CIRCULARLY POLARIZED EMISSION OF TERBIUM(III) SUBSTITUTED BOVINE CARDIAC TROPONIN-C.

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Summary. Upon substitution of Tb(III) for the most easily replaced Ca(II) from bovine cardiac TN-C, irradiation at 280 nm produces an emission at 545 nm from Tb(III) that is partially circularly polarized. Characteristics of these emission spectra produced by energy transfer from a tyrosyl side chain to a juxtaposed Tb(III) are virtually identical to those found in rabbit skeletal muscle TN-C and carp parvalbumin. A single homologous tyrosyl residue occurs in the two troponins and is in turn homologous to a phenylalanyl residue in parvalbumins. Addition of the other troponin subunits, TN-I and TN-T, to Tb(III)-TN-C weakens the total emission and completely quenches the circularly polarized emission.

The sensitivity of muscle contraction to Ca(II) is provided by the protein troponin. Troponin consists of three different subunits, only the lightest of which, TN-C (MW 18,000), binds up to four Ca(II) (1). In the presence of Ca(II), TN-C reverses the effect of the second component, TN-I, which inhibits actomyosin ATPase activity. A third subunit, TN-T, interacts with tropomyosin.

Upon the substitution of a single Ca(II) by terbium(III) in rabbit skeletal muscle TN-C, irradiation in the tyrosyl

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absorption region at 280 nm produces a green emission from Tb(III) at 545 nm (2). Since the tyrosyl to Tb(III) energy transfer process most likely proceeds via a dipole-quadrupole mechanism, its efficiency is expected to exhibit a r^{-8} dependence upon the distance between the donor and acceptor sites. The high efficiency of this process observed for the Tb(III)-TN-C complex clearly suggests that the aromatic side chain of the tyrosyl group must closely overlay the Tb(III). In TN-C the Tb(III) emission is also partially circularly polarized with a negative peak at 544.3 nm and a positive peak at 549.7 nm (3). Like circular dichroism in absorption spectroscopy, circularly polarized emission (CPE) is responsive to fine details of molecular geometry and stereochemistry in a chiral environment.

It is the purpose of this communication to report the emission properties of bovine cardiac TN-C upon substitution of Ca(II) by Tb(III), to describe the effects of added TN-I and TN-T, and to compare the results with those observed for the rabbit skeletal muscle troponin system.

Materials and Methods

Bovine cardiac troponin subunits were prepared as previously described (4,5). Limited quantitites of the subunits precluded more extensive experiments. The concentration of TN-C was 1.5 mgs/ml. The TE and CPE measurements were made on an apparatus constructed at the University of Virginia (6). All experiments were performed at pH 6.5 in a piperazine buffer and at 0.1 M ionic strength controlled with KCl.

Results

Addition of 1 equiv of Tb(III) to bovine cardiac TN-C produces 87% of the total emission (TE) intensity at 545 nm as does addition of 2 equivs of Tb(III). The excitation

spectrum shows that Tb(III) emission in the protein is most strongly enhanced by irradiation at 280 nm, indicating involvement of a tyrosyl residue in energy transfer to Tb(III). The solution with 2 Tb(III) per TN-C exhibits the same ultraviolet circular dichroism down to 220 nm as does TN-C with Ca(II) (4).

The Tb(III) emission from Tb(III)-TN-C is also partially circularly polarized. As previously reported for rabbit skeletal muscle TN-C (3), the TE spectrum in the region of the Tb(III) $^5D_4 \longrightarrow {}^7F_5$ transition exhibits a maximum at 545 nm and a shoulder near 550 nm. The CPE spectrum in this region displays a negative peak at 544.5 nm and a smaller positive peak at 549.8 nm. The spectra may be characterized by the emission dissymmetry factor, g, which is defined by $\Delta I/I = (I_L - I_R)/(I_L + I_R)$, where I_L and I_R are the left- and right-circularly polarized emission intensities respectively. We refer to I as TE intensity and to ΔI as CPE intensity. The g values at the CPE (ΔI) extrema for several Tb(III) substituted troponins are listed in the Table.

In the bovine cardiac system, addition of 0.60 equivs of TN-I to a solution containing TN-C and 2 equivs of Tb(III) reduces the TE intensity at 545 nm by 34% and the g value by 60%. Extrapolation of these results to the addition of 1.0 equiv of TN-I results in a reduction of TE intensity to 43% and to complete quenching of the CPE.

When 0.43 equiv of TN-T is added to a solution containing TN-C, 2 equivs Tb(III), and 0.60 equiv TN-I, the TE intensity is reduced to 44% of that observed in the presence of

Table. Emission Dissymmetry Factors for Tb(III) Emission[a] in Muscle Proteins

	g	nm
Bovine cardiac TN-C	-0.030	544.5
	+0.024	549.8
TN-C + TN-I	0.000	544.7[b]
TN-C + TN-T	0.000	544.6[b]
TN-C + TN-I + TN-T	0.000	544.6[b]
Rabbit skeletal TN-C	-0.029	544.3
	+0.027	549.7
Rabbit skeletal troponin	0.000	544.8[b]
Carp parvalbumin	-0.025	544.9
	+0.029	549.9

a. In region of Tb(III) $^5D_4 \longrightarrow {}^7F_5$ transition.

b. Maximum wavelength in total emission spectrum.

Tb(III)-TN-C alone and the CPE is completely quenched. Thus both TN-T and TN-I appear to be effective in reducing TE and quenching CPE in Tb(III)-TN-C. These results are listed in the Table in terms of integer equivalents of the other troponin components with TN-C.

Also listed in the Table are the results previously reported for rabbit skeletal TN-C and the whole troponin complex when substituted with Tb(III) (3). For the whole troponin complex in both the bovine cardiac and rabbit skeletal muscle systems, CPE does not appear, but TE from Tb(III) is still evident. These identical results on the two systems were obtained on troponin complexes prepared in two different ways.

Bovine cardiac troponin was produced by successive additions of TN-I and TN-T to TN-C, while rabbit troponin was isolated directly from skeletal muscle without separation into troponin components.

Discussion

An induction region for Tb(III) emission is not observed upon addition of Tb(III) to Ca(II) containing TN-C. Thus Tb(III) is bound more strongly than Ca(II) to the protein. Most of the Tb(III) emission is produced by addition of the first equivalent of Tb(III) indicating that of several Ca(II) in TN-C, the one overlaid by a tyrosyl residue is the most easily replaced.

As the Table shows, the wavelengths and emission dissymmetry factors, g, associated with CPE extrema are virtually identical for Tb(III) substituted bovine cardiac TN-C and rabbit skeletal TN-C. In addition a maximum in the excitation spectrum at 280 nm for both proteins identifies a tyrosyl side chain as the donor group in the energy transfer process leading to Tb(III) emission. It is suggested, then, that in both proteins a tyrosyl residue closely overlays the Tb(III) and that the chirality about the Tb(III) binding site in both proteins is nearly identical (as evidenced by the similarity in CPE spectral features). Rabbit skeletal TN-C contains 2 tyrosyl residues and bovine cardiac TN-C 3. The amino acid sequences of both proteins are known and the only homology involving tyrosyl residues occurs at 109 for

rabbit skeletal TN-C and at 111 for bovine cardiac TN-C (7). Thus this single homologous tyrosyl residue is strongly implicated as the one providing energy transfer to Tb(III) in our emission experiments. Further support for this identification comes from the CPE result for Tb(III) substituted carp parvalbumin (Table) (3) which is closely similar to those of the two TN-C molecules. For parvalbumin, however, the excitation spectrum displays a maximum at 259 nm identifying a phenylalanyl side chain as the group participating in energy transfer to Tb(III) (2). In the alignment of amino acid sequences Phe-57 of parvalbumin matches with Tyr-109 of rabbit skeletal muscle TN-C (8). The crystal structure of carp parvalbumin shows the aromatic side chain of Phe-57 overlying Tb(III) in the Ca(EF) site (9). Thus a persistent feature of a Ca(II) binding site of the three multiple Ca(II) proteins from different species is an aromatic side chain overlying the most easily Tb(III) substituted Ca(II) in a site of closely similar chiralities.

The amino acid sequences of TN-C from rabbit skeletal and bovine cardiac muscles differ by 35% (7). In the alignment of parvalbumin sequences with those of rabbit skeletal TN-C only about 30% of the residues are identical (8). The differences among these three sequences suggest that the persistent structural feature of an aromatic side chain overlying the most easily substituted Ca(II) may not be accidental.

Addition of other troponin components to Tb(III)-TN-C reduces the TE and quenches the CPE, indicating complex for-

mation of the other troponin subunits with TN-C. In the three subunit troponin complex from both bovine cardiac and rabbit skeletal muscles, CPE does not appear and the TE, though diminished, is still strong. These changes may reflect an aspect of the stronger binding of Ca(II) to TN-C when the other troponin subunits are complexed (10). The TE and CPE measurements described here provide probes into the microenvironment of Tb(III) in TN-C undergoing subunit interactions with other troponin components. The probes are so sensitive that they may reveal structural alterations that are not apparent at the level of resolution achieved in X-ray diffraction studies of proteins.

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