Structural Study of Human TIFA Protein with X-ray Crystallography

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Forkhead-associated (FHA) domain is a motif with a conserved structural folding of 100-120 amino acid residues and is known to bind directly to phosphothreonine residue(s) of its binding partners *(1)*. This functional domain serves not just a solely protein-protein interaction platform but an important activation/inhibition module that participates in establishing or maintaining several essential cellular mechanisms such as cell cycle checkpoints, DNA repair, signal transduction and transcriptional regulations in both eukaryotes and prokaryotes *(1)*. The objective of this project is to characterize the function and structure of a cancer-related, FHA domain-containing human protein named TIFA (TRAF-interacting protein with a forkhead-associated domain) *(2,3)*. Tumor necrosis factor (TNF) mediated cell survival against tumor is a vital response from the immune system. TNF receptor-associated factors (TRAFs) are key molecules involved in TNF signaling *(4,5)*. TIFA protein has been shown to bind to TRAF family members, TRAF2 and TRAF6 *(2,3)*, and to activate the crucial transcriptional factor NF-κB to promote cell cycle progression *(6,7)* (Fig.1). A comprehensive functional and structural investigation on TIFA will therefore be an important approach to understand the physiological processes involved in the TNF-regulated signaling pathway. Furthermore, since NF-κB is constitutively activated in almost every type of tumor and TIFA is likely an upstream regulator of NF-κB, TIFA could also be a potential novel drug target.

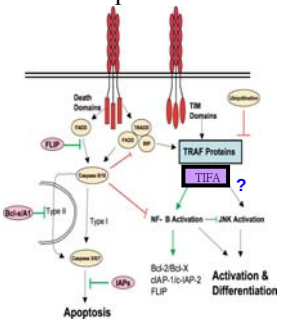
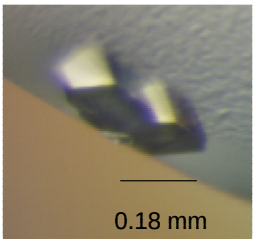


Fig. 1: The proposed molecular mechanism TIFA may be involved in (figure adapted from reference 7).

The primary focus of this project is to obtain the structural information of free TIFA and attributing such information to TIFA's biological functions. To date, all of the solved FHA structures are only the domains themselves or domains complex with short phosphopeptides. None of the current available FHA structures are from a full-length protein. Thus, the achievement of the full-length structure of TIFA will be the first demonstration about how FHA domain fits into a full-length protein.

Full-length human TIFA protein has been successfully expressed in *E.coli* system and purified with the Ni-NTA columns. In solution, TIFA appears as a dimer (overall molecular weight is about 44kD). By far, one crystal form (Fig. 2A) was obtained using the sitting vapour-diffusion method and different cryo-protecting conditions were screened. These crystals have been subjected to several synchrotron radiation shootings at NSRRC BL13B1 and BL13C1 beamlines (Fig. 2B). Data sets have been collected (Table.1) and processed using structural-solving softwares HKL2000, CNS, and MOLREP (CCPi). However, in order to solve the phase problem encountered at this moment, multi-wavelength anomalous dispersion (MAD) method will be applied. Thus, the subsequent experiment will be to purify and crystallize seleno-methionine (Se-Met) labeled TIFA proteins.

A) 

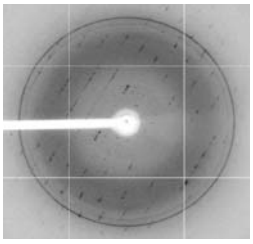
B) 

Fig. 2: A) The crystal was grown in three weeks at 4℃. B) The diffraction pattern of apo TIFA. Parameters for data collection are as follows: oscillation angle is 1.0°, exposure time is 5 sec per frame, and distance between crytal and detector is about 350mm.

	Native TIFA Crystal		
Data collection			
Space group	P321		
Cell dimensions			
a, *b*, *c* (Å)	41.21	41.21	178.91
α, β, γ (°)	90.00	90.00	120.00
Wavelength	1.0		
Resolution (Å)	2.8		
Completeness (%)	98.8		
Redundancy	8.9		

Table 1: Data collection statistics of native TIFA.

References

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