I. INTRODUCTION
ADP-ribosylation is a unique class of posttranslational modifications of proteins using NAD as the donor of the modifying group. As compared with phosphorylation and other modifications, ADP-ribosylation is unique in that the modifying group, ADP-ribose, takes the form of not only monomer but also polymer, and that there are a large variety of enzymes and acceptors [1, 2]. In this presentation, we make a brief review of mono- and poly (ADP-ribosyl)ation, and then report results of our recent research focussed mainly on poly (ADP-ribosyl)ation.

II. MONO- AND POLY (ADP-RIBOSYL)ATION
ADP-ribosylation is classified into two types, mono (ADP-ribosyl)ation and poly (ADP-ribosyl)ation, according to the ultimate length of (ADP-ribose)ₙ chain. Poly (ADP-ribosyl)ation starts with mono (ADP-ribosyl)ation, followed by consecutive transfers of ADP-ribose units, leading to formation of a polymer, termed poly (ADP-ribose). In the cell, the two types of reactions are localized in different compartments, viz. mono (ADP-ribosyl)ation in cytoplasm and poly (ADP-ribosyl)ation in the nucleus. The two differ also in acceptor amino acids; diphthamide, arginine, cysteine, and asparagine are the sites for mono (ADP-ribosyl)ation, whereas glutamic acid, aspartic acid, and C-terminal amino acid (e.g., lysine of histone H1) are the sites for poly (ADP-ribosyl)ation.

Mono (ADP-ribosyl)ation is widely distributed among eukaryotes and prokaryotes (Table 1). Many of the prokaryotic enzymes are known as bacterial toxins. Recently, similar endogenous enzymes have been found in mammalian cells. Arginine-specific, cysteine-specific and asparagine-specific ADP-ribosylations affect functions of G-proteins, including Gₛ, transducin (Gₜ), G₁, Go, rho, and other small Mr GTP-binding proteins.

In contrast, poly (ADP-ribosyl)ation is found only in eukaryotes, and catalyzed almost exclusively by a single enzyme, poly (ADP-ribose) synthetase (also termed polymerase). Many nuclear proteins, including poly (ADP-ribose) synthetase itself, serve as acceptors in vivo and/or in vitro (Table 2). As indicated by this variety of acceptors, poly (ADP-ribosyl)ation has been implicated in various nuclear functions, such as DNA repair, oncogenesis, cell differentiation, and cell cycle events, particularly, chromatin condensation/decondensation [1, 2]. In order to elucidate molecular bases of these intricate functions of poly (ADP-ribosyl)ation, we investigated the primary structure and specific inhibitors of the synthetase [3].

III. STRUCTURE OF POLY (ADP-ROBOS) SYNTHETASE
We investigated the primary structure of the synthetase by cDNA cloning. The nucleotide sequence of cDNA and deduced amino acid sequence of calf thymus poly (ADP-ribose) synthetase [4] indicated that this enzyme consists of 1016 amino acid residues, and is composed of three functional domains, i.e., N-terminal DNA-binding, central automodification, and C-terminal NAD-binding domains.

A comparison of the amino acid sequence of bovine poly (ADP-ribose) synthetase with those of human and mouse enzymes reported by others showed that the whole sequence has been well conserved throughout evolution, the DNA-binding domain being the lowest and the NAD-binding domain the highest in homology. We identified many functional segments that are almost completely conserved among the three species [4]. They are; repeated zinc-binding finger motifs, repeated...
helix-turn-helix structures, and nuclear location signal in the DNA-binding domain, all glutamic acid and aspartic acid residues in the automodification domain, and A and B sites of nucleotide-binding fold in the NAD-binding domain. These conserved segments in the three domains appear to contribute to multiple DNA-enzyme interactions, ADP-ribose acceptance, and strict specificity for the substrate, NAD, respectively.

IV. INHIBITORS OF POLY(ADP-RIbose) SYNTHETASE

From the viewpoint that all known inhibitors currently used for the synthetase, including nicoti-

TABLE 1
Mono(ADP-ribosyl)ation reactions.

| Enzyme                        | Acceptors                          | Reporters                           |
|-------------------------------|------------------------------------|-------------------------------------|
| Diphtheria-specific ADP-ribosyltransferases | Elongation factor 2 | Honjo et al. 1968                     |
| Pseudomonas toxin             | Elongation factor 2 | Iglewski & Kabat 1975                |
| Mammalian cytoplasmic enzyme  | Elongation factor 2 | Lee & Iglewski 1984                  |
| Arginine-specific ADP-ribosyltransferases | RNA polymerase and other | Rohrer et al. 1975                    |
| Cholera toxin                 | $G_\alpha$ protein | Goff 1974                            |
|                              | Microtubule proteins            | Cassel & Pfeuffer 1978              |
|                              | Transducin                     | Gill & Meren 1978                    |
|                              | Myelin basic protein           | Amir-Zaltsman et al. 1982           |
| E. coli enterotoxin LT        | $G_\alpha$ protein             | Hawkins & Browning 1982             |
| Avian erythrocyte enzyme      | $G_\alpha$ protein             | Abood et al. 1982                    |
| Mammalian cytosolic enzyme    | Soluble proteins               | Moss & Richardson 1978              |
| Mammalian membrane enzyme     | $G_\alpha$ protein and other membrane proteins | Moss & Vaughan 1978 |
| Mammalian mitochondrial enzyme | Mitochondrial inner membrane protein | Beckner & Blecher 1981 |
| Avian nuclear enzyme          | Nuclear proteins               | De Wolf et al. 1981                  |
| Botulinum C2 toxin            | Actin                            | Walaas et al. 1981                   |
| Rhodospirillum rubrum enzyme   | Dinitrogenase                   | Adamietz et al. 1981                 |
| Clostridium perfringens iota toxin | Actin                           | Richter et al. 1981                  |
| Clostridium sporiforme toxin  | Actin                            | Shimoyama et al. 1982                |
| Cysteine-specific ADP-ribosyltransferases | | Simpson 1984 |
| Islet-activating protein      | $G_\alpha$ protein             | Ohishi & Tsuyama 1986                |
| (Pertussis toxin)             | Transducin                     | Van der Kerkhove et al. 1987        |
| Human erythrocyte enzyme      | $G_\alpha$ protein             | Katada & Uii 1982                    |
| Porcine skeletal muscle enzyme | $G_\alpha$ protein             | Manning et al. 1984                  |
| Bovine brain cytosolic enzyme | Sarcomplasmic proteins         | Katada et al. 1986                   |
| Asparagine-specific ADP-ribosyltransferases | | Tanuma et al. 1987 |
| Botulinum C1, C3 & D toxins   | Membrane rho & rac proteins     | Soman & Graves 1988                  |
| ADP-ribosyltransferases with unknown amino acid specificity | | Maehama et al. 1991 |
| Pseudomonas aeruginosa exoenzyme S | Elongation factor 1-associated proteins | Iglewski et al. 1987 |
| N4 phage enzyme               | $E. coli$ proteins              | Pesce et al. 1976                    |
| E. coli (noninfected) enzyme  | $E. coli$ proteins              | Skórkó & Kurf 1981                   |
namide and 3-aminobenzamide, are associated with strong side actions in vivo, we carried out a systematic survey of the synthetase inhibitors using an in vitro assay system. From among ca. 350 compounds, we found many strong inhibitors [5] (Table 3). In terms of IC50 values, the top several compounds were 2 or 3 orders of magnitude more potent than 3-aminobenzamide (IC50 = 33 μM) or nicotinamide (IC50 = 210 μM), respectively. All of them shared a common structure, i.e., a benzene ring with an attached carboxamide group, free or extended into a heterocyclic ring.

Among inhibitors were included a number of natural compounds, some of them belonging to the vitamin group [6]. The strongest inhibitors, as judged by IC50 values, were essential fatty acids, particularly, arachidonic and linoleic acids, followed by vitamin K derivatives. The potency of arachidonic acid was comparable to that of 3-aminobenzamide.

Most of strong inhibitors exhibited mixed-type inhibition with respect to NAD [5]. A rather small number of inhibitors, including xanthurenic acid and 5-nitrouracil, acted competitively with NAD.

V. INHIBITORS OF ADP-RIBOSYLTRANSFERASES AND THEIR APPLICATIONS

In parallel to studies of poly(ADP-ribose) synthetase inhibitors, we searched for inhibitors of mono(ADP-ribosyl)transferase [5]. We employed arginine-specific mono(ADP-ribosyl)transferase from hen heterophils, a kind gift from Dr. Shimoyama (Shimane Medical University). The strongest inhibitors we found for this enzyme were vitamin Ks and saturated long-chain fatty acids.

By comparing IC50 values on mono(ADP-ribosyl)transferase and poly(ADP-ribose) synthetase, we classified inhibitors into three groups, i.e., poly-specific, mono-specific, and effective to both (Table 3). It is clear that most of strong inhibitors of poly(ADP-ribose) synthetase are specific for

| Enzyme                              | Acceptor/Protein                  | Reporters                |
|-------------------------------------|----------------------------------|--------------------------|
| **Nuclear enzyme**                  |                                  |                          |
| [Poly(ADP-ribose) synthetase]       | Histones                         | Nishizuka et al. 1968    |
|                                     | Ca++, Mg++-Endonuclease           | Yoshihara 1974           |
|                                     | RNA polymerase I                  | Müller & Zahn 1976       |
|                                     | Poly(ADP-ribose) synthetase       | Yoshihara et al. 1977    |
|                                     | HMG proteins                      | Kawauchi et al. 1978     |
|                                     | A24 protein                       | Okayama & Hayashi 1978   |
|                                     | Actin                             | Kun 1980                 |
|                                     | RNAs                             | Leone et al. 1980        |
|                                     | SV40 T antigen                    | Goldman et al. 1981      |
|                                     | Adenovirus T antigen              | Goding & Russel 1981     |
|                                     | Nucleolar proteins                | Kawashima & Izawa 1981   |
|                                     | Nuclear matrix proteins           | Ueda et al. 1981         |
|                                     | InRNA-associated proteins         | Kostka & Schweiger 1982  |
|                                     | Polyoma virus minichromosome      | Prieto-Soto et al. 1983  |
|                                     | Topoisomerase I                   | Jongstra-Vilen et al. 1983|
|                                     | Stress-induced protein            | Carlsson & Lazarides 1983|
|                                     | DNA replicase                     | Yoshihara et al. 1984    |
|                                     | DNA polymerases α & β             | Yoshihara et al. 1984    |
|                                     | DNA ligase II                     | Yoshihara et al. 1984    |
|                                     | RNA polymerase II                 | Taniguchi et al. 1985    |
|                                     | Terminal deoxynucleotidyl transferase| Yoshihara et al. 1985    |
| **Extranuclear enzymes**            |                                  |                          |
| Microsomal enzyme                   | Histones                         | Roberts et al. 1975      |
| Mitochondrial enzyme                | M-band proteins                   | Kun et al. 1975          |
| Reovirus enzyme                     | Capsid proteins                   | Carter et al. 1980       |
| mRNA-associated protein             | mRNP proteins                     | Thomassin et al. 1985    |
TABLE 3
Inhibitory effects of various compounds on poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase.

| Compounds                        | IC₅₀ (µM) | Poly(ADP-ribose) synthetase (µM) | Mono(ADP-ribosyl)transferase (µM) |
|----------------------------------|-----------|----------------------------------|----------------------------------|
| 6(5H)-Phenanthridinone           | 0.30      | <1000                            | <3333                            |
| 1,5-Dihydroxyisoquinoline        | 0.39      | 890                              | 2282                             |
| 4-Amino-1,8-naphthalimide        | 0.18      | <200                             | <1111                            |
| 3-Hydroxybenzamide               | 9.1       | 9000                             | 989                              |
| 4-Hydroxyquinazoline             | 9.5       | 2600                             | 274                              |
| 2-Nitro-6(5H)-phenanthridinone   | 0.35      | 83                               | 237                              |
| 1-Hydroxyisoquinoline            | 7.0       | 1500                             | 214                              |
| Benzamide                        | 22        | 4500                             | 205                              |
| 2-Methyl-4(3H)-quinazolinone     | 5.6       | 1100                             | 196                              |
| 5-Iodouridine                    | 43        | 7200                             | 167                              |
| 1(2H)-Phthalazinone              | 12        | 510                              | 43                               |
| Benzoyleneurea                   | 8.1       | 200                              | 25                               |
| 1,8-Naphthalimide                | 1.4       | 20                               | 14                               |
| Oleic acid (C18:1, cis-9)        | 82        | 200                              | 2.4                              |
| Linoleic acid (C18:2, cis-9,12)  | 48        | 90                               | 1.9                              |
| Arachidonic acid (C20:4, cis-5,8,11,14) | 44 | 66                              | 1.5                              |
| Linolenic acid (C18:3, cis-9,12,15) | 110 | 110                             | 1.0                              |
| Vitamin K₃ (menadione)            | 420       | 120                              | 0.29                             |
| Novobiocin                        | 2200      | 280                              | 0.13                             |
| Palmitic acid (C16:0)            | >200      | 16                               | <0.08                            |
| Stearic acid (C18:0)             | >500      | 6.1                              | <0.0122                          |
| Vitamin K₁ (phyllquinone)        | 520       | 1.9                              | 0.0037                           |

this enzyme, whereas vitamin K₅s, novobiocin, and saturated long-chain fatty acids are specific for mono(ADP-ribosyl)transferase. Unsaturated fatty acids act on both types of enzyme.

A preliminary experiment showed that one of our new inhibitors, 4-hydroxyquinazoline, was capable of inducing murine teratocarcinoma cell differentiation. It seems that new inhibitors are applicable to various biological studies of mono- as well as poly(ADP-ribosylation) reactions.

SUMMARY

Biological roles of mono- and poly(ADP-ribosylation) reactions are reviewed, along with analysis of functional sequences of poly(ADP-ribose) synthetase and inhibitors of the synthetase compared with mono(ADP-ribose)transferase.

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