Detergent n-octyl-β-D-maltoside (OM) induces both monomerization and oligomerization of Bcl-w

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Introduction

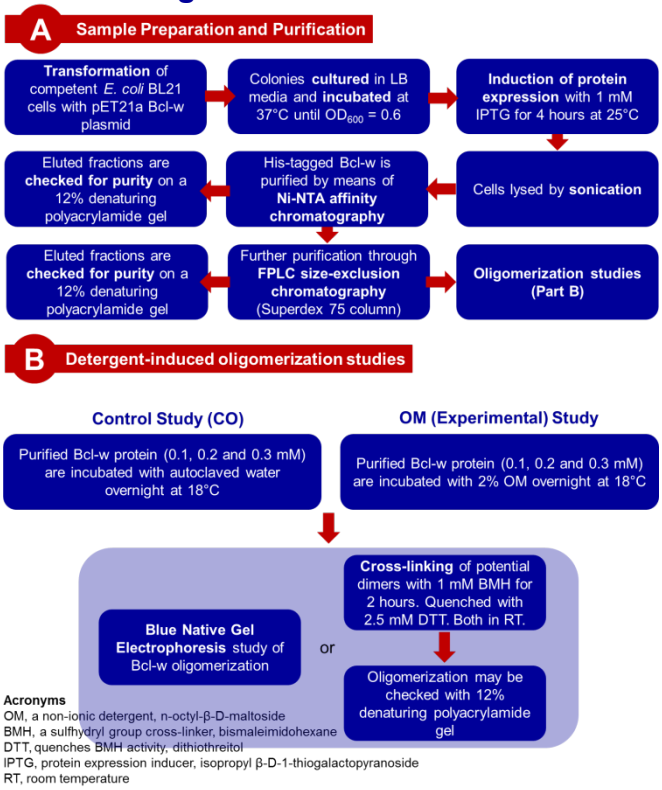
The process of apoptosis, programmed cell death, is principally mediated by the Bcl-2 family of proteins. Some of the Bcl-2 family members promote apoptosis, while others repress it. This protein tug-of-war commands whether the cell continues to live (anti-apoptotic) or is to die (pro-apoptotic).

Bcl-w is an anti-apoptotic member of the Bcl-2 family. Bcl-w-ΔC15 (P117V), the recombinant form of the natural Bcl-w protein being studied, has 8 α-helical domains and has 178 amino acids (ΔC15). The molecular weight of Bcl-w is approximately 20 kDa and its theoretical isoelectric point (pI) is 5.82 [1]. Bcl-w is reported to dimerize naturally – i.e., without the need of inducing the dimers per se [2]. The dimers are described to form by the domain-swapping mechanism. It was also observed that the usually truncated C-terminal residues of the Bcl-w protein play a key role on Bcl-w's biological activity [3].

As the structure of Bcl-w (and its dimerization capacity) is linked to its function, and as Bcl-w was found to be misregulated in Alzheimer's disease [4] and other forms of neuropathy [5], it would be interesting to characterise the oligomerization properties of Bcl-w in different environments.

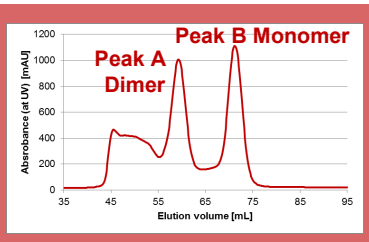
This poster describes the progress of the research into the oligomerization characteristics of the Bcl-w protein. Samples of purified Bcl-w protein was incubated with 2% n-Octyl-β-D-maltoside (OM) detergent overnight and blue-native gel electrophoresis was run to determine the dimerization characteristics of the protein. OM was utilised to mimic the membrane environment that was reported to cause the oligomerization of other members of the Bcl-2 family of proteins [6].

Methodologies



Acronyms
OM, a non-ionic detergent, n-octyl-β-D-maltoside
BMH, a sulfhydryl group cross-linker, bismaleimidohexane
DTT, quenches BMH activity, dithiothreitol
IPTG, protein expression inducer, isopropyl β-D-1-thiogalactopyranoside
RT, room temperature

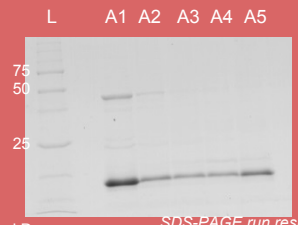
Preliminary Results



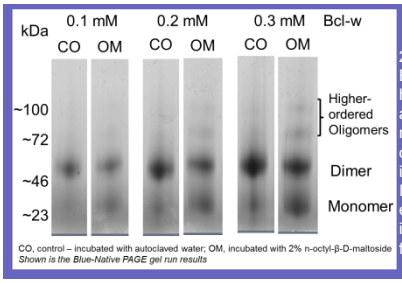
1. Conformational Changes of Bcl-w

Elution chromatograph of size exclusion purification of Bcl-w through FPLC (Superdex 75 column). Dimerization, as reported by Lee et al (2011) is observed.

Confirmation that the Peak A aliquots is an oligomer of the other by denaturing gel electrophoresis. As SDS-PAGE denatures proteins and breaks dimers, having the presence of a more prominent band at the elutes of Peak A indicates that the oligomer can be seprated into a monomer.



SDS-PAGE run results
Peak A: Elutes A1
Peak B: Elutes A2-5



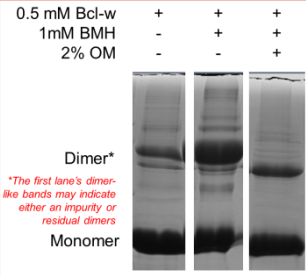
CO, control – incubated with autoclaved water; OM, incubated with 2% n-octyl-β-D-maltoside
Shown is the Blue-Native PAGE gel run results

2. The Detergent Effect

2% OM-incubated 0.2 and 0.3 mM Bcl-w forms more monomers and higher-ordered oligomers (albeit to a lesser extent). Bands of the monomeric Bcl-w intensified, and oligomeric Bcl-w bands appeared in OM-incubated samples. Increased monomerization was not expected as OM is known to induce dimerization in other Bcl-2 family members.

3. Cross-linking Studies

BMH cross-linking studies also suggests that 2% OM induces monomerization of the Bcl-w dimer. As seen here, the addition of 2% OM reduces the amount of Bcl-w cross-linked dimer. Although higher-order oligomeric Bcl-w was not clearly observed, its existence cannot be ruled out as the sulfhydryl groups involved in cross-linking may not be "available" (due to distance or sterical constraints) for BMH to crosslink in these higher-ordered oligomers.



Future Work

- No conclusions may be made from the current results as there are concerns regarding the purity of the Bcl-w samples.
- Optimization of the purification process and identification of the non-specific, dimer-like band (as seen in Cross-linking Studies gel, lane 1)
- More runs to confirm if OM indeed induces Bcl-w monomerization
- Further studies on dimerization properties will be performed (e.g., pH, temperature and ligand-induced dimerizations)

References. [1] E. Gasteiger et al., in The Proteomics Protocols Handbook, J. M. Walker, Ed., Humana Press, 2005, pp. 571-607. [2] E. F. Lee et al., Structure (19), pp. 1467-1676, 12 October 2011. [3] M. G. Hinds, et al., The EMBO Journal (22), no. 7, pp. 1497-1507, 2003. [4] X. Zhu, et al., Journal of Neurochemistry (89), pp. 1233-1240, 2004. [5] S. L. Courchesne, et al., The Journal of Neuroscience (31), no. 5, pp. 1624-1634, 2011. [6] F. Yu, et al., The Journal of Biochemistry (143), pp. 243-252, 2008. [7] C. D. Tiu, Research Report on the URECA Project, Molecular Characterization of Bcl-w, an anti-apoptotic protein, 2013.

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