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*Tc*PARP: A DNA damage-dependent poly(ADP-ribose) polymerase from *Trypanosoma cruzi*

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Abstract

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme present in most eukaryotes and has been involved in processes such as DNA repair and gene expression. The poly(ADP-ribose) polymer (PAR) is mainly catabolised by poly(ADP-ribose) glycohydrolase. Here, we describe the cloning and characterisation of a PARP from *Trypanosoma cruzi* (*Tc*PARP). The recombinant enzyme ($M_r = 65$) required DNA for catalytic activity and it was strongly enhanced by nicked DNA. Histones purified from *T. cruzi* increased *Tc*PARP activity and the covalent attachment of [^{32}P]ADP-ribose moieties to histones was demonstrated. *Tc*PARP required no magnesium or any other metal ion cofactor for its activity. The enzyme was inhibited by 3-aminobenzamide, nicotinamide, theophylline and thymidine but not by menadione. We demonstrated an automodification reaction of *Tc*PARP, and that the removal of attached PAR from this protein resulted in an increase of its activity. The enzyme was expressed in all parasite stages (amastigotes, epimastigotes and trypomastigotes). When *T. cruzi* epimastigotes were exposed to DNA-damaging agents such as hydrogen peroxide or β-lapachone, PAR drastically increased in the nucleus, thus confirming PAR synthesis in vivo and suggesting a physiological role for PARP in trypanosomatid DNA repair signalling.

Keywords: *Trypanosoma cruzi*; PARP; DNA repair signalling; PARG

1. Introduction

Poly(ADP-ribosyl)ation of nuclear proteins, especially histones, is a post-translational modification that was originally found to be induced by DNA strand breaks. This modification establishes a molecular link between DNA damage and chromatin structure remodelling. Poly(ADP-ribose) polymerase (PARP) catalyses the transfer of an ADP-ribose moiety from NAD^+ to a glutamate, an aspartate or a carboxyterminal lysine residue of target proteins. Subsequently, this enzyme is responsible for the extension and branching of poly(ADP-ribose) (PAR) chains, to produce polymers of a length up to 200 units with multiple branching points (Ueda and Hayaishi, 1985; D'Amours et al., 1999; Ame et al., 2004; Diefenbach and Burkle, 2005; Kim et al., 2005; Hassa et al., 2006; Schreiber et al., 2006). The catabolism of PAR is mediated primarily by poly(ADP-ribose) glycohydrolase (PARG), an enzyme with exo- and endoglycosidase activity which generates free ADP-ribose (Brochu et al., 1994; Davidovic et al., 2001; Bonicalzi et al., 2005).

PARPs constitute a large family of at least 17 protein members in humans, encoded by different genes and showing a conserved catalytic domain. According to the amino acid sequence similarity in this domain, Otto et al. (2005) divided the family into five groups. Other classifications group the members using the functional domains as criteria (Ame et al., 2004; Schreiber et al., 2006). The catalytic

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domain comprises a NAD^+-binding fold composed of six β-strands and one α-helix. There is a catalytic triad: H (histidine)–Y (tyrosine)–E (glutamic acid), which is conserved in PARP-1, PARP-2, PARP-3, vPARP and Tankyrases 1 and 2 (Otto et al., 2005).

The structure of PARP-1 has been investigated in depth and comprises an N-terminal DNA-binding domain (DBD), a regulatory domain, a WGR domain and a C-terminal catalytic domain (Otto et al., 2005; Schreiber et al., 2006). PARP-2 is shorter and resembles an N-terminal truncated PARP-1 with a short basic amino terminal. This enzyme displays an automodification reaction and properties similar to those of PARP-1 (Ame et al., 1999; Diefenbach and Burkle, 2005). PARP-1 and PARP-2 are the only members of the family that are highly activated upon DNA damage.

The targets of PARP-1 activity include PARP-1 itself, PARP-2, core histones (mainly H2B), the linker histone H1, high-mobility group (HMG) proteins, DNA topoisomerases 1 and 2, and DNA polymerase β. In addition, X-ray repair cross-complementing factor 1 (XRCC1) and a variety of transcription-related factors interact with PARP-1. A prevailing opinion on the mechanisms leading to PARP activation is that the activity requires the binding of the enzyme to damaged DNA. However, DNA hairpins, cruciform and stably unpaired regions are all effective coactivators of poly (ADP-ribosyl)ation of histone H1 and PARP-1 automodification (Lonskaya et al., 2005; Potaman et al., 2005).

The models that have been proposed to explain the role of PARP during DNA repair in living cells were widely reviewed by D'Amours et al. (1999). PARP is one of the first nuclear factors to recognise lesions in DNA and PAR synthesised at the site of a DNA strand break could dissociate histones from DNA, thus granting the DNA repair machinery access to damaged DNA. Several recent studies have extended the knowledge on the roles played by PAR, PARP-1 and some other PARPs in diverse molecular and cellular processes, including DNA damage detection and repair, chromatin modification, transcription, cell death pathways and a structural role in the mitotic spindles (D'Amours et al., 1999; Ziegler and Oei, 2001; Tulin et al., 2002; Kraus and Lis, 2003; Chang et al., 2004; Kim et al., 2004; Diefenbach and Burkle, 2005; Faraone-Mennella, 2005; Hassa et al., 2005, 2006; Malanga and Althaus, 2005; Petermann et al., 2005; Gagne et al., 2006). In addition, different mechanisms were observed in transcriptional activation. The first of those affects many genes located in the same locus and operates by loosening the chromatin structure after histone and HMG protein modification; the second acts on individual genes through the participation of PARP in promoter/enhancer binding complexes functioning as transcriptional coactivators (D'Amours et al., 1999; Kraus and Lis, 2003; Hassa et al., 2005). Several reports also point out that PARP could be involved in the differentiation state of cells, through its chromatin remodelling capacity and genomic regulation (Schreiber et al., 2006).

We previously reported the presence of PARP in the trypanosomatid *Crithidia fasciculata* (Villamil et al., 2001; Podesta et al., 2004). Here, we present the cloning and biochemical characterisation of a PARP from *Trypanosoma cruzi*, the aetiological agent of Chagas' disease in Latin America. In addition, we demonstrate that the enzyme is activated in vivo in response to DNA damage.

2. Materials and methods

2.1. Materials

All radiochemicals used in this work were purchased from Dupont NEN Life Science Products Inc., Boston, MA and restriction endonucleases were from New England Biolabs Inc., Beverly, MA. Bacto-tryptose and liver infusion were from Difco Laboratories, Detroit, MI. All other reagents were purchased from Sigma Chemical Co., St. Louis, MO. β-lapachone: (3-4-dihydro-2,2-dimethyl-2*H*-napho [1,2-*b*] pyran-5,6- dione) was a gift from Dr. Marta Dubin (School of Medicine, University of Buenos Aires, Argentina).

2.2. Parasites cultures and cell extracts

Trypanosoma cruzi amastigotes and trypomastigotes were kindly supplied by Ms. Berta Franke de Cazzulo from the University of San Martin, Buenos Aires, Argentina. *Trypanosoma cruzi* epimastigote forms (CL Brener strain) were cultured at 28 °C for 7 days in liver infusion tryptose (LIT) medium (5 g l^{-1} liver infusion, 5 g l^{-1} bacto-tryptose, 68 mM NaCl, 5.3 mM KCl, 22 mM Na_2HPO_4, 0.2% (W/V) glucose, and 0.002% (W/V) hemin) supplemented with 10% (V/V) FCS, 100 U ml^{-1} penicillin and 100 mg l^{-1} streptomycin. Cell viability was assessed by direct microscopic examination. Cells were harvested by centrifugation at 750*g* and 4 °C, washed three times with PBS and resuspended at 10 g ml^{-1} of wet weight in buffer A: 50 mM Tris–HCl, pH 8.0, 1.0 mM EDTA, 10% (V/V) glycerol, 10 mM 2-mercaptoethanol, containing protease inhibitors: 1 μg ml^{-1} *trans*-epoxysuccinyl-L-leucylamido(4-guanidino) butane (E-64), 1 mM pepstatine A, 1 mM phenylmethylsulfonylfluoride (PMSF), and 0.1 mM *N*α-*p*-tosyl-L-lysine chloro-methyl ketone (TLCK). Cells were lysed in an Ultrasonic Processor Model W385 Sonicator (Heat Systems-Ultrasonic Inc. Plainview, IL, NY, USA) and centrifuged for 30 min at 27000*g* and 4 °C. The supernatant thus obtained was used as a protein source for Western blot analysis and activity measurement.

2.3. Histone preparation

Frozen epimastigotes (5×10^8 parasites) were resuspended in 1 ml of 10 mM potassium glutamate, 250 mM sucrose, 2.5 mM $CaCl_2$ and lysed by the addition of 0.1% Triton X-100 containing protease inhibitors as described above. The suspension was centrifuged, washed