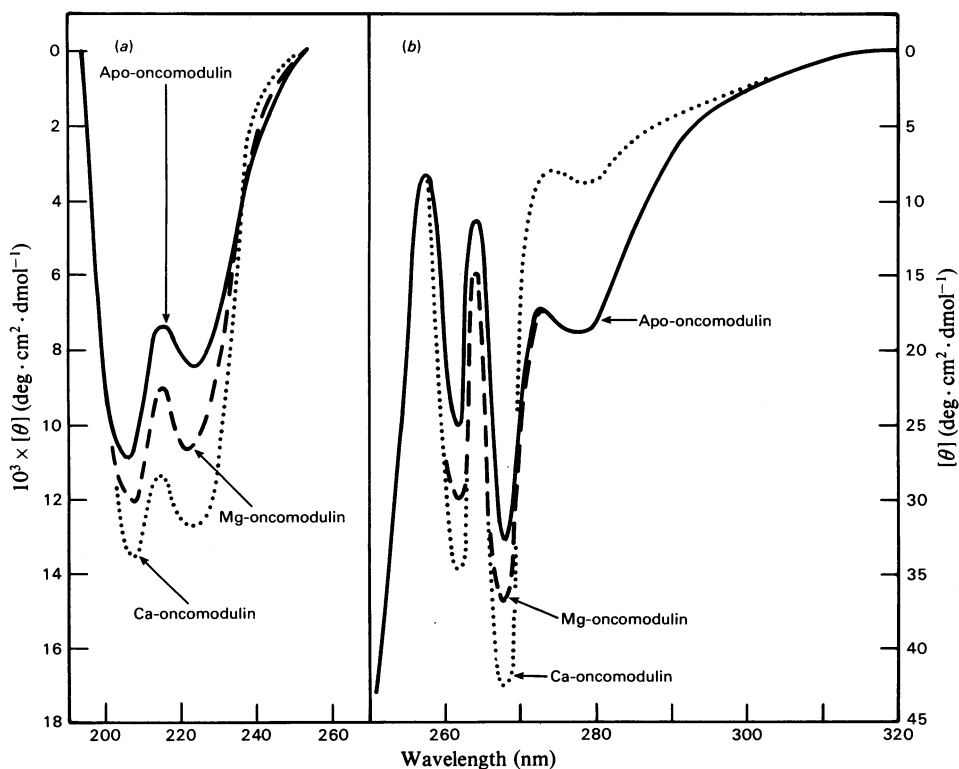


Fig. 2. *C.d. spectra of oncomodulin derivatives*

Oncomodulin was $32 \mu M$ in (a), and $350 \mu M$ in (b); metal ions were added to a final concentration of 5 mM.

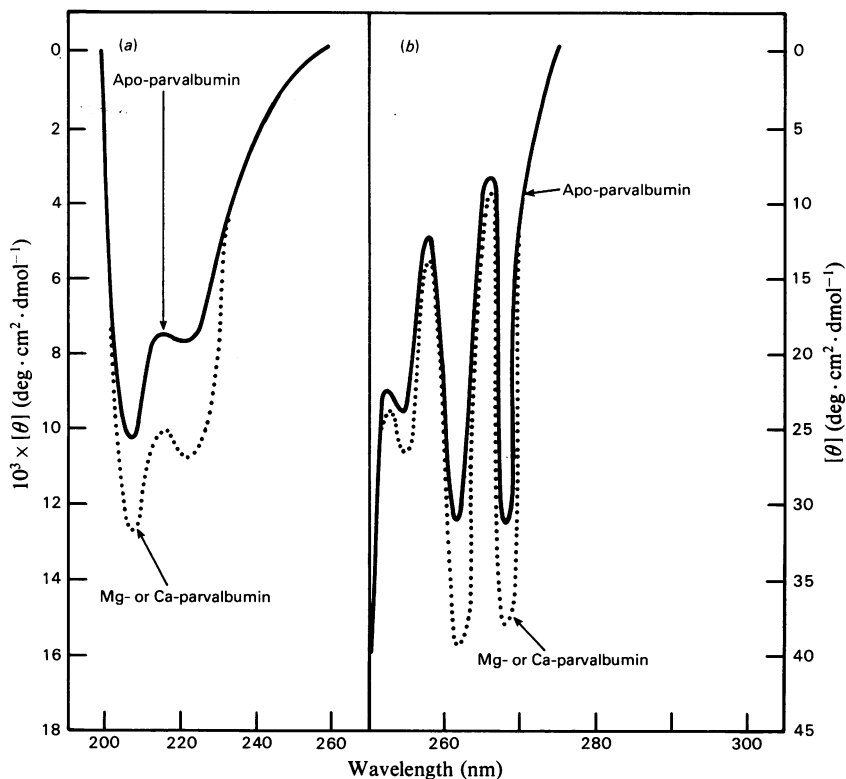


Fig. 3. *C.d. spectra of parvalbumin derivatives*
Parvalbumin was $32\ \mu\text{M}$ in (a) and $330\ \mu\text{M}$ in (b); metal ions were added as in Fig. 2.

1.18 for the Mg- and Ca-parvalbumin. These results suggest that the binding of Mg^{2+} and Ca^{2+} to the two proteins was different. A suggested cause of this difference emerged from titration studies.

When the intensity of the 222nm band of oncomodulin was titrated against increasing bivalent-cation concentrations, the major fraction of the conformational change appeared to saturate at either approx. $2\text{Ca}^{2+}/\text{molecule}$ or $1\text{Mg}^{2+}/\text{molecule}$ (Fig. 4). Evidence of a second, perhaps non-specific, site was seen with $\text{Mg}^{2+}/\text{oncomodulin}$ ratios greater than 1:1. Full saturation of the effect only occurred at metal-ion/protein ratios above 10:1 (Fig. 4). The parvalbumin titration, on the other hand, was monophasic for both metal ions, and gave the appearance of being fully saturated at metal ion/protein ratios of approx. 2:1 (results not shown).

Approximations of secondary structure of these metal-ion-binding proteins were made by using the c.d. spectra in the 190–205nm region and the CONTIN program (Provencher, 1982a,b). Given the qualitative nature of such estimates, the results suggested that the two apoproteins have approxi-

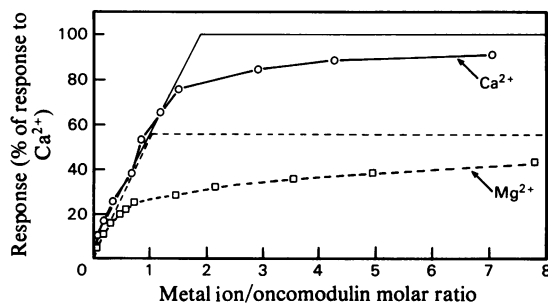


Fig. 4. *Titration of the c.d. intensity change of oncomodulin at 220nm with increasing metal-ion concentration*
The idealized lines were drawn assuming a linear response to metal ion and full saturation of the effect at $2\text{Ca}^{2+}/\text{molecule}$ and $1\text{Mg}^{2+}/\text{molecule}$.

mately the same amount of α -helix, an amount that is in agreement with the parvalbumin helical content estimated by other methods (Cox *et al.*, 1979; Berchtold *et al.*, 1982). The two rat proteins appeared to have different amounts of β -turn, and random structure (Table 1). Addition of metal ions

Table 1. Comparison of estimates of secondary structure of oncomodulin and parvalbumin from c.d.-spectral data. Estimates of secondary structure (based on 40 datapoints) were made by the CONTIN program, version 1 (Provencher, 1982a,b). Probabilities above 0.1 are considered significant.

Derivative	Secondary structure (%)			Random	Probability
	α -Helix	β -Sheet	β -Turn		
Apo-oncomodulin	24	20	20	36	0.305
Mg-oncomodulin	30	13	19	38	0.645
Ca-oncomodulin	35	11	9	46	0.593
Apo-parvalbumin	29	44	9	18	0.779
Mg-/Ca-parvalbumin*	39	30	8	23	0.706

* Spectra were identical (Fig. 3).

caused increases in helical content, and concomitant fall in estimates of β -sheet content. The estimate of β -turn content in oncomodulin decreased after Ca^{2+} addition, whereas no change occurred in parvalbumin.

Tyrosine fluorescence spectra

The addition of an excess of Mg^{2+} to apo-oncomodulin resulted in a 30% increase in the intensity of the fluorescence maximum at 301 nm. The addition of an excess of Ca^{2+} to either apo-oncomodulin or Mg-oncomodulin gave identical spectra, the maximum signal increasing by 40% over the apo form or 8% over the Mg^{2+} form (Table 2).

Comparison of the fluorescence of apo-oncomodulin with that of tyrosine (both spectra normalized at their maxima) indicated an additional contribution to the fluorescence of the apo-protein at longer wavelengths (Fig. 5). Making the assumption that the fluorescence at 301 nm was solely attributable to tyrosine, the two spectra were normalized at 301 nm, and a difference spectrum was produced (Fig. 5 inset) by subtracting the tyrosine spectrum from the apo-oncomodulin spectrum. The maximum of this difference spectrum, centred at 345 nm, was indicative of a tyrosinate contribution to the fluorescence. Similar spectra were obtained for Mg- or Ca-oncomodulin (Fig. 5 inset) but showed a decrease in the tyrosinate content.

The quantum yields of the oncomodulin derivatives were measured by integration of the spectra and using tyrosine as a standard [quantum yield = 0.14 (Chen, 1967)] (Table 2). Although the increases in quantum yields of Mg- or Ca-oncomodulin were parallel to the increase in fluorescence maximum intensity, the fractional increases of the quantum yields of either Mg- or Ca-oncomodulin were not as great. The reason for this discrepancy was because the fluorescence spectra are composites of tyrosine and tyrosinate

Table 2. Quantum yields and fluorescence intensity at 301 nm of oncomodulin derivatives

Derivative	Quantum yield	Intensity
Apo-oncomodulin	0.041 ± 0.001	66.4 ± 0.5
Mg-oncomodulin	0.046 ± 0.001	84.7 ± 0.5
Ca-oncomodulin	0.051 ± 0.001	90.0 ± 0.5

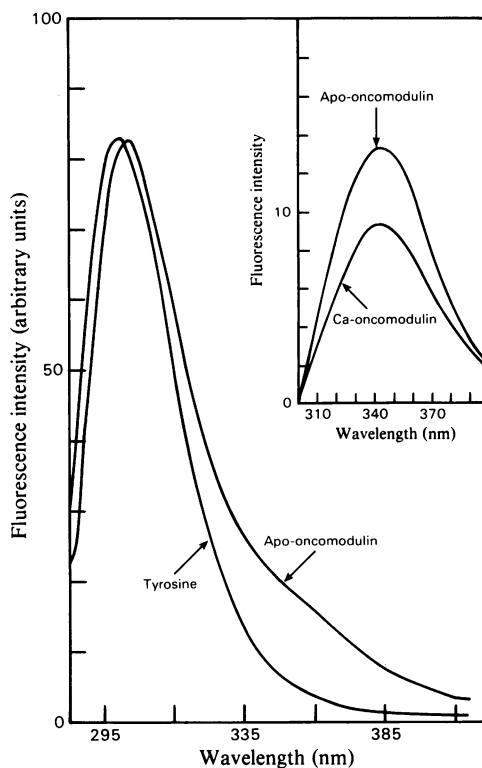


Fig. 5. Comparison of the fluorescence spectrum of apo-oncomodulin with that of tyrosine

Inset: fluorescence difference spectrum of apo-oncomodulin and Ca-oncomodulin when compared with tyrosine, with spectra normalized at 301 nm.

fluorescence, so that while the tyrosine fluorescence was increasing on metal ion bonding (Table 2) the tyrosinate fluorescence was decreased (Fig. 5 inset).

The corrected fluorescence excitation spectra of the apo- and metal-ion-bound oncomodulin were most revealing. These spectra were determined by using an emission wavelength of 310 nm (Fig. 6). When the excitation spectra were normalized to have the same intensity at their identical maxima (276 nm), the excitation spectra of the apo- and Mg-oncomodulin were superimposable (Fig. 6). However, the spectrum of Ca-oncomodulin was significantly different from those of both apo- and Mg-oncomodulin. This spectrum of Ca-oncomodu-

lin had an increased intensity on the high-energy side of the maximum (272 nm), and a different shape. This was indicative of an increase in the energy-transfer efficiency of phenylalanine to tyrosine in the Ca^{2+} form.

Discussion

The troponin C superfamily of bivalent-cation-binding proteins includes the four- Ca^{2+} -site calmodulin, the two- Ca^{2+} -site + two- $\text{Ca}^{2+}/\text{Mg}^{2+}$ -site troponin C and the two- $\text{Ca}^{2+}/\text{Mg}^{2+}$ -site parvalbumins (Barker *et al.*, 1979; Kretsinger, 1980; Klee & Vanaman, 1982; Demaille, 1982; Potter & Johnson, 1982; Wnuk *et al.*, 1982). The same conformational change was caused by binding Ca^{2+} or Mg^{2+} in parvalbumins, when monitored by either fluorescence or c.d. spectroscopy (Cox *et al.*, 1979; Moeschler *et al.*, 1980). This was also found with rat parvalbumin (Figs. 3a and 3b), although some subtle differences have been described in other parvalbumins during investigations with proton n.m.r. or microcalorimetry (Birdsall *et al.*, 1979; Moeschler *et al.*, 1980). On the other hand, in oncomodulin Ca^{2+} produces an easily discernible specific change over and above that induced by Mg^{2+} . This was observed with u.v.-absorption spectroscopy (Fig. 1), with c.d. spectroscopy (Figs. 2a and 2b) or with either emission or excitation fluorescence spectroscopy (Table 2 and Fig. 6). This Ca^{2+} -specific conformational change was observed by Ca^{2+} addition to either apo-oncomodulin or Mg-oncomodulin.

The structural estimates from the c.d. data show that Ca^{2+} induces in oncomodulin an increase in α -helical content over and above that induced by Mg^{2+} (Table 1). In addition, Mg-oncomodulin would seem to have less random structure than Ca-oncomodulin, possibly owing to the smaller Mg^{2+} ion collapsing the binding domain further than Ca^{2+} , leading to a tighter structure. This has been previously suggested from n.m.r. studies on carp parvalbumin (Birdsall *et al.*, 1979).

Comparison of the primary structures of the putative CD and EF binding domains of rat oncomodulin and parvalbumin are shown in Fig. 7. The observed fluorescence can be assigned to that of the two tyrosine residues in oncomodulin, Tyr-57 and Tyr-65. Both these tyrosine residues are in the CD loop, Tyr-57 as a binding ligand (carbonyl group) and Tyr-65 in the flanking helix (Kretsinger, 1980). The Tyr-57 is homologous to the Tyr-99 of the vertebrate calmodulins (Klee & Vanaman, 1982). The large (2–3-fold) increase in calmodulin fluorescence on Ca^{2+} binding has been attributed in the main to Tyr-138 (Klee & Vanaman, 1982). This is supported by the relatively small increase (40%) in the fluorescence of oncomodulin,

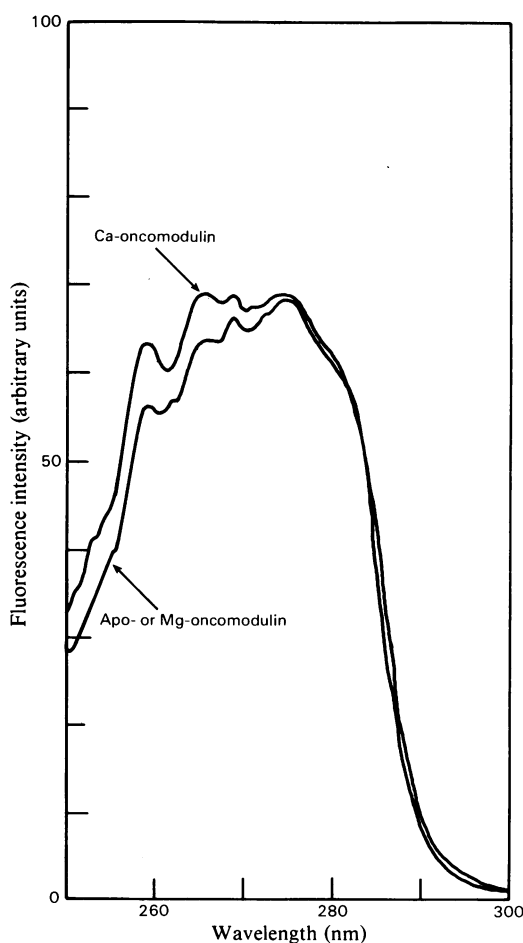


Fig. 6. Fluorescence excitation spectra of oncomodulin derivatives

Oncomodulin was $40 \mu\text{M}$, and metal ions were added as in Fig. 2. ($\lambda_{\text{exc}} = 280 \text{ nm}$, $A_{280} = 0.1$). Spectra were normalized to their identical maxima (276 nm).

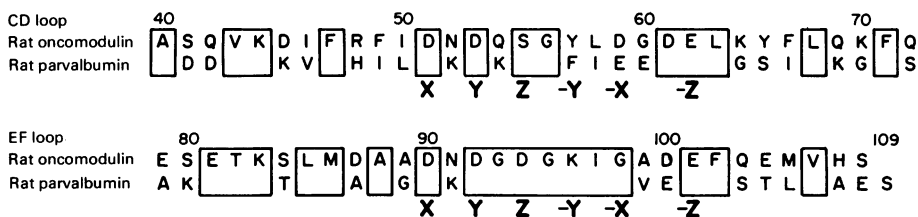


Fig. 7. Comparison of amino acid sequences in the putative metal-ion binding domains (Moews & Kretsinger, 1975) of rat oncomodulin (MacManus *et al.*, 1983a) and rat parvalbumin (Berchtold *et al.*, 1982; MacManus *et al.*, 1983b). The boxes indicate identity in both proteins and the one-letter amino acid code is used (IUPAC-IUB Commission on Biochemical Nomenclature). X, Y, Z, -X, -Y and -Z refer to the metal ion binding ligands (Kretsinger, 1980).

which can be considered to have only the Tyr-99 of calmodulin.

The formation of tyrosinate from the excited state of tyrosine is a fluorescence-quenching mechanism (Rayner *et al.*, 1978). The tyrosinate fluorescence in Fig. 5 can only result from proton removal from an excited tyrosine residue by proximate acid residues such as aspartic acid or glutamic acid. Such residues surround Tyr-57, and are suitable ligands for metal-ion binding (Fig. 7). Therefore the increase in fluorescence intensity on cation binding may occur because these acid residues are no longer available for proton abstraction. However, Tyr-65 may also be involved through interaction with acid residues in other parts of the protein.

The interpretation of the fluorescence excitation spectra involved the Tyr-65 residue. The excitation spectra of apo-oncomodulin and Mg-oncomodulin were superimposable (Fig. 6). The structure in this spectrum observed at wavelengths shorter than the maxima indicated some energy transfer from phenylalanine to tyrosine in these forms. On binding Ca^{2+} there was a change in the high-energy region of the Ca-oncomodulin excitation spectrum compared with the normalized apo-oncomodulin spectrum (Fig. 6). There was an increase in the structure and intensity, which indicated an increase in the energy-transfer efficiency from one or more phenylalanine residues to the tyrosine residues. A model of oncomodulin built on parvalbumin co-ordinates (Moews & Kretsinger, 1975) indicated that Phe-49, or less probably Phe-47, may be well positioned to transfer energy to Tyr-65. On the basis of this model there is no phenylalanine residue with the correct orientation to allow energy transfer to Tyr-57. Thus we concluded that Ca^{2+} induced a specific conformational change that was primarily located in the CD loop.

The differences observed in the excitation spectra of apo- and Ca-oncomodulin assist in the

interpretation of the u.v. difference spectra (Fig. 1). The fluorescence excitation spectra indicated that there must be changes in the orientation and/or the interaction between phenylalanine and tyrosine residues on binding Ca^{2+} . This change in orientation and interaction between residues presumably results in increased electronic interaction between these residues, which is the reason for the observed u.v. difference spectrum on binding Ca^{2+} .

Comparison of the primary structures of the two binding domains of rat oncomodulin and rat parvalbumin gives no hint as to why this Ca^{2+} -specific conformational change occurs (Fig. 7). Indeed, comparison of the sequences of calmodulin, troponin C and parvalbumin do not give any indication of why some sites are Ca^{2+} -specific whereas others bind either Ca^{2+} or Mg^{2+} (Kretsinger, 1980; Klee & Vanaman, 1982; Demaille, 1982; Potter & Johnson, 1982; Wnuk *et al.*, 1982). Changes in the tertiary structure induced by metal-ion binding has been invoked as the source of the specific conformational change, which allows the regulatory protein-protein interaction (LaPorte *et al.*, 1980; Tanaka & Hidaka, 1980; Klee & Vanaman, 1982).

Muscle parvalbumins, which lack large Ca^{2+} -specific conformational changes (Figs. 3a and 3b); Cox *et al.*, 1979; Moeschler *et al.*, 1980) are described as being incapable of protein-protein interaction (Demaille, 1982; Wnuk *et al.*, 1982). Although like a parvalbumin in primary structure (MacManus *et al.*, 1983a,b), oncomodulin has been demonstrated to be capable of such interaction, shown by Ca^{2+} -specific binding of hepatoma proteins to immobilized oncomodulin (MacManus & Whitfield, 1983), by stimulation of some calmodulin-dependent enzymes (MacManus, 1981) or by stimulation of DNA synthesis in Ca^{2+} -deprived cells (Boynton *et al.*, 1982). Thus oncomodulin has calmodulin-like activity and parvalbumin-like primary structure.

The stoichiometry of the metal-ion association suggests that rat parvalbumin binds either 2 Ca^{2+} ions or 2 Mg^{2+} ions per molecule, in agreement with the work of many (Demaille, 1982; Wnuk *et al.*, 1982). On the other hand, oncomodulin binds 2 Ca^{2+} ions or 1 Mg^{2+} ion per molecule (Fig. 4), or, if it binds 2 Mg^{2+} ions per molecule, then the second Mg^{2+} ion does not induce any further major conformational change in the protein. If this latter possibility does in fact occur, it would strengthen the evidence that only Ca^{2+} induces a specific conformational change in oncomodulin. It is to be noted that the evidence obtained from each of the spectroscopic techniques agreed with this suggestion. The binding ligands in the EF loop for both proteins are identical (Fig. 7), and so it is concluded this is a $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding site for oncomodulin, as well as for parvalbumin. The CD loop would therefore be the Ca^{2+} -specific site, a conclusion that is supported by the changes observed in the c.d. spectrum (Fig. 2), the Ca^{2+} -specific tyrosine fluorescence change (Table 2) and the phenylalanine-to-tyrosine energy transfer (Fig. 6). Thus oncomodulin, instead of being analogous to calmodulin, is more akin to troponin C. Indeed, the tumour protein may be more akin again to cardiac troponin C, which has only one Ca^{2+} -specific site in addition to the two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites (Potter & Johnson, 1982). The description of oncomodulin as a 'mini-cardiac-troponin C' would narrow its regulatory ability compared with calmodulin (Klee & Vanaman, 1982; Potter & Johnson, 1982) and would also reconcile the inability of oncomodulin to stimulate all calmodulin-dependent enzymes (MacManus & Whitfield, 1983).

Our thanks are due to Mr. B. Braceland for assistance in purifying proteins, and Mr. D. T. Krajcarski for assistance in obtaining fluorescence spectra. Our thanks are also extended to Dr. L. Bramall, Division of Biomathematics, National Research Council, for running of the CONTIN program, which was kindly provided by Dr. S. W. Provencher, E.M.B.O., Heidelberg, Germany. The assistance of Dr. M. Young, Division of Biological Sciences, National Research Council, in building the oncomodulin model is gratefully acknowledged.

References

- Barker, W. C., Ketcham, L. K. & Dayhoff, M. D. (1979) in *Atlas of Protein Sequence and Structure* (Dayhoff, M. D., ed.), vol. 5, Suppl. 3, pp. 273–283, National Biomedical Research Foundation, Washington
- Berchtold, M. W., Heizmann, C. W. & Wilson, K. J. (1982) *Eur. J. Biochem.* **127**, 381–389
- Birdsall, W. J., Levine, B. A., Williams, R. J. P., Demaille, J. G., Haiech, J. & Pechère, J. F. (1979) *Biochimie* **61**, 741–750
- Boynton, A. L., MacManus, J. P. & Whitfield, J. F. (1982) *Exp. Cell Res.* **138**, 454–458
- Chen, R. F. (1967) *Anal. Lett.* **1**, 35–42
- Cox, J. A., Winge, D. R. & Stein, E. (1979) *Biochimie* **61**, 601–605
- Criss, W. E. & Kakiuchi, S. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2289–2291
- Demaille, J. G. (1982) *Calcium Cell Funct.* **2**, 111–144
- Gillen, M. F. & Williams, R. E. (1975) *Can. J. Chem.* **53**, 2351–2353
- Haiech, J., Derancourt, J., Pechère, J. F. & Demaille, J. G. (1979) *Biochimie* **61**, 583–587
- Haiech, J., Klee, C. B. & Demaille, J. G. (1981) *Biochemistry* **20**, 3890–3897
- Klee, C. B. & Vanaman, T. C. (1982) *Adv. Protein Chem.* **35**, 213–320
- Kretsinger, R. H. (1980) *C.R.C. Crit. Rev. Biochem.* **8**, 119–174
- LaPorte, D. C., Wierman, B. M. & Storm, D. R. (1980) *Biochemistry* **19**, 3814–3819
- MacManus, J. P. (1979) *Cancer Res.* **39**, 3000–3005
- MacManus, J. P. (1980) *Biochim. Biophys. Acta* **621**, 296–304
- MacManus, J. P. (1981) *FEBS Lett.* **126**, 245–249
- MacManus, J. P. & Whitfield, J. F. (1983) *Calcium Cell Funct.* **4**, 411–440
- MacManus, J. P., Whitfield, J. F., Boynton, A. L., Durkin, J. P. & Swierenga, S. H. H. (1982) *Oncodev. Biol. Med.* **3**, 79–90
- MacManus, J. P., Watson, D. C. & Yaguchi, M. (1983a) *Eur. J. Biochem.* **136**, 9–17
- MacManus, J. P., Watson, D. C. & Yaguchi, M. (1983b) *Biosci. Rep.* **3**, 1071–1075
- Moeschler, M. J., Schaer, J. J. & Cox, J. A. (1980) *Eur. J. Biochem.* **111**, 73–78
- Moews, P. C. & Kretsinger, R. H. (1975) *J. Mol. Biol.* **91**, 201–228
- Potter, J. D. & Johnson, J. D. (1982) *Calcium Cell Funct.* **2**, 145–173
- Provencher, S. W. (1982a) *Comput. Phys. Commun.* **27**, 213–227
- Provencher, S. W. (1982b) *Comput. Phys. Commun.* **27**, 229–242
- Provencher, S. W. & Glockner, J. (1981) *Biochemistry* **20**, 33–37
- Rayner, D. M., Krajcarski, D. T. & Szabo, A. G. (1978) *Can. J. Chem.* **56**, 1238–1245
- Tanaka, T. & Hidaka, M. (1980) *J. Biol. Chem.* **255**, 11078–11080
- Wnuk, W., Cox, J. A. & Stein, E. A. (1982) *Calcium Cell Funct.* **2**, 243–278