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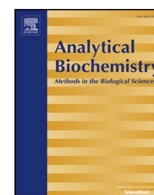

An ELISA method to estimate the mono ADP-ribosyltransferase activities: e.g in pertussis toxin and vaccines

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Abbreviations:
ADP
Adenosine diphosphate
ABTS
2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
BSA
bovine serum albumin
D
diphtheria toxoid
ELISA
Enzyme-linked immunosorbent assay
DTT
dithiothreitol
EU
ADP-Ribosyltransferase enzymatic unit
FHA
filamentous haemagglutinin
Fims2/3
fimbriae type 2 and type 3
F
formaldehyde
Hib
Haemophilus influenzae type b
HPLC
high performance liquid chromatography
His
histidine
HIST
histamine sensitisation test
HRP
horseradish peroxidase
H_2O_2
hydrogen peroxide
IPV
inactivated poliovirus vaccine
LPC
lysophosphatidylcholine

ABSTRACT

ADP-ribosyltransferase activities have been observed in many prokaryotic and eukaryotic species and viruses and are involved in many cellular processes, including cell signalling, DNA repair, gene regulation and apoptosis. In a number of bacterial toxins, mono ADP-ribosyltransferase is the main cause of host cell cytotoxicity. Several approaches have been used to analyse this biological system from measuring its enzyme products to its functions. By using a mono ADP-ribose binding protein we have now developed an ELISA method to estimate native pertussis toxin mono ADP-ribosyltransferase activity and its residual activities in pertussis vaccines as an example. This new approach is easy to perform and adaptable in most laboratories. In theory, this assay system is also very versatile and could measure the enzyme activity in other bacteria such as Cholera, Clostridium, *E. coli*, Diphtheria, Pertussis, Pseudomonas, Salmonella and Staphylococcus by just switching to their respective peptide substrates. Furthermore, this mono ADP-ribose binding protein could also be used for staining mono ADP-ribosyl products resolved on gels or membranes.

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mAb
Monoclonal antibody
NAD
nicotinamide adenine dinucleotide
NAAD
nicotinic acid adenine dinucleotide
OD
optical density
OVA
ovalbumin
PRN
pertactin
PTd
Pertussis toxoid
PTx
Pertussis toxin
PBS
phosphate buffered saline
SHD
single human dose
T
tetanus toxoid
TBS
Tris buffer saline

Introduction

ADP-ribosyltransferase activity has been observed in many prokaryotic and eukaryotic species, viruses and bacterial toxins [1–4]. Its action is to add one (mono) or more (poly) ADP-ribose moiety from NAD+ to acceptor proteins and is a reversible post-translational modification that is involved in many cellular processes, including cell signalling, DNA repair, gene regulation and apoptosis [5–10]. Haag and Buck [10] provided a good review on many approaches that have been used to analyse this biological system from measuring its enzyme products to its cellular function: (a) using labelled NAD+ for identification of the ADP-ribosylated products, (b) determining the specificity of ADP-ribosylation sites, (c) detection of endogenous ADP-ribosylated proteins, (d) using anti-ADP-ribosyl antibodies and ADP-ribosyl binding proteins [10]. Karras and colleagues [11] exploited a family of specific ADP-ribosyl binding proteins, termed macro domains, to identify a series of cellular targets resulting from the actions of ADP-ribosyltransferases and toxins by “pulling down” the ADP-ribosylated products from a reaction mixture and analysing them by mass spectrometry. Macro domains are high-affinity ADP-ribose binding proteins and are associated with a diverse range of cellular processes such as transcriptional regulation, chromatin remodelling and developmental processes [12–17]. However, these methods have not been widely adopted.

Using a recombinant substrate, Venkannagari et al. [18] established a 96-well activity-based inhibition assay for human mono-ADP-ribosyltransferases ARTD7/PARP15 andARTD10/PARP10 aimed at screening and profiling inhibitors for these two enzymes to evaluate NAD+ analog drugs which could be used for cancer treatment [18]. ARTD10/PARP10 ADP-ribosylates core histones and also interacts with the oncoprotein MYC [19,20]. It has been suggested to have a role in cell proliferation, which makes it a possible target for inhibitor development against cancer [21,22]. This assay measures the amount of NAD + present after chemically converting it to a fluorescent analog.

During our long standing quest for an assay system to detect and measure the quantities of mono-ADP ribosylated protein/peptide products, we have tried a chemical fluorescence-labelling approach and in-house prepared polyclonal antibodies against mono to poly ADP-ribose. They worked but lacked sensitivity and were also a challenge to attain the required consistency (unpublished results). There is an anti-poly ADP-ribose (anti-PAR) monoclonal antibody which is commercially available for use in ELISA and western blots. Unfortunately, in our hand, this antibody has only weak affinity in our assay system which requires the detection of mono-ADP-ribosyl products.

In this report, we describe the use of a recombinant macro domain AF1521 from *Archaeoglobus fulgidus* [12] as a new and novel approach to develop a 96-well enzymatic-ELISA (E-ELISA) assay system to analyse the activity of mono-ADP ribosyltransferase in bacterial toxins and their residual activities in vaccines using pertussis toxin as an example.

Pertussis vaccines contained detoxified pertussis toxin (PTx) and the current safety test is the *in vivo* histamine sensitisation test (HIST) [23]. HIST is a lethal test and large numbers of animals are used. In addition to ethical problem with HIST, this assay has large assay variability and is difficult to standardize [24]. Therefore an *in vitro* biochemical test system has been developed as a potential alternative to HIST based on measuring the residual activities of both of the functional domains of PTx: a carbohydrate-binding assay to measure the host-cell binding activity of the B-oligomer and an enzyme coupled-HPLC (E-HPLC) to determine the mono-ADP-ribosyltransferase activity of the toxin in vaccines [25]. Although this approach to determine bacterial toxin activities has been shown to be valuable [26], the requirement of HPLC to measure the enzyme activity has proved to be less efficient compared to the conventional 96-well ELISA system of the carbohydrate binding assay. Therefore it would be very advantageous if there is a 96-well plate based method to detect the mono-ADP-ribosylated enzyme product.

Materials and methods

Production of AF1521

The DNA sequence encoding the AF1521 (192 residues; 7-stranded β sheet sandwiched by 4 α helices) [12] with a thrombin cleavable N-terminal 6-histidine (HIS) tag, optimised for *E. coli* k12 expression, was synthesised (IDT, Belgium) and cloned into pET-21a (Novagen). The AF1521/pET-21a plasmid was transformed into BL21 (DE3) *E. coli* cells (Novagen, 70235-3). Cells were grown in M9Y medium at 37 °C to an OD_{600nm} of 0.6 and then AF1521 expression was induced by the addition of 1 mM final IPTG and cultures grown at 37 °C for a further 4 h. Cells were lysed by homogenisation in a glass/Teflon homogeniser (10 passes) after re-suspension in xTractor buffer (Clontech, 635671) with 1 mg/ml final lysozyme and 25 units/ml final Benzonase nuclease (Novagen, 70746-3). The His-tagged AF1521 was then purified using TALON resin (Clontech, 635504) following the manufacturers ‘native conditions’ protocol and the eluted protein was dialysed into 50 mM Trizma, 150 mM NaCl pH 8.0.

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