CALCIUM BINDING SITES OF RABBIT TROPONIN AND CARP PARVALBUMIN

Theodore L. MILLER, Donald J. NELSON, Harry G. BRITTAIN,
Frederick S. RICHARDSON, R. Bruce MARTIN
Chemistry Department, University of Virginia, Charlottesville, Virginia 22901, USA

and

Cyril M. KAY
Biochemistry Department, University of Alberta, Edmonton, Alberta, Canada

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1. Introduction

Transient fluxes of calcium ion regulate muscle contraction; vertebrate muscle contain troponin, a calcium ion modulated protein believed to be critical in triggering contraction by its association with other muscle proteins. Troponin consists of three different polypeptide chains, only the lightest of which, TN-C (mol. wt 17 846), binds up to four Ca (II) [1]. The amino acid sequence of rabbit muscle TN-C [2] exhibits homologies with parvalbumin mol. wt ~ 11 500), a protein from the white muscle of fish and amphibians that binds two Ca (II) [3]. Loss of Ca (II) reduces the helical content of both proteins [4–6]. The three dimensional X-ray structure of a carp parvalbumin has been determined [7,8], but the TN-C structure has not been solved. Of the two Ca (II) of parvalbumin, Ca (EF) is overlaid within 0.5 nm by the aromatic side chain of Phe-57. Upon substitution of Ca (II) by terbium (III) and irradiation at 259 nm a characteristic green Tb (III) emission appears, due to energy transfer from the aromatic side chain of Phe-57 to Tb (EF) [6,9]. Tb (III) emission also occurs upon substitution of the lanthanide ion for Ca (II) in TN-C. However, in this protein the excitation spectrum identifies one of the two tyrosyl residues as being involved in energy transfer to Tb (III) [6]. In the alignment of the amino acid sequences Tyr-109 (108) of TN-C matches with Phe-57 of parvalbumin [2]. In this communication we characterize more completely by emission titration and circularly polarized/emission (CPE) spectroscopy similarities of the Ca (II) binding sites in two proteins upon Tb (III) substitution. We also report the total emission (TE) and CPE spectra of TN-C containing Tb (III) in the whole troponin trimer (troponin complex).

2. Materials and methods

The molar absorptivity of TN-C [5] was taken as 4100 at 277.5 nm. The three protein troponin complex was prepared from freshly killed rabbits [1,10]. The purification was carried to the stage of precipitation with $(NH_4)_2SO_4$ at 70% saturation. Carp parvalbumin was the same sample as employed in a recent study [9]. The molar absorptivity was taken as 2000 at 259 nm.

Most TE and all CPE measurements were made on an apparatus constructed in these laboratories [11]. The emission experiments were conducted at pH 6.5 employing a 0.1 M KCl-piperazine buffer for TN-C and parvalbumin, and a 5 mM Tris, 0.1 mM $CaCl_2$, 0.1 mM dithiothreitol buffer for the troponin complex.

3. Results

The effects of added increments of Tb (III) at

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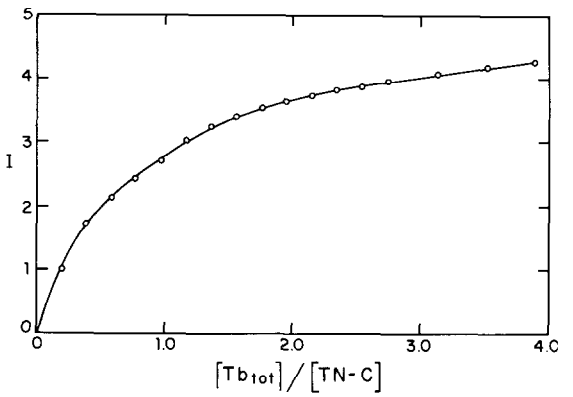

Fig.1. Relative emission intensity at 545 nm upon addition of Tb (III) to TN-C.

pH 6.5 on the total emission intensity at 545 nm upon irradiation of TN-C at 280 nm is plotted in fig.1. The total emission (TE) and circularly polarized emission (CPE) spectra obtained for TN-C at pH 6.5 to which 2.0 equivs of Tb (III) had been added are shown in fig.2. Both spectra were obtained with 280 nm irradiation of the sample. The maximum in the TE spectrum is matched by a minimum in the CPE spectrum. The CPE spectrum also shows two maxima, one on each side of the minimum, which correspond to shoulders in the TE spectrum.

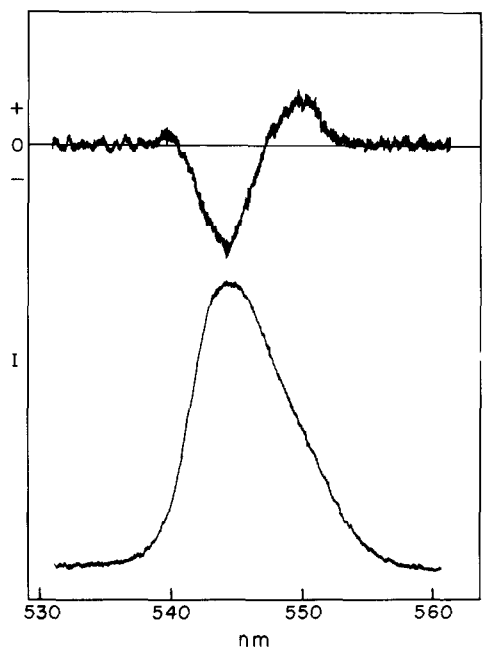

Fig.2. Circularly polarized emission (upper) and total emission (lower) spectra of TN-C after addition of 2.0 equivs of Tb (III). Both spectra were taken at 0.2 nm resolution.

The TE and CPE spectra of carp parvalbumin upon addition of 2 equivs of Tb (III) at pH 6.5 are virtually identical in shape to those of TN-C in fig.2. This result confirms the earlier parvalbumin CPE spectrum [6] except that the signs should be reversed in the previous study. The emission (TE and CPE) intensities for TN-C are more than twice those of parvalbumin. The near identity of the spectra from the two proteins is also indicated by the emission dissymmetry factor, g, of CPE to TE intensities. At the extrema in the CPE spectra, $g = -0.029$ at 544.3 nm and $g = +0.027$ at 549.7 nm for TN-C, while $g = -0.025$ at 544.9 nm and $g = +0.029$ at 549.9 nm for parvalbumin.

When Tb (III) is added to the troponin complex, strong Tb (III) luminescence appears with a maximum at 544.5 nm as shown in fig.2 for TN-C. The excitation spectrum exhibits a maximum at 280 nm, again indicating that a tyrosyl residue is involved in energy transfer to a nearby Tb (III). In contrast to TN-C, however, the troponin complex with Tb (III) does not display an observable CPE.

4. Discussion

The characteristic terbium luminescence reported in this study depends upon a dipole–quadrupole energy transfer mechanism between the irradiated aromatic side chain and the emitting Tb (III). The probability of such an intramolecular energy transfer exhibits an r^{-8} dependence upon the distance between the donor and acceptor sites. Thus, observation of Tb (III) emission upon irradiation of the appropriate aromatic side chains indicates that Tb (III) is juxtaposed to a tyrosyl group in TN-C and a phenylalanine ring in parvalbumin.

Emission (phosphorescence) in the 535–555 nm region originates with Tb (III) transitions derived from the $^5D_4 \rightarrow {}^7F_5$ free ion radiative process. The $^5D_4 \rightarrow {}^7F_5$ transition is magnetic dipole allowed and one may anticipate, therefore, that it will exhibit substantial optical activity when Tb (III) resides

in a chiral ligand environment. The degeneracy of this transition will, of course, be lifted in the presence of low symmetry ligand fields and one may further expect to observe splittings in the associated spectral observables. The CPE spectrum displayed in fig.2 reveals three components split out of the $^5D_4 \rightarrow {}^7F_5$ Tb (III) transition in the Tb (III): TN-C complex.

The near identity in CPE lineshape and dissymmetry factors for TN-C and parvalbumin indicates closely similar Tb(III) binding sites. The complexity of the weak Tb (III) transitions assures that even small variations in geometry lead to CPE spectra of differing shapes and intensities. Indeed, Tb (III) binding near an aromatic residue need not produce an observable CPE spectrum if the Tb (III) is not experiencing a chiral environment.

In the experiment with the troponin complex, Tb (III) TE appears with tyrosyl excitation as in TN-C, but loss of an observable CPE in the trimer indicates a much reduced asymmetry of the Tb (III) binding site. This modification of the metal ion binding site upon association of TN-C with two other protein components may be related to the stronger Ca (II) binding of the troponin complex compared to TN-C [12].

Addition of 1.0 equivs of Tb (III) produces 63% of the intensity at 4.0 equivs in the TN-C emission titration curve of figure 1. This result indicates that the Ca (II) overlaid by Tyr-109 is easily substituted by Tb (III) as it is replaced more easily than other calcium ions. Like parvalbumin [6,9], Ca (II) in TN-C is bound less strongly than Tb (III).

The total emission titration curve for TN-C in fig.1 exhibits a continuing increase in intensity as additional amounts of Tb (III) are added. In contrast the corresponding plot for parvalbumin displays a maximum at 1.4 to 1.8 equivs of Tb (III) with the relative intensity at 4 equivs only about half that at the maximum [6,9]. This reduction in emission intensity upon addition of excess Tb (III) to parvalbumin may have at least two origins. First, it might be due to indirect action of excess Tb (III) inducing a conformational change that alters the critical spatial requirements for intramolecular energy transfer. Second, it may be due to association of Tb (III) with a water molecule that is bound to Tb (EF) and radiationless energy dissipation from Tb (EF) through the bound water to the associated Tb (III) which is highly hydrated. Arguments have been presented in favor of the latter alternative [9]. The absence of quenching in TN-C by excess Tb (III) implies that a water molecule does not occur at the corresponding site or if water does occur the environment is not inducive to binding of excess Tb (III). Though TN-C and parvalbumin exhibit strong similarities in their TE and CPE lineshapes and dissymmetry ratios, they differ in that excess Tb (III) quenches only the parvalbumin emission.

Substitution of Tb (III) for Ca (II) in proteins and examination of TE and CPE spectra is a sensitive monitor of the microenvironments of Ca (II) binding sites with nearby aromatic side chains. Observation of emission from the Tb (III) probe and determination of the excitation spectrum upon irradiation in the aromatic region identifies a nearby aromatic side chain. The CPE spectrum characterizes the asymmetry of the binding site. It will be interesting to learn whether similar TE and CPE spectra are obtained from parvalbumins and TN-C proteins of other species, from TN-C when associated with other troponin components and muscle proteins, and from related proteins.

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