POLY-ADP-RIBOSYLATION OF PROTEINS RESPONDS TO CELLULAR PERTURBATIONS

F. H. A. SCHNEEWEISS[1] and R. N. SHARAN[2]

[1]Institute of Medicine, Research Center Jülich, 52425 Jülich, Germany (f.schneeweiss@fz-juelich.de); [2]Radiation & Molecular Biology Unit, Department of Biochemistry, North-Eastern Hill University, Shillong 793 022, India (ms08@hotmail.com)

BACKGROUND

Adenosine-5'-diphosphate ribosylation (ADP-ribosylation) represents a process by which cellular proteins are post-translationally modified. It is an ubiquitous process in a wide range of living systems spanning from the simplest of prokaryotes to complex eukaryotes. The modification is an enzyme catalyzed reaction and is completely reversible. The reaction involves condensation of one or more of ADP-ribose moieties from cellular NAD^+ (nicotinamide adenine dinucleotide) onto amino acid residues, predominantly glutamic acid residues, of a protein by

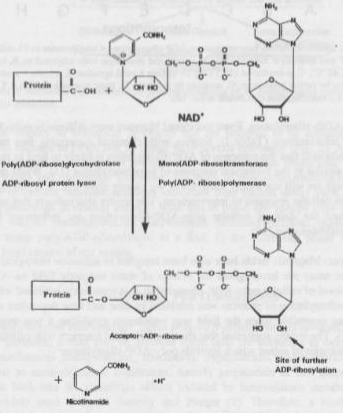

Figure 1: Schematic representation of ADP-ribosylation reaction.

glycosidic linkages. A transfer of one ADP-ribose unit indicates a mono-ADP-ribosylation and in case of more than one unit this process is designated as poly-ADP-ribosylation. Since each unit of ADP-ribose contains two negative charges (from two phosphate groups) a protein modification by addition of ADP-ribose moieties leads to an increase of at least two negative charges of the target protein (Fig. 1). Synthesis and degradation of poly-ADP-ribosylated proteins influence biological processes. Various aspects of poly-ADP-ribosylation and its biological implications have been reviewed by several authors (1-6). For the sake of clarity, we shall attempt to briefly summarize relevant aspects of the process in the next paragraphs.

Target proteins for this post-translational modification are varied. Histones are involved in the poly-ADP-ribosylation in eukaryotes which suggests that this process may be of importance in chromatin organization. Some non-histone chromosomal proteins as high mobility group (HMG) proteins, low mobility group proteins, topoisomerases, DNA polymerases, DNA ligases, RNA polymerases, nucleases (both endo- and exonucleases), different nuclear scaffold proteins, and ribonucleoproteins are also reported to be poly-ADP-ribosylated. Poly-ADP-ribose polymerase which mainly catalyzes the poly-ADP-ribosylation reaction is poly-ADP-ribosylated by itself. This process, referred to as automodification, has been shown to inhibit the enzyme activity and therefore it seems as a self regulatory process for cellular ADP-ribosylation.

The mono-ADP-ribosyl transferase catalyzes mono-ADP-ribosylation of proteins. This reaction has been shown to be connected with signal transduction in eukaryotes and in expression of bacterial toxins mediated effects in prokaryotes. Poly-ADP-ribose polymerase catalyzes the process of poly-ADP-ribosylation of cellular proteins which accounts for up to 95% of known ADP-ribosylation reactions in eukaryotes. Essentially two enzymes are reported to be involved in degradation or removal of the ADP-ribose units from a poly-ADP-ribosylated protein. The main enzyme is poly-ADP-ribose glycohydrolase which breaks the chemical bonds between successive monomer units sequentially. The chemical bond by which an ADP-ribose unit is bound to the target protein is removed by enzyme ADP-ribosyl protein lyase. Involvement of a third enzyme, a phosphodiesterase with pyrophosphatase activity, has also been reported in the degradation process of poly-ADP-ribose from a modified protein. It is obvious that a balance between the synthetic and degradative activities of the enzymes determine the level of poly-ADP-ribosylated protein in a given cell or living system.

Poly-ADP-ribose is a homopolymer of ADP-ribose units of variable length. It is essentially organized like nucleic acid and may be unbranched, chain-like polymer or a complex branched structure comprising up to 200 ADP-ribose units on a target protein. The most favoured targets for this modification reaction are chromosomal proteins, especially histones. Since histones are crucial in organization of DNA in nucleosomes the process of poly-ADP-ribosylation has significant influence on the structural organization of chromatin. Because of the bulky size of ADP-ribose the interaction with the target protein is not only affected by the higher negative charges but also due to the higher mass of the added ADP-ribose molecules (Fig. 1). Therefore, hyper-poly-ADP-ribosylated histone proteins shall interact weakly with DNA in organization of chromatin leading to creation of a relaxed chromatin. The degree of relaxation of chromatin shall directly depend on the extent of poly-ADP-ribosylation of histone proteins. Viewing the whole process in reverse, the chromatin will progressively become more condensed when enzymatic degradation of ADP-ribose units from the modified histone protein occurs leading to hypo-poly-ADP-ribosylated histones. Thus, this post-translational modification unlike other metabolic avenues, such as phosphorylation, acetylation, methylation, or adenylation not only alters charge status of target protein but has influence of the bulk of the modification. Since the structure of

chromatin has bearing on the functional abilities of chromatin poly-ADP-ribosylation assumes significance as a possible regulator of a wide variety of biological processes, e.g. repair of DNA damage (7), regulation of cell cycle (2,8), cell differentiation and gene expression (2), control of adenylate cyclase dependent metabolic processes (9).

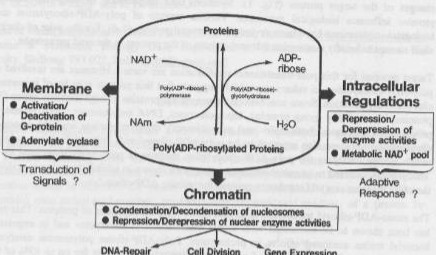

Figure 2: Schematic representation of possible influences of ADP-ribosylation reaction on different cellular processes.

Among the variety of biological processes that are believed to be influenced by poly-ADP-ribosylation damage to DNA and its repair have special relevance (Fig. 2). All living forms are constantly exposed to a variety of perturbations or interventions which may include radiations, magnetic forces and chemicals. Therefore, the study of poly-ADP-ribosylation of proteins as a biomarker of cellular response becomes important from human safety view point as well as a predictive parameter in clinical therapy. With this in mind, our groups have taken up studies of effects of various perturbations or interventions including different qualities and doses of radiation, using poly-ADP-ribosylation as a tool. In the following pages we strive to bring forward a brief review of our results.

GENERAL PERTURBATIONS

Physical and chemical interventions: Since poly-ADP-ribosylation is involved in a wide array of biological reponses, it may also be affected when cells are subjected to external interventions, such as mechanical manipulations, irradiation, hyperthermia, trypsinization or gravitational forces. Due to methodological constraints, namely preparation of cell suspension, it was not possible to look into the metabolic effects induced by interventions mentioned above by the hitherto widely used assay of Surowy and Burger (8). Therefore, a modified method was developed which used confluent monolayer cells and ^{32}P-NAD$^+$ for the study (10). With this we

have been able to precisely quantify the influences exerted by interventions listed above on human kidney T1-cells growing as a confluent monolayer culture. Fig. 3 summarizes the results. They clearly show that interventions routinely used in laboratories may significantly influence

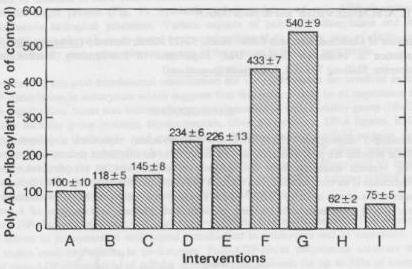

Figure 3: Effects of various interventions on ADP-ribosylation of total proteins in T1-cell assayed by ^{32}P-NAD$^+$ and monolayer method (10). A, control and monolayer cells subjected to: B, hyperthermia (30 min , 42 °C); C, γ-irradiation (16.5 Gy); D, trypsin + mild agitation; E, trypsin + severe agitation; F, scraping by rubber policeman; G, scraping by microtip; H, static magnetic field (1.4 T, 30 min); I, 130-460 x g centrifugation (see details in ref. 10).

cellular poly-ADP- ribosylation. Even individual histones were differently poly-ADP-ribosylated after various interventions (Table I). Studies with chemical generating free radicals in living system also indicated that the response of ADP-ribosylation is likely to lead to structural changes in chromatin similar to the biological outcome of hyperacetylation (11). While the consequences of these findings are still not totally understood, we clearly show that ADP-ribosylation reactions are involved in cellular response to interventions. The results also indicate that unless monolayer method is used the data of cellular poly-ADP-ribosylation are influenced by normal cell preparation methodologies (10).

Magnetic forces: Magnetic fields have also been reported to influence biological systems. Using the monolayer assay we investigated the effects of static magnetic field on ADP-ribosylation (12,13). The level of cellular poly-ADP-ribosylation was transiently inhibited wherein the levels of poly-ADP-ribosylation of proteins was inhibited in dose and time dependent manner (Fig. 4). The effect was reversible when the field was withdrawn exhibiting a non-monotonous retreat course (12,13). The results suggested that the magnetic field interacts with cellular membrane by affecting the transfer of signal which controls poly-ADP-ribosylation.

Table I: Levels of ADP-ribosylation of different histone proteins in T1-cells (mean ± SD of areas [absorption OD x mm] of peaks of densitometric scans) in monolayer cells (ML) and in suspension of single cells (S), after mechanical manipulations (MM), after 15 min recovery incubation (RI), and in presence of the inhibitor 3-aminobenzamide (AB). Bold-face numbers represent factors by which a given area differs from the ML value of that particular histone (10).

	H1A	H1B	H2A	H2B	H3	H4
ML	0.41 ±.18	0.39 ±.17	0.35 ±.09	1.31 ±.38	0.48 ±.09	0.54 ±.30
S	1.09 ±.23 **2.7**	0.93 ±.30 **2.4**	0.77 ±.02 **2.2**	2.43 ±.15 **1.9**	1.74 ±.06 **3.6**	1.23 ±.03 **2.3**
ML+AB	0.10 ±.03 **0.2**	0.10 ±.02 **0.3**	0.14 ±.02 **0.4**	0.12 ±.10 **0.1**	0.07 ±.03 **0.2**	0.16 ±.14 **0.3**
S+AB	0.04 ±.05 **0.1**	0.13 ±.05 **0.3**	0.11 ±.11 **0.3**	0.22 ±.03 **0.2**	0.06 ±.06 **0.1**	0.04 ±.03 **0.1**
ML+MM	2.36 ±.08 **5.8**	1.99 ±.27 **5.1**	2.03 ±.10 **5.8**	3.48 ±.40 **2.7**	4.31 ±1.08 **9.0**	3.28 ±.18 **6.1**
ML+MM +AB	0.05 ±.01 **0.1**	0.08 ±.04 **0.2**	0.18 ±.20 **0.5**	0.46 ±.07 **0.4**	0.14 ±.04 **0.3**	0.20 ±.04 **0.4**
ML+MM +RI	2.27 ±.62 **5.5**	2.09 ±.09 **5.4**	1.67 ±.06 **4.8**	4.20 ±.40 **3.2**	1.75 ±.43 **3.6**	3.05 ±.21 **5.6**
ML+MM +RI+AB	0.09 ±.06 **0.2**	0.12 ±.05 **0.3**	0.25 ±.02 **0.7**	0.19 ±.07 **0.1**	0.17 ±.22 **0.4**	0.18 ±.06 **0.3**

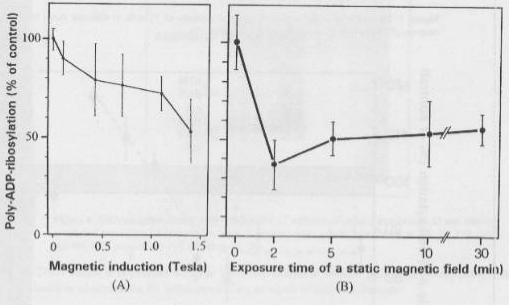

Figure 4: Effect of static magnetic field on poly-ADP-ribosylation (mean ± SD) in T1-cells: (A) effect of strength of magnetic field and (B) effect of time of exposure at 1.4 T (see details in ref. 13).

Membrane active drugs: Cellular membranes, especially plasma membrane, represents the first cellular target for external interventions inducing protein poly-ADP-ribosylation. To specify this influence of membranes, we have incorporated various membrane active substances, e.g. chloropromazine, an inhibitor of acetylcholine esterase and Na^+/K^+-ATPase, γ-linolenic acid, a

drug altering membrane fluidity, and ouabaine, an inhibitor of Na^+/K^+-channel activity. While 100 µmol chloropromazine and 220 µmol γ-linolenic acid increased cellular poly-ADP-ribosylation in T1-cells, 1 µmol chloropromazine and 340 µmol ouabaine inhibited it (Table II).

Table II: Effect of membrane active drugs on poly-ADP-ribosylation of proteins in T1-cell (n = 4-8).

Membrane active drugs	*Poly-ADP-ribosylation (% of control)*
Chloropromazine (1 µmol)	74.8 ± 3.2
Chloropromazine (100 µmol)	236.5 ± 3.6
γ-linolenic acid (220 µmol)	175.5 ± 6.9
Ouabaine (340 µmol)	77.3 ± 4.0

Poly-ADP-ribosylation of individual histones – primarily the core histones – reflected similar effects (Fig. 5). The reasons for these effects on poly-ADP-ribosylation are among others the blocking of membrane Ca^{++}-channels which inhibits the Ca^{++}-induced activation of poly-ADP-ribose polymerase (1 µmol chloropromazine and 340 µmol ouabaine) and the stimulation of the intracellular signal transduction which finally activates protein kinases increasing the poly-ADP-ribosylation (100 µmol chloropromazine and 220 µmol γ-linolenic acid).

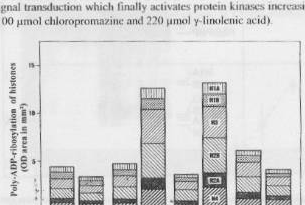

Figure 5: Effect of membrane active drugs on poly-ADP-ribosylation of individual histones in T1-cells after incubation with chloropromazine, γ-linolenic acid and ouabaine. Segments of bars represent peak areas of densitometric scans in mm² of newly synthesized poly-ADP-ribosylated histones after the treatment with drugs; C = untreated control.

RADIATIONS

Elucidation of mechanisms of radiation effects on living systems is of great importance and relevance to human safety. Radiations are omnipresent and are continuously interacting with

cells at molecular level. The damages inflicted by different qualities of radiations on DNA and their repair are subject to accessibility of DNA to damaging species and repair systems. Since poly-ADP-ribosylation of chromosomal proteins is one key factor in organization of DNA in chromatin, it is obvious that the changes of the levels of poly-ADP-ribosylation will influence the accessibility of DNA. With this in mind our groups have been studying the effects of various qualities of radiation on poly-ADP-ribosylation in an effort to understand molecular mechanisms of repair of damages. A brief outline of our findings are presented below.

Gamma radiation: T1-cell suspension subjected to ^{137}Cs-γ-irradiation in the dose range of 1.8 to 486 cGy (Fig. 6A, lanes 2-6) and after repair incubation (Fig. 6B, lanes 2-6) showed a different pattern compared with that of the controls (Fig. 6A & B, lane 1). Whereas the γ-induced patterns are still visible, the control pattern does not show any band after repair (Fig. 6B) leading to the assumption that the trigger of γ-induced poly-ADP-ribosylation is different from the trigger of control pattern which only represent the poly-ADP-ribosylation after cell preparation (10,14). Furthermore, the poly-ADP-ribosylation of histones H3, H1 (H1A + H1B) and H2B were predominantly enhanced with increasing radiation dose (Fig. 6 and in more details ref. 14). In addition, chromatographic experiments showed lower affinity of poly-ADP-ribosylated histone

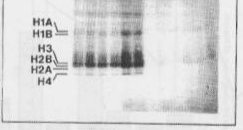

Figure 6: Autoradiogram of poly-ADP-ribosylated T1-cell proteins after γ-irradiation (A) and after γ-irradiation and repair incubation (B). Lane 1, control; lanes 2 to 6, γ-irradiated at 1.8, 30, 120, 240 and 486 cGy, respectively (see details in ref. 14).

to DNA which is consistent with the view that radiation induced poly-ADP-ribosylation causes relaxation of chromatin for better possibility of repair of induced damages.

Neutron radiation: In contrast to γ-radiation induced effect on poly-ADP-ribosylation of proteins in T1-cell suspension 6 MeV neutrons inhibited the reaction up to 0.37 Gy and regained the normal level in a dose dependent manner at 3 Gy (15). Fig. 7 shows the comparative results. Analyses of cellular NAD^+ pools in T1-cell following n- and γ-irradiations suggest that the two qualities of radiations affected the ADP-ribosylation reaction differently. Expectedly, neutrons were several folds more efficient.

Alpha radiation: Recognizing potential application of high-LET α-emitter, particularly ^{211}At, in human cancer therapy (16) we have studied the effect of extracellular irradiation of human glioblastoma (HG) cells on poly-ADP-ribosylation with α-particles generated by ^{211}At. The monolayer cultures of the cells were exposed to α-particle fluxes of 1.5×10^2 to 3×10^6 α/cm^2. In

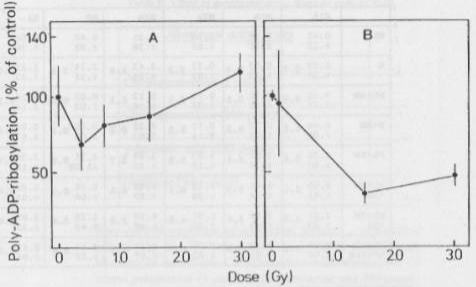

Figure 7: Poly-ADP-ribosylation (mean ± SE) of proteins of T1-cells at different doses following neutron- (A) and γ- (B) irradiations (see details in ref. 15).

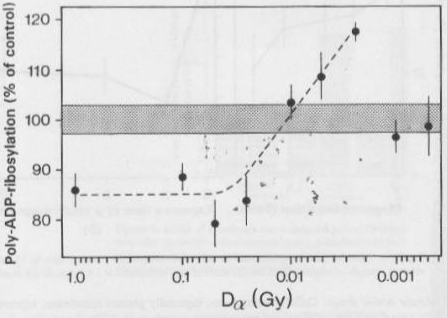

Figure 8: Poly-ADP-ribosylation of total proteins of HG-cells as a negative logarithmic function of absorbed α-particle from ^{211}At. The level of poly-ADP-ribosylation of control cells has been taken as 100%.

this condition the absorbed dose was calculated to be in the range of 0.0005 to 1 Gy over 1 hour exposure time at 37 °C. The total cellular poly-ADP-ribosylation and poly-ADP-ribosylation of histones of HG-cells were assayed by the monolayer method immediately after exposure (10). Fig. 8 shows the relative total cellular poly-ADP-ribosylation of HG-cells. The elevated level of total poly-ADP-ribosylation at relatively low dose of α-irradiation (< 0.01 Gy) was progressively inhibited with increasing dose and remained below control level in the dose range of 0.1 to 1.0 Gy (Fig. 8). Level of poly-ADP-ribosylation has been correlated with repair of DNA damage (1,5-7) wherein elevated level of poly-ADP-ribosylation poises DNA for repair due to relaxation of chromatin as is the case in the dose range of 0.002 to 0.01 Gy (low dose α-radiation) (Fig. 8). However, at relatively higher doses (> 0.01 Gy) the level of poly-ADP-ribosylation was inhibited suggesting radiation induced deactivation of poly-ADP-ribose polymerase. This is in line with experimental observations of non-repaired DNA inflicted by high-LET α-radiations (17).

SUMMARY

From the results presented above it is quite obvious that poly-ADP-ribosylation reaction is a sensitive parameter to monitor cellular responses to a wide variety of perturbations. Having developed a monolayer assay system using ^{32}P-NAD$^+$ as a marker, it has become possible to measure levels of cellular ADP-ribosylation more precisely (10). It has been demonstrated that the trigger of poly-ADP-ribosylation reaction may involve different cellular components for different perturbations (10-13). In this, membrane has been found to be important (12,13). The study has been particularly informative in the realm of DNA damage and repair following qualitatively different radiation assaults. As poly-ADP-ribosylation in eukaryotic cells primarily affects chromosomal proteins, notably histones (2), the reaction is strongly triggered in response to single and double strand breaks in DNA (7). Therefore, level of cellular poly-ADP-ribosylation can potentially be used as a biosensor of radiation induced strand breaks and can be specially useful in clinical monitoring of progress of radiotherapy. The assay of poly-ADP-ribosylation, however, requires use of radiolabelled tracer, e. g. ^{32}P-NAD$^+$. Due to this, study of poly-ADP-ribosylation can not be extended to monitor effects of incorporated radionuclides. In order to overcome this shortcoming and to make the assay more sensitive and quick, a Western blot immunoassay has been developed (18). The preliminary indications are that the immunoassay of poly-ADP-ribosylation will fulfil the requirements to use poly-ADP-ribosylation as a sensitive, convenient and clinically applicable biosensor of cell response not only to radiations but also to different perturbations.

ACKNOWLEDGEMENTS: The work has been carried out under an Indo-German Collaborative Research Program.

REFERENCES

1. K. Ueda and O. Hayaishi (1985) ADP-ribosylation, Ann. Rev. Biochem., 54, 73-100.

2. F. R. Althaus and Ch. Richter (1987) *ADP-ribosylation: Enzymology and Biological Significance*, Springer-Verlag, Berlin.

3. T. Alderson (1990) New targets for cancer chemotherapy – poly(ADP-ribosylation) processing and polyisoprene metabolism, Biol. Rev., 65, 623-641.

4. T. Boulikas (1991) Relation between carcinogenesis, chromatin structure and poly(ADP-ribosylation (review), Anticancer Res., 11, 489-527.

5. G. De Murcia and M. De Murcia (1994) Poly (ADP-ribose) polymerase: a molecular nick sensor, Trends Biochem. Sci., 19, 172-176.

6. A. Nagele (1995) Poly(ADP-ribosyl)ation as a fail-safe, transcription-independent, suicide mechanism in acutely DNA-damaged cells: a hypothesis, Radiat. Environ. Biophys., 34, 251-254.

7. S. Shall (1984) ADP-ribose in DNA repair: A new concept of DNA excision repair, Adv. Radiat. Biol., 11, 1-69.

8. C. S. Surowy and N. A. Berger (1983) Unique acceptors for poly(ADP)-ribose in resting, proliferating and DNA damaged human lymphocytes, Biochim. Biophys. Acta, 740, 8-18.

9. J. Moss and M. Vaughan, Eds. (1990) *ADP-ribosylating Toxins and G Proteins: Insights into Signal Transduction*, American Society for Microbiology, Washington DC.

10. F. H. A. Schneeweiss, R. N. Sharan and L. E. Feinendegen (1995) Change of ADP-ribosylation in human kidney T1-cells by various external stimuli, Indian J. Biochem. Biophys., 32, 119-124.

11. L. Böhm, F. H. A. Schneeweiss, R. N. Sharan and L. E. Feinendegen (1997) Influence of histone acetylation on the modification of cytoplasmic and nuclear proteins by ADP-ribosylation in response to free radicals, Biochim. Biophys. Acta, 1334, 149-154.

12. F. H. A. Schneeweiss, F. Xia, R. N. Sharan and L. E. Feinendegen (1991) *Strahlenschutz fur Mensch und Umwelt*, Vol. 1, pp. 434-438.

13. F. H. A. Schneeweiss, F. Xia, R. N. Sharan and L. F. Feinendegen (1993) A strong static magnetic field inhibits the poly-ADP-ribosylation of proteins in human kidney T1-cells. Bioelectrochem. Bioenerg., 30, 111-117.

14. R. N. Sharan, F. H. A. Schneeweiss, J. R. Saikia and L. E. Feinendegen (1998) Poly-ADP-ribosylation of histone proteins of human kidney T1-cells *in vitro* following γ-irradiation, Indian J. Biochem. Biophys., 35, 97-102.