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DISASSEMBLY OF THE VITAMIN B_{12} TRANSPORT COMPLEX FROM *E. COLI*

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ATP-binding cassette (ABC) transporters constitute one of the largest membrane protein families, and prevail in all domains of life. These proteins actively transport their substrates across the lipid bilayer, and are directly related to human disease and multidrug resistance.

In E. coli, the inner membrane ABC transporter BtuCD interacts with the periplasmic binding protein BtuF to form the vitamin B12 transport complex, BtuCD–BtuF. The conventional model assumes that BtuF binds the substrate and then associates with BtuCD. Consequently, BtuCD–BtuF complex must dissociate and re-associate in a cyclic manner to resume vitamin B12 transport into the cytoplasm. However, in vitro, BtuCD–BtuF complex is extremely stable ($K_D = 1.16\times10^{-13}$ M). Hence we ask how the complex disassembles.

We aim to first assemble BtuCD–BtuF complex in vitro, and then to dismantle it. As was shown in vitro, either vitamin B12 or ATP reduces the affinity between BtuF and BtuCD. Moreover, when both ligands are present, there is no complex formation. For this reason, the substrate and the nucleotide are the prime candidates for BtuCD–BtuF complex disassembly. Another possibility is that a different molecule of BtuF (pre-loaded with vitamin B_{12}) is necessary for disassembly of the present transport complex and assembly of the next one. Also, we should take other factors – yet to be identified – into consideration.

In any case, we expect to gain insights as to how the vitamin B_{12} transport complex falls apart; and this will further our understanding of complex recycling.

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RESONANCE ASSIGNMENT OF PSBP FROM PHOTOSYSTEM II OF *SPINACIA OLERACEA*

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The PsbP is a 23 kDa extrinsic protein from Photosystem II which is located in the so called oxygen evolving center. This is a place where the fundamental reaction of photosynthesis, the water splitting, takes place. PsbP together with other extrinsic proteins regulates the concentration of the necessary cofactors – Ca^{2+} and Cl^-. The PsbP protein also induces structural changes in the thylakoid membrane upon binding which are necessary for a stable oxygen production[1]. However the mechanism of these interactions has not yet been clarified. Recent X-ray crystallographic structure has brought detailed information about the structure of PsbP. Unfortunately the N-terminus and dynamic loops are missing[2]. PsbP is therefore an ideal candidate for the study by NMR.

PsbP was expressed in *E. coli* as 6x His-tagged protein and purified using various chromatographic techniques (affinity, ion - exchange, size - exclusion). In order to study protein by NMR, one needs the complete assignment of its resonances. A set of 2D and 3D NMR experiments was recorded: ^{15}N HSQC, ^{13}C HSQC, HNCACB, CBCA(CO)NH, HNCO, HCCH - COSY, H(CC)(CO)NH, (H)CC(CO)NH, (H)CCH – TOCSY and ^{15}N NOESY – HSQC. Using these spectra, 91% of the backbone and non-aromatic side chain chemical shifts have been assigned up to this date. Another step is to derive 3D solution structure of PsbP and continue with the study of interactions between Psb extrinsic proteins.

1. Bricker et al., *Biochim Biophys Acta*, **2012**, 1817, 121 - 142.
2. Kopecky et al, *PloS One*, **2012**, 7, e46694.

This research was supported by the Austrian Science Funds (project M1404 to KC), and the European Union through the EFRE INTERREG IV ETC-AT-CZ program (project M00146 "RERI-uasb") and by OEAD through the Aktion program AT-CZ (project nr. 69p26 to NM and JK).