SH2/SH3/SH2 composite domains of GAP120 complexed to a diphosphorylated peptide

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The p120 GTPase-activating protein (GAP) functions at a key relay point in signal transduction pathways that control cell proliferation by acting as a negative regulator of p21 Ras. In addition to binding to Ras, GAP associates with other proteins that are potentially important in its mechanism of regulation, including a 190-kDa Rho GTPase-activating phosphoprotein (p190). To mediate these protein-protein interactions, GAP is composed of several recognized domains including an SH3 domain flanked by 2 SH2 domains, a pleckstrin homology domain, a putative calcium-dependent binding domain and a C-terminal catalytic domain. It is interesting to note that the structure of the SH3 domain is actually modified by binding of the p190 target peptide to the flanking SH2 domains. In this project, we intend to elucidate the structure of the GAP SH3 and SH2 domains complexed with a double-phosphotyrosine peptide from p190 representing the target sequence containing Tyr1087 and Tyr1105, each of which binds to one of the two SH2 domains of GAP.

Due to a most unfortunate airline incident, during which all our flash-frozen crystals shipped in an IATA-approved container were removed from the plane and exposed to room temperature long enough to destroy most of them and to at least melt and re-freeze some of the surrounding liquid resulting in strong diffraction rings from the ice or salt crystals formed during the process. This mishap prohibited us from collecting the full equivalent of MAD data and we had to settle for data collection at the peak of the anomalous absorption curve determined during a previous synchrotron visit.

The high brilliance of the BL41XU line resulted in our best data set so far. The space group is P2, a=63.8Å, b=91.3Å, c=86.0Å; β =96.0°. Data were collected to a resolution of 2.8 Å, with an overall R-sym of 12.8% and 28% in the highest resolution shell and the completeness is 86% with 81.2% from 2.9Å to 2.8Å; I/σ is 12.2. We are presently using these data in an effort to apply molecular replacement techniques to solve the structure of this interesting complex.

As our supply of GAP crystals was so depleted we also collected data on an Fab fragment (2F5) specific for gp41 of HIV-1. The high intensity of the beam combined with the long read-out times (replacement detector) led to high radiation damage. The data were merged with those collected at the NSLS and have resulted in an excellent electron density map.