Metal-dependent energy transfer in parvalbumin

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The parvalbumins studied here appeared straightforward objects for energy transfer measurements due to their amino acid sequences providing simple donor/acceptor situations: In pike II parvalbumin there is a single tyrosine in position 47 to transfer absorbed energy onto a Tb^{3+} ion in the CD calcium binding site. In whiting III parvalbumin a single tyrosine/tryptophan couple in postions 26 and 102 lends itself to follow metal-induced conformational changes.

A. Pike parvalbumin (PAP). Although the tyrosine is located in close proximity of the CD site (1), its fluorescence sees the binding of Ca^{2+} to both the CD and the EF site. Occupation of both sites is also required to bring about the full change in circular dichroism. The Ca-induced CD change is much smaller at physiological background concentrations of Mg^{2+} than in the metal-free case. Titration of Ca_2PAP with Tb^{3+} followed by the increase of fluorescence at 545 nm results in a characteristic binding curve also observed with carp parvalbumin (2). Whereas in rabbit skeletal troponin C and calmodulin the increase in Tb fluorescence is accompanied by the expected decrease of the donor emission, the tyrosine fluorescence of PAP remains constant over the whole titration range. Correspondingly, the decay of the tyrosine fluorescence which is purely single-exponential, does not change. The CD spectrum of PAP in the presence and absence of Ca^{2+} and/or Tb^{3+} is the same. Possible explanations for the missing decrease of the donor emission are discussed.

B. Whiting parvalbumin (WPA). The striking feature of calcium binding to WPA is a dramatic change in the intensity and position of the emission band and in the circular dichroism of the tryptophan residue (3,4). These phenomena were interpreted as a locking of the side-chain in an extremely rigid position. The donor/acceptor separation in the dissolved molecule is calculated on the basis of the Förster mechanism. The value for the Ca_2 form is compared with the corresponding distance in crystalline carp parvalbumin (1) in order to examine the compatibility of these structures. The evaluation of the metal-free situation shows the extent of the distance modification which is inaccessible by X-ray crystallography.

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