METABOLISM AND FUNCTION OF POLY(ADP-RIBOSE) IN NUCLEI OF RAT PERITONEAL MACROPHAGES

TAISUKE INAGAKI, KUNIHIRO UEDA* and TAKASHI MURACHI**

Department of Agricultural Chemistry, Faculty of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591, and *Department of Medical Chemistry and **Department of Clinical Science, Faculty of Medicine, Kyoto University, Sakyo, Kyoto 606, Japan

ABSTRACT

The presence of poly(ADP-ribose) in nuclei of rat peritoneal macrophages was demonstrated by immunofluorescence staining technique using antiserum specific to poly(ADP-ribose). Isolated nuclei of the macrophages were found to show measurable activities of poly(ADP-ribose) synthetase, poly(ADP-ribose) glycohydrolase and ADP-ribosyl histone hydrolase. When the nuclei were incubated with [ade-U-¹⁴C]NAD, ¹⁴C-labeled poly(ADP-ribose) was synthesized with an average chain length of approximately 7 ADP-ribosyl units. These observations indicate that both synthesis and degradation of poly(ADP-ribose) are actually occurring *in vivo* in chromatin of the macrophages. When isolated chromatin was incubated under such conditions that either synthesis or degradation of poly(ADP-ribose) could be separately suppressed, a significant increase or decrease, respectively, in activity of a neutral protease was observed. These results suggest that poly(ADP-ribose) plays some role in regulation of the activity of a chromatin-bound protease, through metabolic fluctuation of its local level.

KEY WORDS poly(ADP-ribose) / neutral protease / rat peritoneal macrophage / chromatin

Since the discovery of poly(ADP-ribose) synthesis in liver nuclei (2, 16, 24) and the finding of its association with chromatin (15), this polymer has been expected to play some regulatory role in nuclei. It has been known that two enzymes are related to the metabolism of the polymer *in vivo*: one is poly(ADP-ribose) synthetase (18, 20) and the other is poly(ADP-ribose) glycohydrolase (12, 13, 29). Although the discovery of these enzymes indicates that synthesis and degradation of poly(ADP-ribose) may be possible *in vivo*, precise function of the polymer has not yet been elucidated. A number of observations have so far been reported suggesting a close correlation of poly(ADP-ribosylation) of nucleoproteins to DNA replication, DNA repair (1, 5, 23), cell proliferation, cell differentiation (9, 11, 21), or the decreased affinity of histones for DNA (14, 22). None of these obser-

vations, however, have been conclusive, because of a lack of direct methods to examine *in vivo* events.

Recently, Suzuki and Murachi (26) found that an authentic preparation of poly(ADP-ribose) had a potency to inhibit *in vitro* a chromatin-bound protease of rat peritoneal macrophages. They also reported that a specific protease inhibitor existed in association with chromatin of rat peritoneal macrophages, and that the inhibitor was a poly(ADP-ribose)-like compound(s). At that time, the inhibitor was purified only to a limited extent and the supporting evidence was rather indirect (26). We then purified the inhibitor and identified it, on the basis of more direct evidence, as poly(ADP-ribose) (8).

The chromatin-bound protease was shown to be a chymotrypsin-like enzyme which could hydrolyze histones (26). Suzuki and Murachi (26) have speculated that this protease plays some role in the biological functions of mac-

** To whom correspondence should be addressed

rophages as triggered by a catabolic modification of histones in chromatin, which could in turn be under regulation by poly(ADP-ribose) also present in chromatin.

The present study was therefore undertaken in order to achieve: (1) cytological demonstration of the existence of poly(ADP-ribose) in nuclei of rat peritoneal macrophages, (2) determination of the activities of the enzymes related to the poly(ADP-ribose) metabolism in these nuclei, and (3) demonstration of the correlation of the polymer to regulation of chromatin-bound protease, using isolated chromatin preparation. The results of the experiments to be presented below show that biosynthesis and degradation of poly(ADP-ribose) are actually occurring *in vivo* in nuclei of rat peritoneal macrophages, and that the level of the polymer in chromatin seems to be a regulatory factor on the activity of chromatin-bound protease.

MATERIALS AND METHODS

Chemicals

Thymidine 5'-monophospho-*p*-nitrophenyl ester was purchased from Sigma Co.; 3', 5'-cyclic AMP and β -NAD were from Kohjin Co., Tokyo; fluorescein isothiocyanate (FITC)-labeled swine anti-rabbit immunoglobulin(IgG) (F/P ratio, 1.48) was from Dakopatts, Denmark; RPMI 1640 medium was from Nissui Seiyaku Co., Tokyo; newborn calf serum and EDTA solution, composed of 10 mM potassium-sodium phosphate buffer, pH 7.0, 0.8% NaCl, 0.02% KCl and 0.02% EDTA, were from Institute for Microbial Diseases, Osaka University, Osaka; kanamycin was from Banyu Seiyaku Co., Tokyo. Other reagents were purchased from Nakarai Chemical, Ltd., Kyoto.

Radiochemicals

[Ade-U- 14 C]NAD was purchased from the Radiochemical Centre, Amersham. [Ade-U- 14 C]poly(ADP-ribose) was prepared from labeled NAD, as described by Ikai *et al.* (7). Labeled poly(ADP-ribose) used in this work had an average chain length of approximately 20 ADP-ribosyl units. [Ade-U- 14 C]ADP-ribosyl histone H2B was prepared by the method of Okayama *et al.* (19).

Preparation of Anti-Poly(ADP-Ribose) Antiserum

Antiserum was raised against poly(ADP-ribose)

in a rabbit and prepared by Kanai's method as modified by Ikai *et al.* (7).

Preparation of Macrophages

Peritoneal exudate cells were obtained from male adult rats of Wistar strain by intraperitoneal injection of medium RPMI 1640 containing kanamycin (60 μ g/ml). Cells obtained were washed with the same medium 3 times and suspended in the same medium with 20% newborn calf serum. After an incubation in tissue culture flask (Falcon No. 3013) for 1 hr at 37°C and in 5% CO₂, non-adherent cells were removed by washing the flask 6 times with the culture medium. The adherent cells were detached from the flask by adding EDTA solution and incubating for 90 min on ice. More than 90% of adherent cells were macrophages. The non-adherent cell fraction was subfractionated into lymphocytes and granulocytes, by the method of Harris and Ukaejiofo (3), with higher than 90% and approximately 70% purity, respectively.

Isolation of Nuclei and Chromatin

Cells were homogenized by three strokes in a Potter-Elvehjem homogenizer with a medium, which contained 0.25 M sucrose, 0.5% (v/v) Triton X-100 and 3.3 mM CaCl₂. Nuclei were separated as described previously (26). Chromatin was isolated from the nuclei according to the method of Huang and Huang (4).

Purification of Chromatin-Bound Protease

Chromatin-bound protease was separated from chromatin of rat peritoneal macrophages on a Sephadex G-75 column in the presence of 1 M NaCl by the method of Suzuki and Murachi (25). Protease-active fractions were pooled, concentrated with Amicon PM 10 membrane and dialyzed against distilled water.

Immunostaining Technique

Cells smeared on a slide glass were fixed with ethanol and preincubated at room temperature for 30 min with or without NAD. The cells were then washed 3 times with 10 mM phosphate buffer, pH 7.0, in saline and were stained with indirect immunofluorescence technique of Ikai *et al.* (7). The control was carried out with preimmune serum.

Enzyme Assays

Poly(ADP-ribose) synthetase was assayed with 0.1 mM [ade-U-¹⁴C]NAD (500 cpm/pmol) as substrate in 0.1 M Tris-HCl buffer, pH 8.0, containing 10 mM MgCl₂, and 1 mM dithiothreitol by the method of Okayama *et al.* (18). Poly(ADP-ribose) glycohydrolase was assayed with 1.6 μ M (as ADP-ribose residues) [ade-U-¹⁴C]poly(ADP-ribose) (100 cpm/pmol) as substrate in 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol by the method of Ueda *et al.* (29). ADP-ribosyl histone hydrolase was assayed with [ade-U-¹⁴C]-ADP-ribosyl histone H2B (6 cpm/pmol) in 0.1 M potassium phosphate buffer, pH 6.0, containing 1 mM dithiothreitol and 10 mM EDTA by the method of Okayama *et al.* (19). Phosphodiesterase was assayed with 0.4 mM thymidine 5'-monophospho-*p*-nitrophenyl ester as substrate in 40 mM Tris-acetate buffer, pH 8.0, containing 10 mM MgCl₂ by the method of Oka *et al.* (17). Protease activity was assayed with 2% casein as substrate in 50 mM borate buffer, pH 8.5, according to the method of Suzuki and Murachi (25).

Determination of an Average Chain Length of Poly(ADP-Ribose)

Nuclei isolated from rat peritoneal macrophages (5×10^6 cells) were incubated with 200 μ l of 0.1 M Tris-HCl buffer, pH 8.0, containing 10 mM MgCl₂, 1 mM dithiothreitol and 0.1 mM [ade-U-¹⁴C] NAD (20,000 cpm/ μ l) for 5 min at 25°C, then precipitated with 20% trichloroacetic acid and resuspended in 50 μ l of 0.1 M Tris-HCl buffer, pH 8.0. The suspension was treated first with trypsin (0.5 mg/50 μ l) and then with snake venom phosphodiesterase (Worthington Co., 10.6 units/ μ l) by the method of Ueda *et al.* (28). The mixture was applied on filter paper (Whatman 3 MM) and subjected to chromatography in a solvent system: isobutyric acid, 1M NH₄OH, 0.1 M EDTA (100:60:1.6, v/v/v), pH 8.0. The paper sheet was cut into strip and examined for radioactivity. The average chain length was calculated from the radioactivities associated with the cleavage products, AMP and *iso*ADP-ribose, according to the equation: (AMP + *iso*ADP-ribose)/AMP (5).

Chemical Analysis

Protein was determined by the method of Lowry *et al.* (10) using bovine serum albumin as

standard.

RESULTS

Distribution of Poly(ADP-Ribose)

Existence of poly(ADP-ribose) in rat peritoneal exudate cells was studied using an immunofluorescence technique. With control (preimmune) serum, nonspecific fluorescence was observed in cytoplasm of granulocytes and lymphocytes while the fluorescence in macrophages was hardly detectable (Fig. 1a). When the cells were treated with antiserum, the nuclei of lymphocytes and macrophages emitted fluorescence (Fig. 1b); the emission from macrophages was weaker than that from lymphocytes, indicating that poly(ADP-ribose) content was less in macrophage than in lymphocyte. In granulocytes, the nuclei appeared darker than the cytoplasm, indicating the absence of poly(ADP-ribose) in nuclei. Fig. 1c shows that the fluorescence in macrophages and lymphocytes was markedly intensified by preincubation with NAD, suggesting that poly(ADP-ribose) was produced from exogenously added NAD in the cells which had been fixed with ethanol.

Poly(ADP-Ribose) Synthetase in Macrophages

The presence of poly(ADP-ribose) synthetase in macrophage was confirmed by assaying *in vitro* the activity of isolated nuclei. At pH 8.0 and 25°C, the reaction proceeded linearly for several minutes. K_m value for NAD was calculated to be 38 μ M. The synthetase activity was slightly inhibited by ammonium sulfate as reported with rat liver nuclei (18). Table I shows the distribution of the enzyme in peritoneal exudate cells. The specific activity was highest in macrophage fraction, followed by lymphocyte fraction, and lowest in granulocyte fraction. Since the purity of granulocytes was only approximately 70%

Fig. 1 Intracellular localization of poly(ADP-ribose) in rat peritoneal exudate cells. Exudate cells were obtained by the method described under Materials and Methods. Cells obtained were washed with saline 6 times. May-Giemsa staining of an aliquot showed: 41%, macrophage; 30%, lymphocyte; 23%, granulocyte; 6%, others. Cells were smeared, fixed, incubated (a) with control (preimmune) serum after a preincubation with NAD, (b) with antiserum, (c) with antiserum after a preincubation with NAD, and stained with FITC-labeled swine anti-(rabbit IgG) IgG. M, macrophage; L, lymphocyte; G, granulocyte

Fig. 1

Table 1 *Poly(ADP-Ribose) Synthetase Activity in Rat Peritoneal Exudate Cells*

Cell fraction ^a	Specific activity (units ^b /mg protein)
Peritoneal exudate cells	0.51
Macrophage	0.47
Lymphocyte	0.38
Granulocyte	0.15

^a Macrophage, lymphocyte and granulocyte fractions were separated from rat peritoneal exudate cells (4.3×10^7 cells) as described in Materials and Methods. Poly(ADP-ribose) synthetase was assayed using nuclei isolated from the respective cells.

^b One unit is defined as the amount of enzyme which catalyzes incorporation of 1 nmol of ADP-ribose into acid-insoluble fraction per min at 25°C.

Table 2 *Enzymatic Activities for Synthesis and Degradation of Poly(ADP-Ribose) in Rat Peritoneal Macrophages*

Enzyme ^a	Specific activity (units/mg protein)	Recovery of total activity (%)
Poly(ADP-ribose) synthetase		
Macrophage homogenate	0.45	100
Macrophage nuclei	0.69	84
Macrophage chromatin	2.04	47
Rat liver nuclei (18)	1.30	—
Poly(ADP-ribose) glycohydrolase ^b		
Macrophage nuclei	0.90	—
Calf thymus homogenate (12)	0.89	—
ADP-ribosyl histone hydrolase ^c		
Macrophage nuclei	1.08	—
Phosphodiesterase ^d		
Macrophage nuclei	0.12	—

^a Rat peritoneal macrophages (5.1×10^7 cells) were used for the assays. The methods of enzyme assays are given in Materials and Methods.

^b One unit is defined as the amount of enzyme which liberates 1 nmol of ADP-ribose into acid-soluble fraction per min at 37°C.

^c One unit is defined as the amount of enzyme which hydrolyzes 1 nmol of ADP-ribosyl histone per min at 37°C.

^d One unit is defined as the amount of enzyme which causes an increase of 17 $A_{400\text{ nm}}$ per min at 25°C.

Table 3 *Effects of Various Compounds on Poly(ADP-Ribose) Synthetase in Rat Peritoneal Macrophage Nuclei*

Addition (10 mM)	Relative activity (%)
None	100
Nicotinamide	30
Thymidine	48
3', 5'-Cyclic AMP	88
AMP	108
ADP-ribose	136

(see above) the synthetase activity found in granulocyte fraction could have arisen from contaminating macrophages and lymphocytes. Table 2 shows the intracellular distribution of several enzymes involved in poly(ADP-ribose) metabolism. The synthetase activity of the

homogenate was mainly recovered in the nuclear fraction, and approximately 55% of the activity in nuclei was recovered in chromatin. The specific activity in chromatin was about five-fold higher than that in the homogenate. The specific activity in macrophage nuclei was much

lower than that in rat liver nuclei. Table 3 shows the effects of various compounds on the synthetase activity of rat peritoneal macrophage nuclei. The synthetase was inhibited by nicotinamide and thymidine, while much less or no inhibition was noted with cyclic AMP or AMP, respectively. ADP-ribose appeared to slightly activate the synthetase; this effect was presumably due to the inhibition of poly(ADP-ribose) degrading enzymes (12, 13, 19).

Degradative Enzymes of Poly(ADP-Ribose) in Macrophage

It is well known that poly(ADP-ribose) glycohydrolase plays the principal role in degradation of the polymer *in vivo* (12, 29). ADP-ribosyl histone hydrolase, which splits the ADP-ribosyl histone linkage, has also been found in rat liver (19). Table 2 contains the data showing that these two enzymes are present in nuclei of rat peritoneal macrophages. Specific activity of poly(ADP-ribose) glycohydrolase was almost the same as that from calf thymus homogenate. Phosphodiesterase, which splits the pyrophosphate bond, was also present in nuclei of the cells.

Average Chain Length of Poly(ADP-Ribose)

Fig. 2 shows paper-chromatographic separation of *iso*ADP-ribose and AMP produced by snake venom phosphodiesterase digestion of poly(ADP-ribose) which had been synthesized in

Fig. 2 Separation of *iso*ADP-ribose and AMP by paper chromatography. After the polymer synthesized in macrophage nuclei was digested by phosphodiesterase, the mixture was chromatographed on filter paper according to the method described in Materials and Methods. ADP-ribose (ADPR), AMP and adenosine (Ado) as markers were also chromatographed.

nuclei. The radioactive material which moved slightly ahead of the ultraviolet-absorbing spot of ADP-ribose was identified as *iso*ADP-ribose by conversion to ribosyl adenosine with alkaline phosphatase treatment (28). Average chain length of poly(ADP-ribose) was calculated from the radioactivities of *iso*ADP-ribose and AMP to be 7.0 in terms of ADP-ribosyl units.

The Effects of Poly(ADP-Ribose) Metabolism on Chromatin-bound Protease

It is well known (1) that poly(ADP-ribose) is synthesized from NAD (5), and (2) that cyclic AMP, nicotinamide and AMP are potent inhibitors of poly(ADP-ribose) glycohydrolase, poly(ADP-ribose) synthetase and nuclear phosphodiesterase, respectively (27). We first confirmed that the protease purified from macrophage chromatin was not affected by NAD, cyclic AMP, nicotinamide and AMP, and then exam-

Fig. 3 Effects of preincubation with NAD and related compounds on the neutral protease in chromatin. Reaction mixture contained 0.1 M Tris-HCl buffer, pH 8.0, 10 mM MgCl₂, 1 mM dithiothreitol and an appropriate amount of chromatin suspension. The preincubation was carried out in the presence or absence of effectors, and aliquots taken at intervals were examined for the activity of chromatin-bound protease. Δ, control; ○, in the presence of 1 mM NAD; ●, in the presence of 1 mM NAD, 10 mM 3', 5'-cyclic AMP and 10 mM AMP; ◐, in the presence of 1 mM NAD and 10 mM nicotinamide; ▲, in the presence of 0.25 M NaCl

ined whether an incubation of chromatin with these effectors would affect chromatin-bound protease.

Fig. 3 shows the time course of the protease activity in chromatin. A decrease of approximately 25% of the activity was caused by 1 hr incubation with NAD, although such decrease was not observed in chromatin incubated without NAD. Chromatin incubated with NAD in the presence of both cyclic AMP and AMP showed a decrease in protease activity to approximately 40% after 1 hr. In contrast, chromatin incubated with NAD in the presence of nicotinamide resulted in an increase of approximately 50% of protease activity. When assayed in the presence of 0.25 M NaCl, the protease activity was markedly enhanced as previously reported (25). Such activation was known to be due to the dissociation of the inhibitor, i.e., poly(ADP-ribose), from the protease in the presence of a high concentration of salt (8).

DISCUSSION

In the present study, the existence of poly(ADP-ribose) in rat peritoneal macrophages was demonstrated by an immunocytochemical technique (Fig. 1). The technique had been thoroughly studied by Ikai *et al.* (6, 7), who established its high sensitivity and specificity toward poly(ADP-ribose). The absence of the polymer in rat granulocytes was also shown, which agreed with the reported result in the case of rat and human peripheral blood (6, 7). A weak emission found in nuclei of macrophages suggests a low content of poly(ADP-ribose), supporting our earlier conclusion, drawn from quantification of protease inhibitor, that macrophage chromatin contained only a smaller amount of the polymer as compared with hepatocytes (8). The presence of poly(ADP-ribose) synthetase, poly(ADP-ribose) glycohydrolase and ADP-ribosyl histone hydrolase, which are known to be active in poly(ADP-ribose) metabolism *in vivo*, was confirmed by assaying the isolated nuclei of the cells. This confirmation clearly indicates that both biosynthesis and degradation of the polymer take place at ribosyl-ribose linkages and/or ribosyl-histone linkages in chromatin of rat peritoneal macrophages, as was the case for rat liver nuclei (27). Furthermore, the present experiment showed that rat peritoneal macrophage nuclei are able to synthesize poly(ADP-ribose) with an average chain length of about 7 ADP-ribosyl units from NAD (Fig. 2).

An incubation of chromatin with NAD, particularly in the presence of cyclic AMP and AMP, caused a decrease in chromatin-bound protease activity, whereas an increase was brought about by an incubation with NAD in the presence of nicotinamide (Fig. 3). These phenomena can be interpreted as follows: (1) The protease, which had not been associated with poly(ADP-ribose) in chromatin, became inhibited by poly(ADP-ribose) newly synthesized from NAD. The inclusion of cyclic AMP and AMP into the incubation mixture caused further decrease of chromatin-bound protease activity by inhibiting degradation of the polymer (27). (2) The inhibition of poly(ADP-ribose) synthetase by nicotinamide (Table 3) would have caused a relative predominance of the action of poly(ADP-ribose) glycohydrolase in nuclei, which, in turn, resulted in a decrease in the level of poly(ADP-ribose) and hence in activation of the protease. In summary, the present result strongly suggests that synthesis and degradation of poly(ADP-ribose) regulate the activity of chromatin-bound protease *in vivo*. This regulatory function should be at least one of the still unexplored functions of poly(ADP-ribose) as far as rat peritoneal macrophages are concerned.

We wish to thank Professors Osamu Hayaishi and Kazuo Miura for their constant supports to the present investigation. This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

Received for publication 14 June 1980

REFERENCES

1. BURZIO L. and KOIDE S. S. (1970) A functional role of polyADPR in DNA synthesis. *Biochem. Biophys. Res. Commun.* **40**, 1013–1020
2. CHAMBON P., WEILL J. D., DOLY J., STROSSER M. T. and MANDEL P. (1966) On the formation of a novel adenylic compound by enzymatic extracts of liver nuclei. *Biochem. Biophys. Res. Commun.* **25**, 638–643
3. HARRIS R. and UKAEJIOFO E. O. (1970) Tissue typing using a routine one-step lymphocyte separation procedure. *Brit. J. Haematol.* **18**, 229–235
4. HUANG R. C. C. and HUANG P. C. (1969) Effect of protein-bound RNA associated with chick embryo chromatin on template specificity of the chromatin. *J. Mol. Biol.* **39**, 365–378
5. HAYAISHI O. and UEDA K. (1977) Poly(ADP-ribose) and ADP-ribosylation of proteins. *Ann. Rev. Biochem.* **46**, 95–116
6. IKAI K., UEDA K., FUKUSHIMA M., NAKAMURA T. and HAYAISHI O. (1980) Poly(ADP-ribose)

- synthesis, a marker of granulocyte differentiation. *Proc. Natl. Acad. Sci. USA* **77**, 3682–3685
7. IKAI K., UEDA K. and HAYAISHI O. (1980) Immunohistochemical studies on poly(ADP-ribose). *J. Histochem. Cytochem.* **28**, 670–676
 8. INAGAKI T., MIURA K. and MURACHI T. (1980) Identification of a protease inhibitor from rat peritoneal macrophages as poly(ADP-ribose). *J. Biol. Chem.* (in press)
 9. KIDWELL W. R. and MAGE M. G. (1976) Changes in poly(adenosine diphosphate-ribose) and poly(adenosine diphosphate-ribose) polymerase in synchronous HeLa cells. *Biochemistry* **15**, 1213–1217
 10. LOWRY O. H., ROSEBROUGH N. J., FARR A. L. and RANDALL R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
 11. MILLER E. G. (1975) Stimulation of nuclear poly(adenosine diphosphate-ribose) polymerase activity from HeLa cells by endonuclease. *Biochim. Biophys. Acta* **395**, 191–200
 12. MIWA M., TANAKA M., MATSUSHIMA T. and SUGIMURA T. (1974) Purification and properties of a glycohydrolase from calf thymus splitting ribose-ribose linkages of poly(adenosine diphosphate-ribose). *J. Biol. Chem.* **249**, 3475–3482
 13. MIYAKAWA N., UEDA K. and HAYAISHI O. (1972) Association of poly ADP-ribose glycohydrolase with liver chromatin. *Biochem. Biophys. Res. Commun.* **49**, 239–245
 14. MULLINS D. W., GIRI C. P. and SMULSON M. (1977) Poly(adenosine diphosphate-ribose) polymerase: The distribution of a chromosome-associated enzyme within the chromatin substructure. *Biochemistry* **16**, 506–513
 15. NISHIZUKA Y., UEDA K., HONJO T. and HAYAISHI O. (1968) Enzymic adenosine diphosphate ribosylation of histone and poly(adenosine diphosphate-ribose) synthesis in rat liver nuclei. *J. Biol. Chem.* **243**, 3765–3767
 16. NISHIZUKA Y., UEDA K., NAKAZAWA K. and HAYAISHI O. (1967) Studies on the polymer of adenosine diphosphate ribose. I. Enzymic formation from nicotinamide adenine dinucleotide in mammalian nuclei. *J. Biol. Chem.* **242**, 3164–3171
 17. OKA J., UEDA K. and HAYAISHI O. (1978) Snake venom phosphodiesterase: Simple purification with Blue Sepharose and its application to poly(ADP-ribose) study. *Biochem. Biophys. Res. Commun.* **80**, 841–848
 18. OKAYAMA H., EDSON C. M., FUKUSHIMA M., UEDA K. and HAYAISHI O. (1977) Purification and properties of poly(adenosine diphosphate-ribose) synthetase. *J. Biol. Chem.* **252**, 7000–7005
 19. OKAYAMA H., HONDA M. and HAYAISHI O. (1978) Novel enzyme from rat liver that cleaves an ADP-ribosyl histone linkage. *Proc. Natl. Acad. Sci. USA* **75**, 2254–2257
 20. OKAZAKI H., NIEDERGANG C. and MANDEL P. (1976) Purification and properties of calf thymus poly(adenosine diphosphate-ribose) polymerase. *FEBS Lett.* **62**, 255–258
 21. ROBERTS J. H., STARK P., GIRI C. P. and SMULSON M. (1975) Cytoplasmic poly(ADP-ribose) polymerase during the HeLa cell cycle. *Arch. Biochem. Biophys.* **171**, 305–315
 22. SMULSON M., GIRI C. P., SUDHAKAR S. and MULLINS D. (1976) In *Proc. 4th Int. Symp. Poly(ADP-ribose) and ADP-ribosylation of Protein* (ed. HILT H.) pp. 7–12
 23. SOLAO P. B. and SHALL S. (1971) Control of DNA replication in *Physarum Polycephalum*. I. Specific activity of NAD pyrophosphorylase in isolated nuclei during the cell cycle. *Exp. Cell Res.* **69**, 295–300
 24. SUGIMURA T., FUJIMURA S., HASEGAWA S. and KAWAMURA Y. (1967) Polymerization of the adenosine 5'-diphosphate ribose moiety of NAD by rat liver nuclear enzyme. *Biochim. Biophys. Acta* **138**, 438–441
 25. SUZUKI Y. and MURACHI T. (1977) The occurrence of a neutral protease and its inhibitor in rat peritoneal macrophages. *J. Biochem.* **82**, 215–220
 26. SUZUKI Y. and MURACHI T. (1978) A chromatin-bound neutral protease and its inhibitor in rat peritoneal macrophages. *J. Biochem.* **84**, 977–984
 27. UEDA K., HAYAISHI O., KAWAICHI M., OGATA N., IKAI K., OKA J. and OKAYAMA H. (1979) *Modulation of Protein Function* (ed. ATKINSON D. E. and FOX C. F.), Academic Press, New York, pp. 47–58
 28. UEDA K., KAWAICHI M., OKAYAMA H. and HAYAISHI O. (1979) Poly(ADP-ribosylation) of nuclear proteins: Enzymatic elongation of chemically synthesized ADP-ribose histone adducts. *J. Biol. Chem.* **254**, 679–687
 29. UEDA K., OKA J., NARUMIYA S., MIYAKAWA N. and HAYAISHI O. (1972) Poly(ADP-ribose) glycohydrolase from rat liver nuclei, a novel enzyme degrading the polymer. *Biochem. Biophys. Res. Commun.* **46**, 516–523