Expression, purification and characterisation of novel bacterial ADP-ribosyltransferases

Abdula Habib[1], M.Sc. Moona Sakari[2], Ph.D. Arto Pulliainen[2]

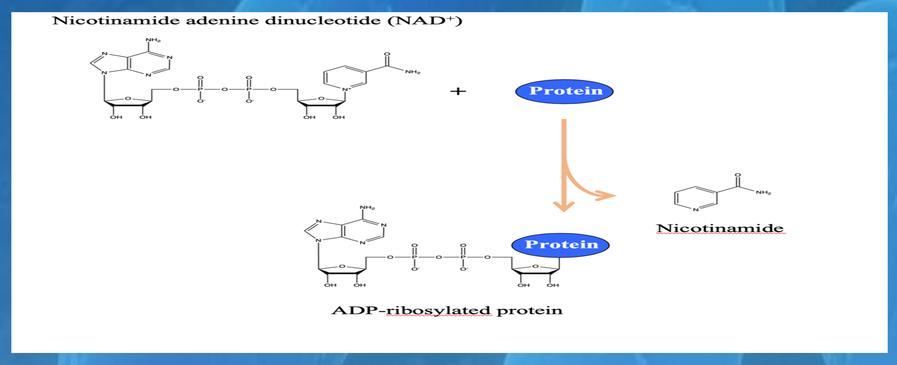
[1]Department of Life Technologies, University of Turku

[2]Institute of Biomedicine, University of Turku

MOLECULAR BIOSCIENCES, BIOCHEMISTRY

1. Introduction

- ADP-ribosylation is a chemical modification of covalently adding adenosine diphosphate ribose (ADPr) to target substrates using the cofactor NAD^+ (Scheme 1.).
- It was identified as a mechanism for bacterial pathogens to attack their hosts though secretion of exotoxins. These toxins are enzymes called bARTTs for bacterial ADP-ribosyltransferase toxins.
- They are comprised of A and B subunits; B subunit recognizes cell surface receptors and mediate host cell entry while A subunit is the enzyme responsible for catalyzing the attachment of ADPr to its target substrate inside the cell.
- Our laboratory utilizes shotgun metagenomics to identify new bARTTS.
- We have succeeded in identifying two new enzymes, CtxA from *Bartonella* and YER from *Yersinia.*

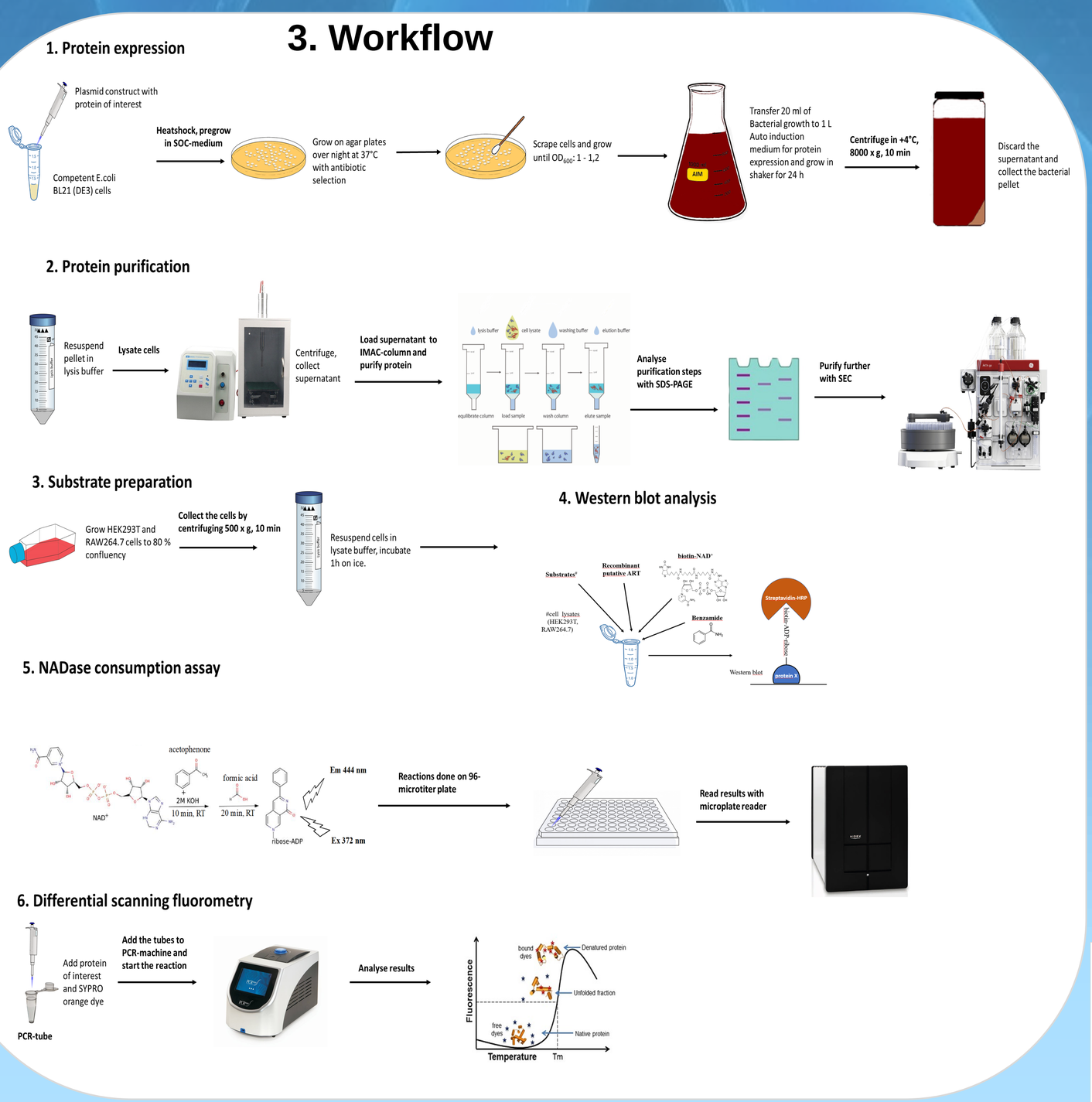


Scheme 1. ADP-ribosylation reaction. ADP-ribosyltransferases catalyse the attachment of ADPr to target proteins using NAD^+. Nicotinamide gets cleaved off.

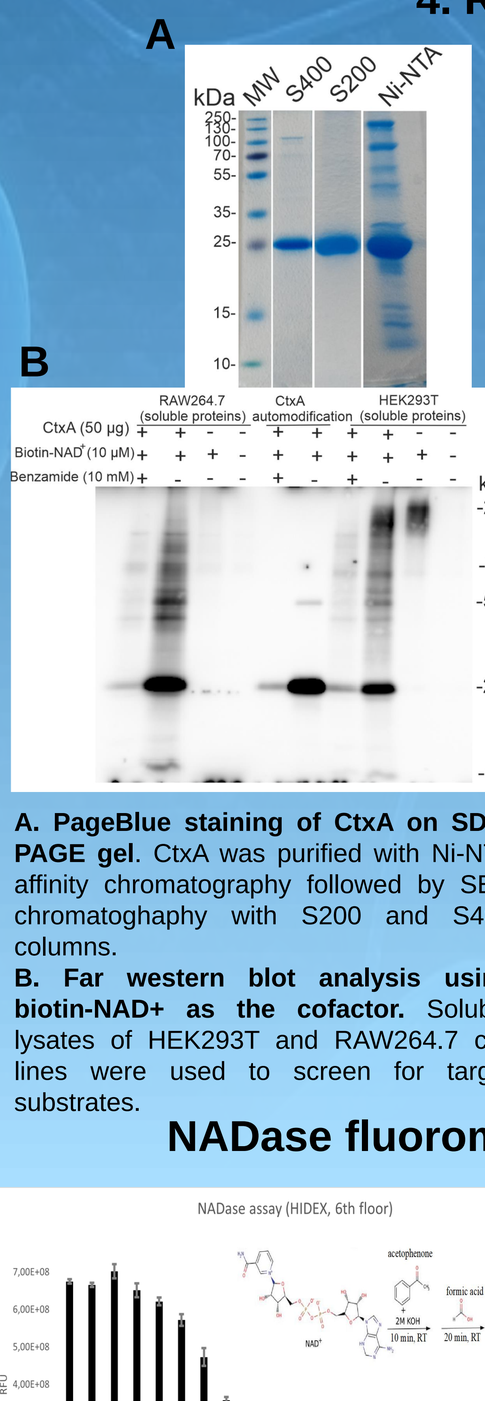
2. Aim of the study

1) Express in *E.coli* and purify the His-tagged CtxA and YER proteins with Ni-NTA affinity chromatography followed by size-exclusion chromatography.
2) Study the fold of the proteins with differential scanning fluorometry (DSF).
3) Study auto and trans-ADP-ribosylation activity of the proteins with western blot assays
4) Study NAD^+ consumption of the proteins with end point fluorometric homogenous assay

3. Workflow



4. Results



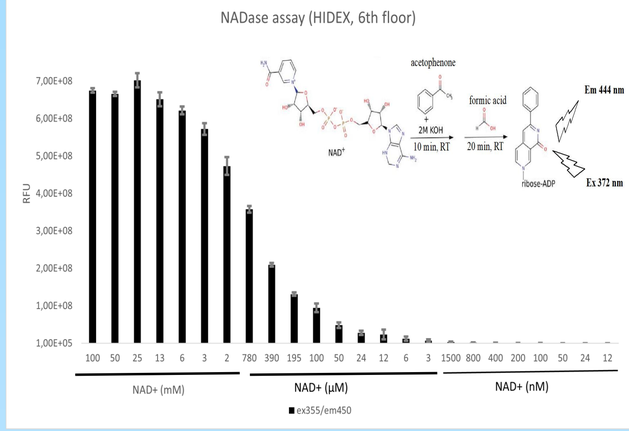
A. PageBlue staining of CtxA on SDS-PAGE gel. CtxA was purified with Ni-NTA affinity chromatography followed by SEC chromatoghaphy with S200 and S400 columns.

B. Far western blot analysis using biotin-NAD+ as the cofactor. Soluble lysates of HEK293T and RAW264.7 cell lines were used to screen for target substrates.

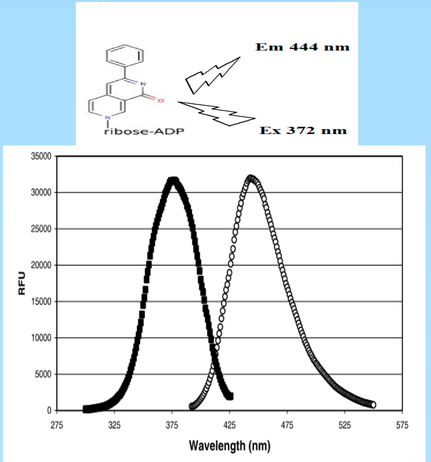
C. PageBlue staining of YER on SDS-PAGE gel. YER was purified with Ni-NTA affinity chromatography

D. Far western blot analysis using biotin-NAD+ as the cofactor. Soluble lysates of HEK293T and RAW264.7 cell lines were used to screen for target substrates.

NADase fluorometric assay



NADase assay reaction with no enzymes added. The end-point assay is based on converting NAD^+ to a stable detectible fluorophore with emission maximum at 444 nm. Since the optimal optimal filters were not found, we read the plate using three instruments with different exitation and emission filters. Above is an example how the plate looks on Hidex Sense –plate reader with emission maximum at 450 nm.



The chemical structure, fluorescence exitation (black curve) and emission (white curve) of the the fluorophore. NAD^+ is converted to 3-phenyl-2,7-naphthyridin-1(7H)-one with acetophenone in base and stabilizing the fluorophore with formic acid.

5. Conclusions & future aspects

- Both YER and CtxA have robust automodification activity. The ADP-ribosylation reaction could not be inhibited with benzamide in the case of YER. This could be due to the structure of the catalytic site.
- CtxA show substrate activity in mammalian cells incidating that it has potential target substrates.
- In the future we will study the fold of the proteins and NAD^+ consumption with microplate read-out. Also we are on our way to express and purify mutated forms of CtxA for further analysis