## Analysis of the weak myosin-binding site on actin

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We recently reported that an 18-residue peptide, comprising residues 77–95 of the actin structure, forms part of the myosin binding site on actin (Alessi *et al.* (1992) *J. Muscle Res. Cell Motil.* **13**, 220). In actin, this region is largely α-helical, with an unwinding of the helix at the C-terminus (α-helix → 5-turn helix), and is located at the top and 'side' of subdomain 1. This peptide binds equally well to S1 alone and to an S1-ATP analogue state, suggesting it forms a contact point in the weak binding acto-S1–ATP complex which is consistent with recent image reconstruction studies (Milligan and Holmes, unpublished data). Extensive analysis of this peptide in solution and when bound to S1 has been carried out by NMR and its structure determined from NOESY spectra using the simulated annealing protocol of XPLOR. In 50% aqueous trifluoroethanol (TFE), the peptide was essentially α-helical over most of its length with both the backbone and side chains aligned. The helix was slightly curved and further analysis showed that there was a discontinuity between $H_{87}$ and $H_{88}$ that was most apparent in the Ramachandran plots and seemed to originate at a break in the regular organization of the side chains; up to $H_{87}$ the side chains were aligned C → N and after $H_{88}$ they were aligned N → C. In most α-helices the side chains align either N → C or C → N without the break. In 90% $H_2O$, the helical character of the backbone is still apparent although the side chains are more flexible and the C-terminal end unwinds out of the α-helix, i.e. in some respect it is more like the structure found in actin. Using the transferred NOESY method, the structure of the peptide bound to S1 in 90% $H_2O$ was also determined. This was found to be much more like that in 50% TFE as evidenced by the larger number of transferred NOEs than NOEs found in water in the absence of S1. The backbone was mostly helical and the side chains more ordered. In particular, residues $W_{86}$-$H_{87}$ were held relatively rigid indicating that these two amino acids were the major contact points with S1. In actin, $H_{87}$ (and $H_{88}$) are readily available for interaction with S1 but $W_{86}$ is buried. However, limited molecular dynamics calculations based on minor changes in winding of the C-terminal region of this helix show that $W_{86}$ can be exposed (and $H_{88}$ buried) at this discontinuity point in the helix. This could be a means whereby S1 could deform the actin structure and transmit information through the molecule.

As controls it has shown that peptides containing the α-helical region 113–125 also retain their structure in solution but do not bind to S1. This is the major surface feature of the 'back' of the subdomain 1. We are presently investigating peptides containing the 338–348 helix, which is a dominant feature of the 'front' of subdomain 1.