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ADP-ribosylation of guanosine by SCO5461 protein secreted from *Streptomyces coelicolor*

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The *Streptomyces coelicolor* A3(2) genome encodes a possible secretion protein, SCO5461, that shares a 30% homology with the activity domains of two toxic ADP-ribosyltransferases, pierisins and mosquitocidal toxin. We found ADP-ribosylating activity for the SCO5461 protein product through its co-incubation with guanosine and NAD^+, which resulted in the formation of N^2-(ADP-ribos-1-yl)-guanosine (^{ar2}Guo), with a K_m value of 110 μM. SCO5461 was further found to ADP-ribosylate deoxyguanosine, GMP, dGMP, GTP, dGTP, and cyclic GMP with k_{cat} values of 150–370 s^{-1}. Oligo(dG), oligo(G), and yeast tRNA were also ADP-ribosylated by this protein, although with much lower k_{cat} values of 0.2 s^{-1} or less. SCO5461 showed maximum ADP-ribosylation activity towards guanosine at 30 °C, and maintained 20% of these maximum activity levels even at 0 °C. This is the first report of the ADP-ribosylation of guanosine and guanine mononucleotides among the family members of various ADP-ribosylating enzymes. We additionally observed secretion of the putative gene product, SCO5461, in liquid cultures of *S. coelicolor*. We thus designated the SCO5461 protein product as *S. coelicolor* ADP-ribosylating protein, ScARP. Our current results could offer new insights into not only the ADP-ribosylation of small molecules but also signal transduction events via enzymatic nucleoside modification by toxin-related enzymes.

Abbreviations: MTX, mosquitocidal toxin from *Bacillus sphaericus* SSII-1; dGuo, 2′-deoxyguanosine; Guo, guanosine; ^{ar2}Guo, N^2-(ADP-ribos-1-yl)-guanosine; ^{r2}Guo, N^2-(ribos-1-yl)-guanosine; NAD^+, β-nicotinamide adenine dinucleotide; cGMP, guanosine 3′,5′-cyclic monophosphate.

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1. Introduction

ADP-ribosylation is the post-translational modification of proteins and involves the transfer of an ADP-ribose moiety from β-nicotinamide adenine dinucleotide (NAD^+) to specific residues in target proteins. Mono-ADP-ribosyltransferase activity is well-known to be present in several bacterial toxins that effectively target G proteins, elongation factors, and actins [see (Aktories and Just, 2000) for review]. Emerging studies have also revealed the existence of non-toxic mono-ADP-ribosyltransferases. Nitrogenases in *Azospirillum brasilense*, *Azospirillum lipoferum*, and *Rhodospirillum rubrum* are regulated by dinitrogenase reductase ADP-ribosyltransferase (DraT) and dinitrogenase reductase-activating glycohydrolase (DraG) during nitrogen fixation (Huergo et al., 2009; Masepohl and Hallenbeck, 2010), and vertebrate ecto ADP-ribosyltransferases (ARTs) target human neutrophil peptide-1 and cell surface $P2X_7$ receptors (Scheuplein et al., 2009; Stevens et al., 2009). Poly(ADP-ribose) polymerase 10 (PARP-10/ARTD10) also shows mono-ADP-ribosylation activity towards histones (Messner and Hottiger, 2011).

Some of the ADP-ribosyltransferases also target non-protein molecules. The pierisins, originally identified from *Pieris rapae* and *Pieris brassicae* as proteineous toxin against mice and cell lines (Marsh and Rothschild, 1974; Feltwell, 1982; Watanabe et al., 1999), target the N^2 amino groups of 2′-deoxyguanosine in double stranded DNA, causing mutations and an apoptotic response in cultured cells (Carpusca et al., 2006; Matsumoto et al., 2008; Orth et al., 2011; Yamamoto et al., 2009). The non-toxic CARP-1 from shellfish *Meretrix lamarckii* also target the same bases of DNA *in vitro* (Nakano et al., 2006). In contrast, tRNA 2′-phosphotransferases initially ADP-ribosylate a 2′-phosphate at the splice junction of pre-tRNA, then remove it by forming ADP-ribose 1″-2″ cyclic phosphate, resulting in the formation of a correct tRNA anticodon loop (Kato-Murayama et al., 2005; Sawaya et al., 2005; Steiger et al., 2005). Some small molecules can also be targets for ADP-ribosylation. For example, both Arr and Arr2 from opportunistic pathogens inactivate rifampicin through ADP-ribosylation (Baysarowich et al., 2008). In addition, some ADP-ribosyltransferases show low NAD^+ glycohydrolase activity that targets water molecules.

We observed from a BLAST search that the SCO5461 protein product, annotated as a secretion protein in the genome of *Streptomyces coelicolor* A3(2) (Bentley et al., 2002), shares homology with the activity domains of the pierisins and the mosquitocidal toxin from *Bacillus sphaericus* SSII-1 (MTX). MTX is an NAD^+:arginine ADP-ribosyltransferase that kills mosquito larvae (Schirmer et al., 2002a,b; Thanabalu et al., 1993), whereas pierisins are NAD^+:DNA(guanine-N^2) ADP-ribosyltransferases that induce apoptosis or gene mutation in mammalian cells in culture and *in vivo* (Shiga et al., 2006; Takamura-Enya et al., 2001; Totsuka et al., 2003; Watanabe et al., 2004). *Streptomyces* are gram-positive, soil-bacteria, and are unique organisms in terms of their metabolite profiles, most notably in relation to antibiotics, and in their properties as soil cleaners (Chater et al., 2010; Hodgson, 2000). In our present study, we demonstrated the ADP-ribosylating activity of SCO5461 and found that it has strong activity against the N^2 amino groups of guanine residues in nucleosides and mononucleotides. This is therefore the first report of an ADP-ribosyltransferase that mainly targets nucleosides, mononucleotides, and their 5′-phosphorylated forms. We also discuss the physiological roles of the ADP-ribosylation of nucleosides and mononucleotides.

2. Materials and methods

2.1. Bacterial strains, culture conditions, and a plasmid

S. coelicolor A3(2) M145 ($SCP1^-SCP2^-$) was grown on Tryptic Soy Broth (Difco, Detroit, MI), with shaking in a Sakaguchi-flask at 28 °C. *Escherichia coli* K-12 JM109 (Toyobo, Osaka, Japan) was grown on LB for subcloning; *E. coli* K-12 ER2508 (New England Biolabs, Ipswich, MA) was grown on Terrific Broth for protein expression. A plasmid vector, pMALp2x (New England Biolabs), was used for subcloning and protein expression.

2.2. cDNA subcloning and expression of ScARP

We performed genome DNA extraction, PCR cloning and subcloning of cDNA using standard protocols (Kieser et al., 2000; Sambrook and Russell, 2001). SCO5461 and SCO5461(43–204) genes were ligated into pMALp2x. We introduced point mutations into these genes via overlap-PCR (Nakano et al., 2006). Proteins encoded in pMALp2x vectors were expressed as maltose-binding protein (MBP)-fused products in *E. coli* (Riggs, 1990). Following affinity purification, the MBP tag was cleaved from these recombinant products with factor Xa protease, followed by Mono-S column chromatography. Details of all of these procedures are included with the Supporting information.

2.3. ADP-ribosylation of nucleic acids

The standard reaction conditions employed for nucleosides and mononucleotides were as follows: nucleosides (final 1 mM) were incubated with SCO5461(43–204) protein (final 0.2 nM) and NAD^+ (final 0.01–3 mM) in 200 μl of 50 mM Hepes–NaOH pH 7.0 and 50 mM NaCl, for 10 min at 30 °C. The reaction mixture was immediately injected into an HPLC column. When oligo- or polynucleotides were used as substrates, reacted nucleotides (final 0.1 mg/ml) were injected into HPLC columns after digestion with micrococcal nuclease, phosphodiesterase II, and alkaline phosphatase (Nakano et al., 2006). The products were quantified from the A_{257} values in a standard curve generated using an equimolar mixture of ADP-ribose and Guo. Details of the digestion and HPLC conditions are included with the Supporting information.

2.4. Chemical synthesis of N^2-(D-ribofuranos-1-yl)-guanosine

The chemical synthesis of N^2-(D-ribofuranos-1-yl)-guanosine was performed in accordance with the synthesis route determined previously for N^2-(D-ribofuranos-1-yl)-2′-deoxyguanosine (Takamura-Enya et al., 2001). The