Preliminary Crystallographic Characterization of the Grb2 SH2 Domain in Complex with a FAK-derived Phosphotyrosyl Peptide

Hsiao-Hsin Chen (陳孝信), Cuei-Wen Chen (陳翠紋), Yu-Yung Chang (張裕勇), and Chun-Hua Hsu (徐駿森)

Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

Growth factor receptor-bound protein 2 **(**Grb2) is an adaptor protein with a single SH2 domain that specifically binds to focal adhesion kinase (FAK) when residue Y925 of FAK is phosphorylated. The Grb2/FAK interaction is associated with cellular integrin-activated signal transduction events leading to the activation of the Ras-MAPK pathway. Crystals of the Grb2 SH2 domain in complex with a phosphopeptide corresponding to residues 921-930 of FAK have been obtained using the sitting-drop vapour-diffusion technique.

The purified Grb2 SH2 protein was co-crystallized with the pY925 phosphotyrosyl peptide (NDKVpYENVT G) composed of residues 921–930 of human FAK. A twofold molar excess of the pY925 phosphopeptide was incubated with Grb2 SH2 (10 mg ml^{-1}) before the crystallization trials. Crystals of the Grb2 SH2 in complex with the FAK pY925 phosphotyrosyl peptide were grown by sitting-drop vapour diffusion at 298 K. A typical sitting drop was prepared by mixing 1.0 µl protein solution and an equal volume of reservoir solution.

X-ray diffraction data were collected at beamline BL13C1, equipped with a Q315 area detector, at the National Synchrotron Radiation Research Center (NSRRC) in Taiwan. The crystal was transferred into a cryo-protectant solution containing 20% glycerol in mother liquid for 5 sec, and then flash-cooled in liquid nitrogen to 100 K. Diffraction images were indexed, integrated and scaled using *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997). The crystal belongs to the orthorhombic space group $P3_121$ with unit-cell parameters $a = b = 102.7$ Å, $c = 127.6$ Å and $\alpha = \beta = 90.0°$, $\gamma = 120.0°$. Assuming the presence of six molecules of 13 kDa protein in the asymmetric unit, the calculated Matthews coefficient (V_M) value is 2.49 $Å^3$ Da^{-1}, (Matthews, 1968). The solvent content of the crystal was calculated to be 50.6%. A complete data set has been obtained to 2.49 Å, corresponding to an R_{merge} of 4.5%. Details of the data-collection statistics are summarized in Table 1.

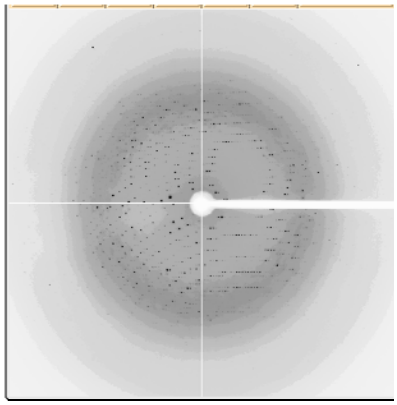

Fig. 1: The X-ray pattern of a Grb2 SH2 in complex with with a FAK-derived phosphotyrosyl peptide crystal diffracting to a resolution of 2.49 Å

Table 1 Data collection statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P3_121$
Unit cell parameters	
	102.7, 102.7, 127.6
(α, β, γ)	90°, 90°, 120°
Resolution (Å)	30-2.49 (2.56-2.49)
Unique reflections	27719 (2701)
Completeness (%)	99.8 (99.4)
R_{merge} (%)[a]	3.25 (49.7)
Average I/ σ (I)	40.7 (3.6)

[a] $R_{merge} = \Sigma_{hkl}\Sigma_i \mid I_i(hkl) - \langle I(hkl) \rangle \mid / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ are the intensities of the individual replicates of a given reflection *hkl* and $\langle I(hkl) \rangle$ is the average intensity over all replicates of that reflection.

References

[1] Z. Otwinowski and W. Minor, Methods Enzymol. **276**, 307 (1997).
[2] B. W. Matthews, J. Mol. Biol. **33**, 491 (1968).