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Localization of β-secretase through interaction of its cytosolic C-terminal tail with GGA-VHS

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Introduction

BACE, β-site amyloid precursor protein (APP) cleaving enzyme is a novel membrane-bound aspartic protease with the β-secretase function in the production of β-amyloid (Aβ) peptide leading to the Alzheimer's disease. Its cytosolic tail has a characteristic acidic-cluster dileucine (ACLL) motif recognized by the VHS domain of adaptor proteins GGA (Golgi-localizing, γ-adaptin ear homology domain, ARF-interacting) for exit from the *trans*-Golgi network (TGN). Here we show that BACE is colocalized with GGAs in the TGN and peripheral structures, and phosphorylation of a serine residue in the cytoplasmic tail enhances interaction with the VHS domain of GGA1 by about three folds. To reveal the difference of the affinity between phosphorylated and unphosphorylated BACE against GGA1-VHS, we report the crystal structure of the VHS domain of GGA1 in complex with a phosphorylated or unphosphorylated peptide of the BACE-tail [1].

Materials and Methods

Crystals of the GGA1 VHS domain complexed with the unphosphorylated BACE peptide were grown against a reservoir containing 20 % (w/v) PEG 5000 MME, 0.2 M $(NH_4)_2HPO_4$ and 0.1 M Tris-HCl (pH 8.4 – 9.0). Crystals of the GGA1 VHS domain complexed with the phosphorylated BACE peptide were grown against a reservoir containing 15 % (w/v) PEG 5000 MME, 0.2 M NH_4I, 0.3 M 1,6-hexanediol and 0.1 M MES-NaOH (pH 6.0 – 6.2).

A data set of the complex with unphosphorylated peptide was collected at PF-NW12. Data processing gave an R_{merge} of 0.059 for intensities and these data were 99.5 % complete (50 – 2.6 Å). A data set of the complex with phosphorylated peptide was collected at PF-BL6A. Data processing gave an R_{merge} of 0.055 for intensities and these data were 99.2 % complete (50 – 1.9 Å).

The crystal structures of the GGA1 VHS domain complexed with unphosphorylated and phosphorylated BACE peptide were solved by the molecular-replacement with the human GGA1 VHS domain structure as a search model. The final model of unphosphorylated complex has an *R*-factor of 24.4 % and an R_{free} of 29.5 %. The final model of phosphorylated complex has an *R*-factor of 21.3 % and an R_{free} of 24.5 % for the resolution range between 40 and 1.9 Å. Data processing and refinement statistics are summarized in Table 1.

Results and discussion

The X-ray crystal structures of the complex between the GGA1-VHS domain and the BACE C- terminal peptides (unphosphorylated; BACE and phosphorylated; BACE-P) illustrate a similar recognition mechanism as mannose 6-phophate receptors (MPRs) except that a glutamine residue closes in to fill the gap created by the shorter BACE peptide. The serine and lysine of the BACE peptide point their side chains towards the solvent (Fig. 1). However, phosphorylation of the serine affects the lysine side chain and the peptide backbone resulting in one additional hydrogen bond and a stronger electrostatic interaction with the VHS domain, hence the reversible increase in affinity.

Table 1 Data processing and refinement statistics

	BACE	BACE-P
Space group	*I*4	$P2_12_12_1$
Cell constants (Å)		
a	114.8	48.5
b	114.8	72.3
c	53.6	103.1
Wavelength (Å)	1.00	0.97
Observed	77,496	189,494
Unique	10,872	28,983
Resolution (Å)	50 – 2.6	50 – 1.9
R_{merge} (%)	5.9	5.5
$I/\sigma(I)$	20.7	17.6
Completeness (%)	99.5	99.2
	BACE	BACE-P
Resolution (Å)	40 – 2.6	40 – 1.9
Reflections	10,857	28,939
R_{factor} (%)	24.4	21.3
R_{free} (%)	29.5	24.5

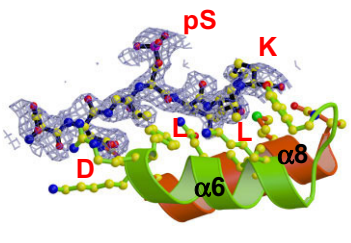

Fig. 1 Electron density map of BACE-P

References

[1] T. Shiba, et al., Traffic, 5, 437, (2004)
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