High-resolution Structure of Titin Kinase from Crystals with Very Thin Plate Morphology

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The high-resolution structure of the serine kinase domain of the giant muscle protein titin was solved from EMBL/DESY Hamburg synchrotron data using crystals with very thin plate morphology. Titin forms the third filament system in striated muscles of vertebrates and plays a key role in muscle differentiation and development. It has a molecular weight of about 3 MDa and a length of about 1 µm, spanning half of the sarcomeric unit from the Z disc to the M line. The sequence contains 248 domains of which about 90% are predicted to contain either an Ig-like or FN-III fold. Only one catalytic domain is present, a serine kinase close to the C-terminus of the molecule. Crystals from human, cardiac titin kinase grew from diverse crystallization conditions as extremely thin plates. The crystal size was optimized by a combination of macroseeding and the use of oil layers over the reservoirs. The thickness of these plates, however, never exceeded 5 µm, making the use of synchrotron radiation essential for x-ray data collection. The structure solution of this kinase was hampered over a time of four years despite several unsuccessful attempts to collect x-ray data at different synchrotron facilities.

We finally were able to collect a 2.0-Å data set at the wiggler beam line BW7B at EMBL/DESY, Hamburg, with good statistics. The structure of titin kinase was subsequently solved by a combination of MR and NCS averaging. The resulting model has revealed the basis for the novel signalling role of this kinase in muscle differentiation. The structure shows the enzyme in a dual autoinhibited conformation, reflecting the requirement for both calcium/calmodulin and phosphorylation for activation (M. Gautel, unpublished results). This is the first structure of a non-RD kinase activated by phosphorylation. In contrast to other kinases so regulated, phosphorylation does not occur in the activation loop but in the P+1 loop, accounting for both activation and substrate specificity.