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CHARACTERIZATION OF CALCIPHORIN BY LASER-EXCITED EUROPIUM LUMINESCENCE

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There is some question whether the calcium binding characteristics of calciphorin are due to contaminating phospholipids. To differentiate protein ion binding from ion binding by phospholipids or contaminating detergent, we describe here the use of Eu(III) as a metal-binding-site probe, and characterize the interaction of Eu(III) with calciphorin, cardiolipin, deoxycholate, and digitonin. The luminescence excitation pattern of Eu(III) bound to the calciphorin preparation clearly differentiates it from Eu(III) interactions with the possible contaminants. In addition, the effect of the luminescence decay constant of Eu(III) bound to calciphorin on the mole fraction of H_2O in a mixture of $H_2O/^2H_2O$ indicates that all except approximately 0.8 of the 9 to 10 water molecules coordinating Eu(III) in solution are stripped off upon binding to calciphorin. This also contrasts with the data for the possible contaminants.

Introduction

It is well-established that mitochondria take up calcium ions in an energy-dependent manner. This process is thought to be electrophoretic, driven by the electrochemical potential across the mitochondrial inner membrane, where the calcium carrier is thought to reside [1–4]. Mn(II) is transported by the same carrier as Ca(II) [4–7]. Using Mn(II) as a Ca(II) analog and assaying bound Mn(II) by electron paramagnetic resonance spectroscopy, we recently isolated a candidate for the mitochondrial inner membrane Ca(II) carrier [7–10], named calciphorin. This 3-kDa protein exhibits high-affinity Ca(II) binding (approx. 10 μM) as assayed by both organic solvent extraction and flow dialysis and it transports Ca(II) in response to a pH gradient across the organic phase of a bulk-phase transport cell ('Pressman cell'). Both functions are strongly inhibited by La(III) and Ruthenium red, which are known inhibitors of mitochondrial Ca(II) transport. We have further shown that calciphorin mediates voltage-stimulated Ca(II) transport when reconstituted into lipid vesicles [11].

Under certain conditions, acidic phospholipids can exhibit high-affinity calcium binding in organic solvent extraction and even in flow dialysis measurements. Cardiolipin, in particular, shows high affinity in these experiments and can even transport Ca(II) in a bulk phase transfer experiment [12]. We emphasize, however, that cardiolipin is not able to mediate Ca(II)-transport in vesicles or in planar bilayer membranes [12]. Since cardiolipin is co-purified in the primary purification of calciphorin, there was some concern that this phospholipid could account for the properties of the protein. We have shown, however, that calciphorin delipidation on a Sephadex LH-20 column retains only 0.3 mole total phosphorus/mole

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protein and still maintains high-affinity binding [13].

In order to further differentiate ion binding by calciphorin from that of contaminants, we have used Eu(III) as a luminescent probe of the Ca(II) site. Using the recently developed techniques of laser-excited Eu(III) luminescence spectroscopy [14,15], we show here that the calciphorin binding of Eu(III) can be clearly differentiated from Eu(III) binding to phospholipids. Calciphorin binds Eu(III) with very high affinity and dehydrates the ion except for approximately 0.8 water molecule. The binding also results in almost complete neutralization of the charge on Eu(III). These observations agree with the hypothesis that calciphorin is a carrier.

Experimental

$EuCl_3$ (99.9%) was obtained from Alfa/Ventron. Piperazine, EDTA, and ^2H_2O were obtained from Sigma Chemical. All other chemicals were reagent grade. $EuCl_3$ stock solutions were calibrated by placing 100 μl of nominally 0.5 M $EuCl_3$ on a small, well-washed column of Aminex 50-X2 (H^+ form) bead (Bio-Rad Labs), followed by about two column volumes of H_2O. The amount of H^+ released into the eluate was determined by titration with NaOH standards, using an Orion pH meter.

Preparation of calciphorin. Mitochondria were isolated from calf or bovine heart, or in some cases from rat liver, and inner mitochondrial membranes prepared, according to Schnaitman and Greenawalt [16]. These were partially solubilized with deoxycholate, centrifuged, and the supernatant fractionated on a Sephadex G-50 column as described previously [9,10]. The peak identified as calciphorin was concentrated and analyzed by gel electrophoresis. This preparation contained approx. 100 moles phosphate/mole protein. Total phosphate was determined as reported by us previously [9,10] and protein by the method of Lowry. A molecular weight of 3000 was assumed [9]. Delipidation was carried out on a Sephadex LH-20 column as described [10]. The material was stored at $-80\,^{\circ}C$ in 1:1 chloroform/methanol. Before use, the solvent was evaporated under a N_2 stream and the protein suspended in aqueous buffer (see Results) by sonication.

Luminescence measurements. The excitation source was a Molectron DL-12 Dye laser pumped by a Molectron UV-12 nitrogen laser. The light was reflected into the sample compartment of a fluorescence lifetime instrument built by Photochemical Research Associates (London, Ontario, Canada). The dye laser was equipped with a grating drive for scanning and had a line width of about 0.01 nm at 580 nm. Light was collected by a Hamamatsu photomultiplier tube used in the photon counting mode, with pulses detected by a constant fraction discriminator. The laser dye was Rhodamine 6G in ethanol, providing about 0.4 mJ/pulse at 580 nm. The pulse rate was 10 pulses/s. Data were collected on a Trancor/Norther TN-1750 multichannel analyzer used in multichannel scaler mode, and transferred to a MINC-23 Computer (Digital Equipment Corp.) for analysis. Alternately, some of the excitation spectra were collected directly on an analog *X-Y* plotter interfaced to a 30 MHz counter (Ortex 776) by a digital-to-analog circuit built by us.

Two types of measurements were made. One is the measurement of time-averaged total luminescence as a function of excitation wavelength, and the other is measurement of luminescence lifetimes at fixed wavelengths. As described by Horrock's group, the former allows differentiation of environments (sites) occupied by Eu(III) while the lifetime measurements enable calculation of the numbeer of water molecules coordinating the Eu(III) ion [14,20].

Excitation at 578–580 nm excites the $^7F_0 \rightarrow {}^5D_0$ transition. Since this is a transition between nondegenerate states, each state unambiguously represents a different ionic environment. However, the absorption at this wavelength is low ($\epsilon \sim 0.001$ $M^{-1} \cdot cm^{-1}$), imposing a lower limit of detection of the aquo (fully hydrated) ion of approx. 10^{-5} M in our system. Lower concentrations of bound Eu(III) are detectable because of increased quantum yield.

Method of analysis

All least-square fit procedures used in the analysis of data reported here were done on a PDP

11/23 computer using the algorithm of Koeppe and Hamman, (1980) [17]. The goodness of fit of experimental data to theoretical equations was estimated by calculating the generalized correlation coefficient r according to the equation

$$r = \left\{ \left(\sum (y_i - \bar{y})^2 - \sum (y - \hat{y})^2 \right) / \sum (y_i - \bar{y})^2 \right\}^{1/2}, \quad (1)$$

where $\hat{y}$ = values of y calculated from the theoretical equation for best values of the parameters, y_i = observed values of y, and $\bar{y}$ = mean of y_i values.

When the theoretical equation represents a straight line Eqn. 1 becomes equivalent to the correlation coefficient in linear regression (refer any standard statistics textbook). For the theoretical equations considered in this paper, value of r deviated from 1 either in the positive or negative direction, but the deviation decreased when the sum of deviations squares between the theoretical and experimental values decreased.

In all the luminescence decay curves we observed an initial spike due to experimental artifacts (Fig. 1). The lifetime of this initial spike was determined in the following manner.

Decay of uncomplexed Eu(III) in a solution of different proportions of $H_2O/^2H_2O$ was taken at different dwell times of the multichannel analyser. These decays were then least-squares fit to the double exponential

$$I = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \quad (2)$$

and the lifetime τ_f of the fast component was extracted for different dwell times.

All fluorescence decay curves reported here were analyzed between times t_s and t_e where $t_s = 3\tau_f$ (refer Fig. 1) where τ_f is the lifetime of the fast component of Eu(III) decay at the same dwell time. t_e is selected at a position in the decay curve which is slightly above the background noise level.

The number of H_2O molecules coordinated to Eu-calciphorin and Eu-cardiolipin complexes were determined from the slopes of $k(=1/\tau)$ against χ(= mole fraction of H_2O in a $H_2O/^2H_2O$ mixture) plots in the following manner.

The original data of Δk_{obs} $(= k^{H_2O} - k^{^2H_2O})$ experimentally observed by Horrocks et al. (1979) [14] for complexes of Eu of known number of coordinated H_2O molecules $(=q)$ were least-squares fit to the equation

$$\Delta k_{obs} = b \cdot q \quad (3)$$

This gives a value of b of 0.987 with a standard deviation σ_b of the slope of 0.298. The data fit Eqn. 3 with a correlation coefficient of 0.99. The number of H_2O molecules q coordinated to Eu-calciphorin and Eu-cardiolipin complexes and the corresponding standard deviation σ_q were calculated using the formulae

$$q = \frac{\Delta k}{b} \quad (4)$$

and

$$\frac{\sigma_q^2}{q^2} = \frac{\sigma_{\Delta k}^2}{\Delta k^2} + \frac{\sigma_b^2}{b^2} \quad (5)$$

where Δk and $\sigma_{\Delta k}$ are the slope and the standard deviation of the slope, respectively, of the least-squares straight line of the correlation between k and χ.

Excitation spectra of samples having both Eu-calciphorin and Eu-cardiolipin complexes were

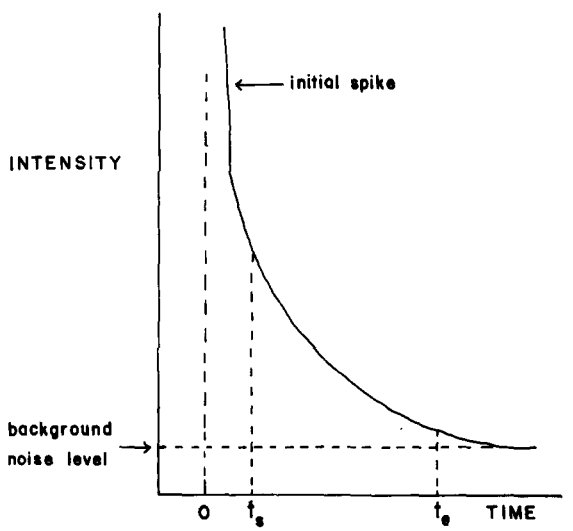

Fig. 1. Rough sketch of a luminescence decay curve used to demonstrate the method of analysis of data. t_s and t_e are defined in text.

least square fit to a double Gaussian given by

$$I = \sum_{i=1}^{2} A_i \exp\{-\ln 2(\lambda - \lambda_i)^2/\sigma_i\} \quad (6)$$

If the first and second components correspond to cardiolipin and calciphorin respectively, then λ_1 (= 579.0 nm) is the position of the Eu-cardiolipin peak and λ_2 (= 579.3) nm is the position of the Eu-calciphorin peak. These values were obtained by independent experiments (see Figs. 2 and 5). A_i is the amplitude of the ith component of the Gaussian and σ_i is the half bandwidth squared of the ith component. The least squares fit values of A_i are taken as proportional to the concentration of the corresponding Eu-ligand complex.

Results and Discussion

1. Eu(III)-calciphorin complex

Fig. 2 shows the excitation spectrum of 20 μM Eu(III) in the presence of calciphorin, contrasted with that of the aquo ion. Three different calciphorin preparations are represented: a calf heart preparation which has been delipidated (see Methods), and two rat liver preparations, one delipidated and one not delipidated. Two effects are evident: the large enhancements of the luminescence peak and the shift of the peak from 578.65 nm (the aquo position) to about 579.35 nm.

The enhancement of the peak reflects the increase in quantum yield due to coordinated H_2O molecules being removed from Eu(III) when it complexes with calciphorin. This can be characterized well from measurements of luminescence lifetime. An example of a luminescence lifetime measurement is given in Fig. 3. The free Eu(III) has a lifetime of 112 μs and when it is bound to

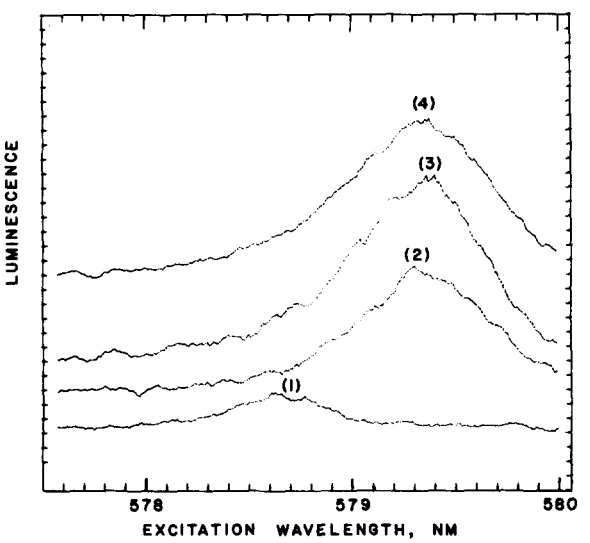

Fig. 2. Excitation spectra of 20 μM Eu(III) alone and in the presence of calciphorin preparations. All solutions contained 5 mM piperazine-HCl at pH 6.6 (1) 20 μM Eu(III) only; (2) with 43 μg/ml delipidated calf heart calciphorin; (3) with 52 μg/ml delipidated rat liver calciphorin; (4) with 100 μg/ml nondelipidated rat liver calciphorin. The luminescence scale is in arbitrary units, and the data has been smoothed digitally with a 9-point averaging program. Curves are vertically spaced for clarity.

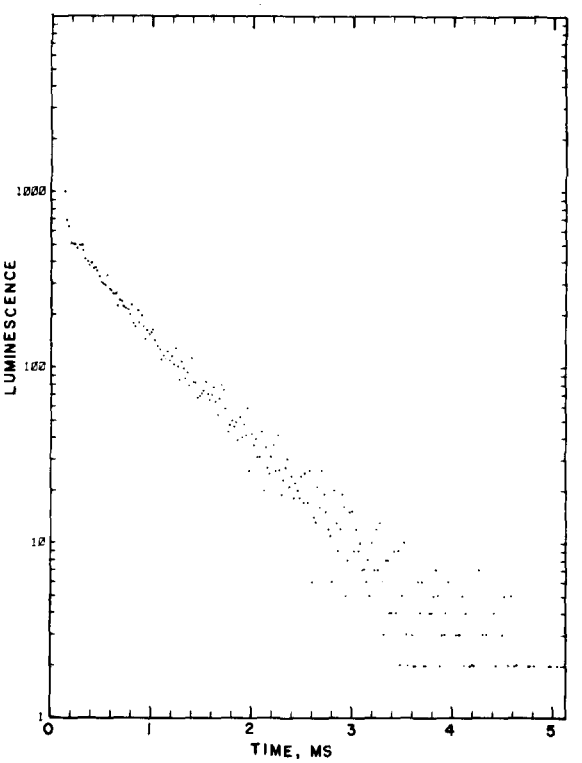

Fig. 3. Representative luminescence decay of Eu(III) complexed with calciphorin. The luminescence scale is arbitrary. The sample contained 20 μM $EuCl_3$, 50 μM calciphorin and 2 mM piperazine-HCl, pH 6.8. The excitation wavelength was 579.30 nm. The decay fits a double exponential having amplitudes 321 and 350 and corresponding lifetimes of 0.36 and 0.85 ms with a generalized correlation coefficient of 0.99.

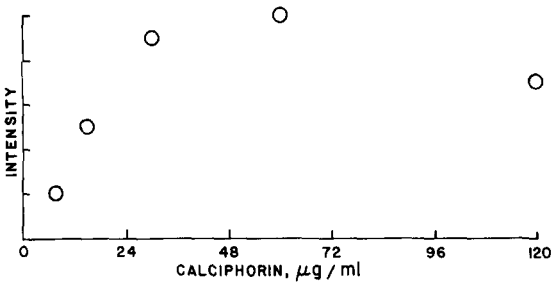

Fig. 4. Titration of 40 μM $EuCl_3$ with delipidated rat liver calciphorin. The total intensity (in arbitrary units) of Eu(III) emission at 618 nm is plotted on the vertical axis. The excitation wavelength is 579.30 nm. 2 mM piperazine-HCl, pH 6.8 was present in all samples.

calciphorin the lifetime increases to about 800 μs because of the dehydration by calciphorin.

The shift in the excitation peak to the red has been empirically shown to roughly reflect a change in the overall charge of the Eu(III)-ligand complex [17]. In model compounds, the shift is about 7.5 cm^{-1} per negative charge unit. The shift from 578.65 nm (17281.6 cm^{-1}) to 579.35 nm (17260.7 cm^{-1}) would indicate a change in electrostatic charge around the ion of 2.8; i.e. a change from +3 (aquo) to 0 or +1 for the complex.

A titration of 40 μM $EuCl_3$ with calciphorin is shown in fig. 4. The emission at first rises with increasing calciphorin concentration, reflecting a loss of quenching by water when the protein binds Eu(III). The intensity levels off at approx. 40 to 60 μg/ml calciphorin. Assuming a molecular weight of 3000 for calciphorin [9,10] we arrived at a rough estimate of the Eu(III) to protein monomer binding ratio of 2:1. At high protein concentrations the solutions became turbid due to precipitation and this could be the reason for the low intensity observed at these points.

We acknowledge the inherent weakness of this method in the determination of the binding ratios. However, we wish to mention that this procedure has been used by Rhee et al. [18] to determine the stoichiometry of binding of Eu(III) to human prothrombin. In previous studies we obtained Ca(II) to calciphorin binding ratios ranging between 0.5 and 1.0 depending on the method and the sample used [8,9]. Lanthanides usually bind to calcium binding proteins with a stoichiometry similar to that of calcium. We are unable at the present time to explain the discrepancies in the values of the binding ratios obtained by the different methods used. We hope by the development of better techniques we should be able to arrive at an accurate and reliable result.

2. Comparison with possible contaminants

There has been some concern that the ionophoric activity demonstrated by calciphorin is due to contaminating phospholipids or detergents used in the isolation procedure. We will examine this possibility in light of the information provided by Eu(III) luminescence.

Cardiolipin is co-purified in the primary steps of calciphorin isolation. Its excitation spectrum is shown in Fig. 5. The peak of the Eu(III)-cardiolipin complex is at 579.0 nm, which is smaller than 579.35 nm, the value observed for the Eu(III)-

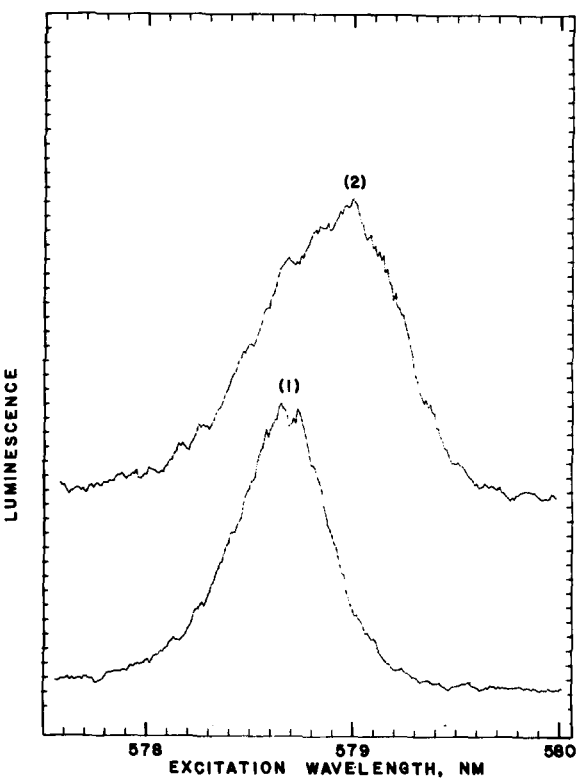

Fig. 5. Excitation spectrum of 0.10 mM Eu(III), (1) in the absence and (2) in the presence of 150 μM cardiolipin. All solutions contained 2.0 mM piperazine-HCl, pH 6.6. Five-point smoothing has been applied to the data. The luminescence scale is arbitrary. Curves are vertically spaced for clarity.

TABLE I

RESULTS OF DOUBLE EXPONENTIAL FITS TO CALCIPHORIN- AND CARDIOLIPIN-Eu COMPLEX DECAY CURVES IN $H_2O/^2H_2O$ MIXTURES AND THE DETERMINATION OF COORDINATED H_2O MOLECULES.

r, generalized correlation coefficient defined by Eqn. 1 for the double exponential fits.

χ (mole fraction of H_2O)	Calciphorin		Cardiolipin	
	slow component k (ms^{-1})	r	slow component k (ms^{-1})	r
1.0	1.225	0.992	1.414	1.005
0.875	0.955	0.995	–	–
0.75	0.80	0.997	1.188	0.983
0.50	0.648	0.987	0.957	1.000
0.25	0.37	0.984	0.629	1.000
0.04	–	–	0.365	1.000
0.015	0.309	0.995	–	–
Linear correlation between k and χ				
Slope	0.77 ± 0.08		1.09 ± 0.05	
Intercept	0.25 ± 0.05		0.35 ± 0.03	
Correlation coefficient	0.984		0.996	
Standard deviation of points	0.056		0.042	
Coordinated H_2O molecules	0.78 ± 0.11 [a]		1.11 ± 0.15 [a]	

[a] Standard errors calculated according to text, all other errors are standard deviations.

calciphorin complex. It means that the net charge on the Eu-calciphorin complex is smaller than that on the Eu-cardiolipin complex by about 1.4 units.

(a) Water molecules coordinated to complexes

We estimated the number of H_2O molecules coordinated to Eu-calciphorin and Eu-cardiolipin complexes according to Rhee et al. [18] and those explained in the experimental section. All decay curves reported here fit the double exponential (Eqn. 2) with a generalized correlation coefficient (Eqn. 1) deviating from 1 by less than 0.02. For both cardiolipin and calciphorin the faster component (larger k, $k = 1/\tau$) of the double exponential fit did not show a correlation between $k(=1/\tau)$ and χ_{H_2O} (= mole fraction of H_2O). Since for all ligands that has previously been studied [14,17–29] there is a good correlation between k and χ, this fast component could be due to experimental artifacts. As Table I shows, the correlation between k and χ for the slow component is better than 0.98 for both lines (see Fig. 6). Therefore the slow component was taken as the representative value of the corresponding complex.

The point corresponding to 100% H_2O ($\chi = 1$)

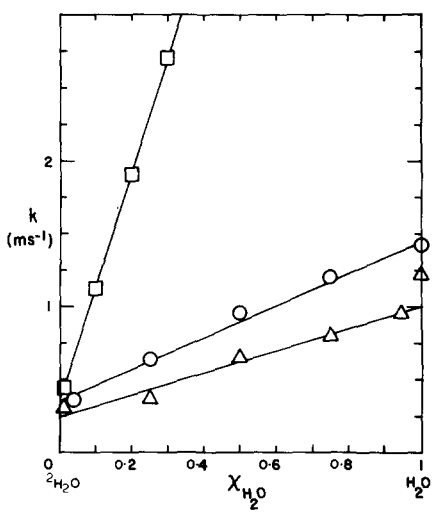

Fig. 6. The slow component decay constant k for Eu(III) bound to cardiolipin (○), and to calciphorin (△), plotted as a function of mole fraction χ_{H_2O} of H_2O in a $H_2O/^2H_2O$ mixture. The corresponding values for free Eu(III) (□) are also plotted for comparison. Conditions of the experiments are, for (□): 0.1 mM Eu, for (○): Eu = cardiolipin = 0.1 mM, for (△): 40 μM Eu, 60 μg/ml calciphorin. All solutions were buffered with 2 mM piperazine-HCl, pH 6.8. The solid lines are least-square straight lines. In (△) the $\chi = 1$ point is excluded in the regression because it is more than three standard deviations away from the line.

for calciphorin in the k against χ plot (Fig. 6) deviates from the least-squares straight line by more than three times the standard deviation of the data and therefore this point was excluded from the analysis.

k and χ values for calciphorin and cardiolipin fit a straight line with correlation coefficients of 0.984 and 0.996, respectively, and the number of water molecules coordinated calculated using Eqn. 4 are 0.78 ± 0.11 and 1.11 ± 0.15, respectively (Table I). The errors are standard errors calculated using Eqn. 5. The difference between the number of H_2O molecules for the two complexes is significant at a confidence level of 0.1 on a Student's t one tail test. Similar analysis shows that the slope of the line for cardiolipin is greater than that of calciphorin at a significance level much smaller than 0.0005 when the 0% ^2H_2O point is excluded from calciphorin data, and is significant at the level of 0.005 when this point is included in the data.

Note that although the value of k at 100% ^2H_2O lie close to each other they need not necessarily be equal. For further comments regarding this see Ref. 13.

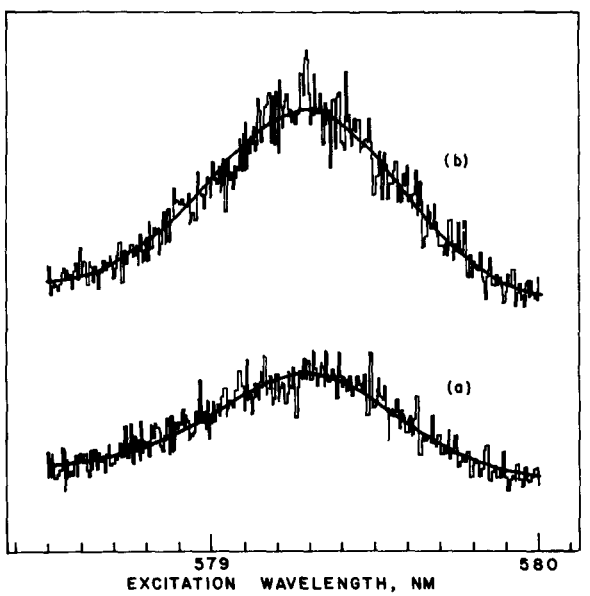

Fig. 7. Excitation spectra of a calciphorin preparation in excess of Eu(III) before (a) and after (b) delipidation. Solutions were buffered with 2 mM piperazine-HCl, pH 6.8. Solid lines represent double Gaussian curves (Eqn. 6) for values of parameters given in Table II obtained by a least-squares curve fit procedure.

(b) Resolution of peaks in a mixture

Figs. 7 (a) and (b) show the excitation spectra of Eu-calciphorin complex before and after delipidation, respectively, in excess of Eu(III) (same calciphorin preparation was used). Although in these samples Eu was in excess we do not observe any evidence of a shoulder at the position of the free Eu(III) peak (578.65 nm). Therefore we would expect that most of the cardiolipin and calciphorin present will be bound to Eu.

In separate experiments we find that Eu-cardiolipin complex peak lies at 579.0 nm (Fig. 5) and the Eu-calciphorin complex peak lies at 579.3 nm (Fig. 2). Therefore in order to determine approximately how much of each component is present in these two samples we fitted these two curves to the double Gaussian given in Eqn. 6, with $\lambda_1 = 579.0$ nm and $\lambda_2 = 579.3$ nm. The values of the parameters for which the sum of deviations squared is minimum is given in Table II and the solid lines in the figures represent the theoretical curves whose parameters have the values in Table II. Both curves fit the experimental data with a generalized correlation coefficient which deviates from 1 by less than 0.04.

The results indicate that approximately the ratio of cardiolipin to calciphorin decreased form 1 : 6 to 1 : 89 during delipidation, and that the excitation spectra fitted well to a single Gaussian as it is purified by delipidation.

The evidence is strong, then, that cardiolipin is not contributing to Eu(III) complexation exhibited by our calciphorin preparations.

The evidence against contaminating detergent

TABLE II

RESULTS OF DOUBLE GAUSSIAN CURVE FIT TO RAT LIVER CALCIPHORIN-Eu EXCITATION SPECTRA, BEFORE AND AFTER DELIPIDATION

	Nondelipidated	Delipidated
(1) Peak height at 579.0 nm	1.8	0.25
(2) Half bandwidth squared at 579.3 nm (nm^2)	0.32	$2.4 \cdot 10^{-4}$
(3) Peak height at 579.3 nm	10.9	22.2
(4) Half bandwidth squared at 579.3 nm (nm^2)	0.1	0.12
(5) Ratio of peak height at 579.3/peak height at 579.0 nm	6.1	88.8

contributions to the Eu(III) complexation is even greater. Deoxycholic acid by itself will not complex Eu(III) because it is protonated (and precipitates) at pH values below about 7.6, and experimentally it is necessary to keep Eu(II) solutions below pH 7.0 to prevent the hydroxide of Eu(III) from forming. We can, however, add small amounts of deoxycholate to solutions already containing calciphorin without observing precipitation of deoxycholic acid (DOC). We have titrated Eu(III)-calciphorin complexes up to large (= 50) DOC-to-Eu(III) mole ratios without having seen any significant change in the excitation spectrum. It is safe to say that deoxycholic acid is not responsible for Eu(III) complexation in our calciphorin preparations.

Digitonin is a neutral molecule and we were unable to observe any significant binding of Eu(III) by it in our assay. It is therefore also ruled out as a complexation agent.

Conclusions

Using this recently developed metal ion binding assay, we have characterized the complexation of Eu(III) with calciphorin, a possible calcium carrier isolated in this laboratory. An essential requirement of a metal ion carrier is the facilitation of the incorporation of the ion from a hydrophilic aqueous environment to the hydrophobic membrane phase by effectively neutralizing its charge and removing the coordinated water molecules. Eu(III) complexation is accompanied by the removal of all but approximately 0.8 of the water molecules in the inner hydration shell of Eu(III) and also an almost complete neutralization of its charge. Although cardiolipin has similar properties but to a lesser extent we emphasize that it is not able to mediate ion transport in vesicles or in planar bilayer membranes. These observations, therefore, agree with the hypothesis that calciphorin is a carrier. The observed Eu(III) complexation is not due to contaminating phospholipids or detergents.

Note added in proof: (Received May 21st, 1984)

In a recent paper [21] we describe the isolation of calciphorin from both calf heart and rat liver mitochondria.

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References

1 Lehninger, A.L. and Carafoli, E. (1971) Arch. Biochem. Biophys. 143, 506–515
2 Vinogradov, A. and Scarpa, A. (1973) J. Biol. Chem. 248, 5527–5531
3 Gunter, T.E. and Puskin, J.S. (1972) Biophys. J. 12, 625–635
4 Lehninger, A.L. (1967) Adv. Enzymol. 29, 259–320
5 Rottenberg, H. and Scarpa, A. (1974) Biochemistry 13, 4811–4817
6 Chance, B., Azzi, A. and Meta, L. (1969) in The Molecular Basis of Membrane Function (Tosteson, D.C., ed.) pp. 561–573, Prentice-Hall, Englewood Cliffs, NJ
7 Lehninger, A.L. (1970) Biochem. J. 119, 129–138
8 Jeng, A.Y., Ryan, T.E. and Shamoo, A.E. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2125–2129
9 Jeng, A.Y. and Shamoo, A.E. (1980) J. Biol. Chem. 255, 6897–6903
10 Jeng, A.Y. and Shamoo A.E. (1980) J. Biol. Chem. 255, 6904–6912
11 Shamoo, A.E. and Brenza, J.M. (1980) Ann. N.Y. Acad. Sci. 358, 73–82
12 Sokolove, P.M., Brenza, J.M. and Shamoo, A.E. (1983) Biochim. Biophys. Acta 732, 41–47
13 Shamoo, A.E. and Jeng, A.Y. (1978) Ann. N.Y. Acad. Sci. 307, 235–237
14 Horrocks, W. De W. and Sudnick, D.R. (1979) J. Am. Chem. Soc. 101, 334–340
15 Rhee, M.-J., Sudnick, D.R., Arkle, V.K. and Horrocks, W. De W. (1981) Biochemistry 20, 3328–3334
16 Schnaitman, C. and Greenawalt, V.W. (1968) J. Cell. Biol. 38, 158–175
17 Sudnick, D.R. (1979) Ph.D. Thesis, The Pennsylvania State University, University Park, PA 16802, p. 46
18 Rhee, M.-J., Horrocks,W. De W. and Cosow, D.P. (1982) Biochemistry 21, 4524–4528
19 Koeppe, P. and Hamann, C. (1980) Comput. Programs Biomed. 12, 121–128
20 Horrocks, W. De W., Schmidt, G.F., Sudnick, D.R. Kittrell, C. and Bernheim, A. (1977) J. Am. Chem. Soc. 99, 2378–2380
21 Ambudkar, I.S., Kima, P.E. and Shamoo, A.E. (1984) Biochim. Biophys. Acta 771, 165–170