The structural adaptation of thymosin beta-4 to its interacting partners

Scientific report

The aim of the work

The goal of the project was to gain a detailed picture on how the different interactions of thymosyn beta 4 (Tβ4) are formed. Considering the conformational adaptability of disordered proteins (IDPs), we expected that its structure would be markedly different in the complexes with its different partners.
We planned to compare the structures of the complexes of Tß4-PINCH, Tß4-ILK, Tß4-stabilin-2 and Tß4-hMLH1 with that previously described for G-actin in order to understand how Tß4 chooses between its many interacting partners.

In accord, the proposed work aimed to achieve four goals:

i. in vitro characterization of the interaction of Tß4 with its partners;
ii. defining the exact structure of each complex;
iii. designing specific Tß4 mutants based on the structural data and study Tß4 function in vivo;
iv. testing the in vitro NMR data in living cells, using STINT NMR and in-cell NMR.

Results

i. We cloned and expressed Tβ4 and the interacting regions of its partner proteins (PINCH, ILK and ANK) to characterize their interaction with Tβ4 both from a kinetic and a structural point of view. We used several independent methods to investigate the interaction of Tβ4 with these partners. The methods and their brief results are listed in **Table 1**.

Table 1. Summary of the methods used to characterize the binding of Tβ4 to its partners

	BLI	Steady-state fluo-rescence	Near UV CD	Far UV CD	ITC	NMR	SAXS	Wide-line NMR
Tβ4/PINCH LIM4–5	K_D = 630 μM	Weak interaction	Interaction with local changes	No structural changes	Too low heat of reaction	Interaction without gaining structure	No major structural changes	Interaction with some buried surfaces
Tβ4/ ILK-Ank-GST	K_D = 380 μM	Weak interaction	Interaction with local changes	No structural changes	Too low heat of reaction	Interaction without gaining structure	No major structural changes	Interaction with some buried surfaces
Tβ4/PINCH LIM4–5-ILK-Ank-GST	No information	No information	Interaction with local changes	No structural changes	Too low heat of reaction	Interaction without gaining structure	No information	No information
Tβ4/Stabilin CTD	K_D = 2.8 mM	No information	Interaction with local changes	No structural changes	Small enthalpy and entropy changes	Interaction without gaining structure	Interaction without gain-ing structure	Interaction with some buried surfaces

ii. All the methods capable of detecting the interaction itself gave indication of weak complexes, but no changes could be observed with methods that provide information about the structure of the macromolecules. This observation led us to the conclusion that Tβ4 remains mostly unstructured in its complexes and forms fuzzy complexes with the partners investigated in this study. By descibing the fuzzy type of interactions, we have encountered a new paradigm of multiple fuzzy interactions of a protein in its distinct functions. The structural differences of the binding of Tβ4 to G-actin and the other partnes most probably stem from the fundamental differences between the functional outcomes of these interactions and have a regulatory role.

iii. Since the structural characterization of the fuzzy complexes is particularly difficult, and the interacting region of Tβ4 overlaps siginificantly with the region of G-actin binding, mutating Tβ4 was not feasible. Instead, we concentrated on finding other factors that might influence the binding strength and the structure of the complexes. The most reasonable approach was to express full-length proteins of the partners and in a eukaryotic system in order to reproduce post-translational modifications (PTMs) that may significantly alter the formation of the complexes. We prepared the expression constructs of full-length ILK, PINCH and Tβ4 for insect cell expression and made stably transformed cell lines. The expression of the proteins was confirmed with Western-blot, but the optimization of the purification is still under way. Once we have the proteins in pure form, we can identfy the PTMs using MS, and through the biophysical characterization of the complex formation, we can decide wether these PTMs have significant effect on the binding of Tβ4.

iv. The constructs of the different partners for the STINT NMR measurements have been prepared and cell lines harboring the two expression vectors has been generated. The expression of the two partners under control of different inducing

agents has been confirmed. NMR measurements with Tβ4 alone inside the cell has started and the optimization of the circumstances is under way. This work has not bee finished yet, because we need a good-quality in-cell NMR spectrum of Tβ4 before the in vivo titrations can start.

During the period of the project we carried out other investigations as well in the field of the disordered proteins, and we plan to continue the detailed characterization of the fuzzy type of complexes that Tβ4 forms with its partners.