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edited by

WALDO E. COHN

Oak Ridge National Laboratory

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Abbreviations and Symbols

All contributors to this Series are asked to use the terminology (abbreviations and symbols) recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN) and approved by IUPAC and IUB, and the Editor endeavors to assure conformity. These Recommendations have been published in many journals (*1*, 2) and compendia (3) in four languages and are available in reprint form from the Office of Biochemical Nomenclature (OBN), as stated in each publication, and are therefore considered to be generally known. Those used in nucleic acid work, originally set out in section 5 of the first Recommendations (*1*) and subsequently revised and expanded (2, 3), are given in condensed form (I–V) below for the convenience of the reader. Authors may use them without definition, when necessary.

I. Bases, Nucleosides, Mononucleotides

1. *Bases* (in tables, figures, equations, or chromatograms) are symbolized by Ade, Gua, Hyp, Xan, Cyt, Thy, Oro, Ura; Pur = any purine, Pyr = any pyrimidine, Base = any base. The prefixes S–, H_2, F–, Br, Me, etc., may be used for modifications of these.

2. *Ribonucleosides* (in tables, figures, equations, or chromatograms) are symbolized, in the same order, by Ado, Guo, Ino, Xao, Cyd, Thd, Ord, Urd (Ψrd), Puo, Pyd, Nuc. Modifications may be expressed as indicated in (1) above. Sugar residues may be specified by the prefixes r (optional), d (=deoxyribo), a, x, l, etc., to these, or by two three-letter symbols, as in Ara-Cyt (for aCyd) or dRib-Ade (for dAdo).

3. *Mono-, di-, and triphosphates of nucleosides* (5′) are designated by NMP, NDP, NTP. The N (for "nucleoside") may be replaced by any one of the nucleoside symbols given in II-1 below. 2′-, 3′-, and 5′- are used as prefixes when necessary. The prefix d signifies "deoxy." [Alternatively, nucleotides may be expressed by attaching P to the symbols in (2) above. Thus: P-Ado = AMP; Ado-P = 3′-AMP] cNMP = cyclic 3′:5′-NMP; Bt_2cAMP = dibutyryl cAMP, etc.

II. Oligonucleotides and Polynucleotides

1. Ribonucleoside Residues

(a) Common: A, G, I, X, C, T, O, U, Ψ, R, Y, N (in the order of I-2 above).

(b) Base-modified: sI or M for thioinosine = 6-mercaptopurine ribonucleoside; sU or S for thiouridine; brU or B for 5-bromouridine; hU or D for 5,6-dihydrouridine; i for isopentenyl; f for formyl. Other modifications are similarly indicated by appropriate *lower-case* prefixes (in contrast to I-1 above) (2, 3).

(c) Sugar-modified: prefixes are d, a, x, or l as in I-2 above; alternatively, by *italics* or boldface type (with definition) unless the entire chain is specified by an appropriate prefix. The 2′-*O*-methyl group is indicated by *suffix* m (e.g., -Am- for 2′-*O*-methyladenosine, but -mA- for 6-methyladenosine).

(d) Locants and multipliers, when necessary, are indicated by superscripts and subscripts, respectively, e.g., -m_2^6A- = 6-dimethyladenosine; -s^4U- or -^{4}S- = 4-thiouridine; -ac^4Cm- = 2′-*O*-methyl-4-acetylcytidine.

(e) When space is limited, as in two-dimensional arrays or in aligning homologous sequences, the prefixes may be placed *over the capital letter*, the suffixes *over the phosphodiester symbol*.

2. Phosphoric Residues [left side = 5′, right side = 3′ (or 2′)]

(a) Terminal: p; e.g., pppN . . . is a polynucleotide with a 5′-triphosphate at one end; Ap is adenosine 3′-phosphate; C > p is cytidine 2′,3′-cyclic phosphate (*1*, 2, 3); p < A is adenosine 3′,5′-cyclic phosphate.

(b) Internal: hyphen (for known sequence), comma (for unknown sequence); unknown sequences are enclosed in parentheses. E.g., pA-G-A-C(C_2,A,U)A-U-G-C > p is a sequence with a (5′) phosphate at one end, a 2′:3′-cyclic phosphate at the other, and a tetranucleotide of unknown sequence in the middle. **(Only codon triplets should be written without some punctuation separating the residues.)**

3. Polarity, or Direction of Chain

The symbol for the phosphodiester group (whether hyphen or comma or parentheses, as in 2b) represents a 3′-5′ link (i.e., a 5′ . . . 3′ chain) unless otherwise indicated by appropriate numbers. "Reverse polarity" (a chain proceeding from a 3′ terminus at left to a 5′ terminus at right) may be shown by numerals or by right-to-left arrows. Polarity in any direction, as in a two-dimensional array, may be shown by appropriate rotation of the (capital) letters so that 5′ is at left, 3′ at right when the letter is viewed right-side-up.

4. Synthetic Polymers

The complete name or the appropriate group of symbols (see II-1 above) of the repeating unit, **enclosed in parentheses if complex or a symbol, is either** (a) preceded by "poly," or (b) followed by a subscript "n" or appropriate number. **No space follows "poly"** (2, 5).

The conventions of II-2b are used to specify known or unknown (random) sequence, e.g.,

polyadenylate = poly(A) or A_n, a simple homopolymer;

poly(3 adenylate, 2 cytidylate) = poly(A_3C_2) or $(A_3,C_2)_n$, an *irregular* copolymer of A and C in 3 : 2 proportions;

poly(deoxyadenylate-deoxythymidylate) = poly[d(A-T)] or poly(dA-dT) or $(dA\text{-}dT)_n$ or $d(A\text{-}T)_n$, an *alternating* copolymer of dA and dT;

poly(adenylate,guanylate,cytidylate,uridylate) = poly(A,G,C,U) or $(A,G,C,U)_n$, a random assortment of A, G, C, and U residues, proportions unspecified.

The prefix copoly or oligo may replace poly, if desired. The subscript "n" may be replaced by numerals indicating actual size, e.g., $A_n \cdot dT_{12\text{-}18}$.

III. Association of Polynucleotide Chains

1. *Associated* (e.g., H-bonded) chains, or bases within chains, are indicated by a *center dot* (not a hyphen or a plus sign) separating the *complete* names or symbols, e.g.:

poly(A) · poly(U) or $A_n \cdot U_m$
poly(A) · 2 poly(U) or $A_n \cdot 2U_m$
poly(dA-dC) · poly(dG-dT) or $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_m$.

2. *Nonassociated* chains are separated by the plus sign, e.g.:

2[poly(A) · poly(U)] → poly(A) · 2 poly(U) + poly(A)
or $2[A_n \cdot U_m] \rightarrow A_n \cdot 2U_m + A_n$.

3. Unspecified or unknown association is expressed by a comma (again meaning "unknown") between the completely specified chains.

Note: In all cases, each chain is completely specified in one or the other of the two systems described in II-4 above.

IV. Natural Nucleic Acids

RNA	ribonucleic acid or ribonucleate
DNA	deoxyribonucleic acid or deoxyribonucleate
mRNA; rRNA; nRNA	messenger RNA; ribosomal RNA; nuclear RNA
hnRNA	heterogeneous nuclear RNA
D-RNA; cRNA	"DNA-like" RNA; complementary RNA

mtDNA	mitochondrial DNA
tRNA	transfer (or acceptor or amino-acid-accepting) RNA; replaces sRNA, which is not to be used for any purpose
aminoacyl-tRNA	"charged" tRNA (i.e., tRNA's carrying aminoacyl residues); may be abbreviated to AA-tRNA
alanine tRNA or $tRNA^{Ala}$, etc.	tRNA normally capable of accepting alanine, to form alanyl-tRNA, etc.
alanyl-tRNA or alanyl-$tRNA^{Ala}$	The same, with alanyl residue covalently attached. [*Note:* fMet = formylmethionyl; hence $tRNA^{fMet}$, identical with $tRNA_f^{Met}$]

Isoacceptors are indicated by appropriate subscripts, i.e., $tRNA_1^{Ala}$, $tRNA_2^{Ala}$, etc.

V. Miscellaneous Abbreviations

P_i, PP_i	inorganic orthophosphate, pyrophosphate
RNase, DNase	ribonuclease, deoxyribonuclease
t_m (not T_m)	melting temperature (°C)

Others listed in Table II of Reference 1 may also be used without definition. No others, with or without definition, are used unless, in the opinion of the editor, they increase the ease of reading.

Enzymes

In naming enzymes, the 1978 recommendations of the IUB Commission on Biochemical Nomenclature (*4*) are followed as far as possible. At first mention, each enzyme is described *either* by its systematic name *or* by the equation for the reaction catalyzed *or* by the recommended trivial name, followed by its EC number in parentheses. Thereafter, a trivial name may be used. Enzyme names are not to be abbreviated except when the substrate has an approved abbreviation (e.g., ATPase, but not LDH, is acceptable).

References*

1. *JBC* **241,** 527 (1966); *Bchem* **5,** 1445 (1966); *BJ* **101,** 1 (1966); *ABB* **115,** 1 (1966), **129,** 1 (1969); and elsewhere.†
2. *EJB* **15,** 203 (1970); *JBC* **245,** 5171 (1970); *JMB* **55,** 299 (1971); and elsewhere.†
3. "Handbook of Biochemistry" (G. Fasman, ed.), 3rd ed. Chemical Rubber Co., Cleveland, Ohio, 1970, 1975, Nucleic Acids, Vols. I and II, pp. 3–59.
4. "Enzyme Nomenclature" [Recommendations (1978) of the Nomenclature Committee of the IUB]. Academic Press, New York, 1979.
5. "Nomenclature of Synthetic Polypeptides," *JBC* **247,** 323 (1972); *Biopolymers* **11,** 321 (1972); and elsewhere.†

Abbreviations of Journal Titles

Journals	*Abbreviations used*
Annu. Rev. Biochem.	ARB
Arch. Biochem. Biophys.	ABB
Biochem. Biophys. Res. Commun.	BBRC

*Contractions for names of journals follow.

†Reprints of all CBN Recommendations are available from the Office of Biochemical Nomenclature (W. E. Cohn, Director), Biology Division, Oak Ridge National Laboratory, Box Y, Oak Ridge, Tennessee 37830, USA.

Biochemistry	Bchem
Biochem. J.	BJ
Biochim. Biophys. Acta	BBA
Cold Spring Harbor Symp. Quant. Biol.	CSHSQB
Eur. J. Biochem.	EJB
Fed. Proc.	FP
Hoppe-Seyler's Z. physiol. Chem.	ZpChem
J. Amer. Chem. Soc.	JACS
J. Bacteriol.	J. Bact.
J. Biol. Chem.	JBC
J. Chem. Soc.	JCS
J. Mol. Biol.	JMB
Nature, New Biology	Nature NB
Nucleic Acid Research	NARes
Proc. Nat. Acad. Sci. U.S.	PNAS
Proc. Soc. Exp. Biol. Med.	PSEBM
Progr. Nucl. Acid Res. Mol. Biol.	This Series

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Poly(adenosine diphosphate ribose)

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I. Introduction

The first description of poly(adenosine diphosphate ribose) [poly(ADPR)] polymerase activity was that of Chambon, Weill, and Mandel in 1963 (*1*). They observed that ATP incorporation into an acid-insoluble fraction was enhanced by nicotinamide mononucleotide at least 1000-fold in chicken liver nuclei. Poly(ADPR) was identified as the enzyme product. NAD was shown to be the direct substrate of the poly(ADPR) polymerase (*2–4*). The structure of poly(ADPR) was re-

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ported by Chambon *et al.* (2) and by Doly and Petek (5) and was confirmed by the groups of Hayaishi (6) and of Sugimura (7). Poly(ADPR) polymerase catalyzes the polymerization of the ADPR moiety of NAD. One of the main direct functions of poly(ADPR) polymerase seemed to be covalent ADP-ribosylation of proteins, since poly(ADPR) is generally found to be bound covalently to various proteins, especially nuclear proteins (8). The natural occurrence of poly(ADPR) was first demonstrated *in vivo* in chicken liver nuclei by Doly and Mandel (9).

An enzyme that hydrolyzes poly(ADPR) occurs in calf thymus (10) and in rat liver nuclei (11). The reaction products are ADPR and oligo(ADPR). This poly(ADPR) glycohydrolase does not split the linkage by which the polymer is bound to protein, but an enzyme that splits the bond between ADPR and histone was subsequently found (12). Snake venom phosphodiesterase (EC 3.1.4.1) is often used for chain-length analysis of the polymer. It splits the pyrophosphate bonds, yielding 5′-AMP and 2′-(5″-phosphoribosyl)-5′-AMP (also called PR-AMP, ΨADPR, or isoADPR). Figure 1 illustrates the structure of poly(ADPR) and the catalytic sites of the enzymes involved in ADP-ribosylation and degradation.

Several hypotheses have been formulated concerning the roles of poly(ADPR) and poly(ADPR) polymerase (or ADPR-transferase), and involvement of poly(ADPR) in DNA duplication and in cell prolifera-

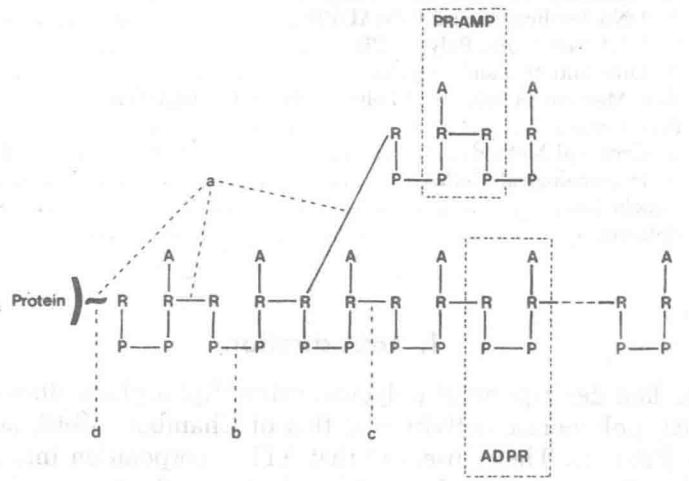

FIG. 1. Structure of poly(ADPR) and enzymology of poly(ADP-ribosylation). Synthesizing enzyme: poly(ADPR) polymerase (a). Degrading enzymes: venom phosphodiesterase (b); poly(ADPR) glycohydrolase (c); ADPR-protein hydrolase (d).

tion has been strongly suggested. Similarly, it was suggested that poly(ADPR) polymerase is involved in DNA repair. Participation in chromatin structure was suggested through ADP-ribosylation of histones, and ADP-ribosylation of other proteins was suggested to be involved in several other mechanisms.

Several comprehensive reviews that cover the details of work on the subject up to 1977 have appeared (*13–16*). The proceedings of the four last international meetings (*17–20*) have been published and are discussed in these reviews. Two brief reviews concerning chromatin structure and ADP-ribosylation of nuclear proteins have been published by Smulson (*21*) and by Hilz *et al.* (*22*), respectively.

This review includes published data from 1977 up to December 1980 and the proceedings of a seminar (*23*) held in October 1979 as well as unpublished results and personal communications kindly provided by many colleagues dealing with the following: higher-order structure of poly(ADPR); poly(ADPR) polymerase purification and its properties; molecular mechanisms of histone- and elongation factor (EF-2)-ADP-ribosylation; DNA-repair as one of the biological functions of poly(ADPR) polymerase; and new techniques for poly(ADPR) studies, including immunological analysis.

II. Poly(adenosine diphosphate ribose)

A. Structure of Poly(ADPR)

Poly(ADPR) is a unique homopolymer synthesized enzymically from NAD by poly(ADPR) polymerase, a ubiquitous enzyme found in the nuclei of all eukaryotic cells tested as well as in some prokaryotic cells (for reviews see *13–16*). The ADPR moieties of NAD are polymerized through ribose–ribose (1″-2′) glycosidic bonds with concomitant release of nicotinamide moieties (Fig. 1). The structure of the ribose–ribose moiety of poly(ADPR) was determined to be that of a 2-*O*-α-D-ribofuranosyl-D-ribofuranose, from the ^{13}C chemical shifts of methyl-α- and methyl-β-D-ribofuranosides, and of the downfield displacement of ^{13}C NMR signals by glycosidic bond formation (*24*).

Poly(ADPR) is generally found covalently bound to various nuclear proteins. After digestion with Pronase and deproteinization with phenol, poly(ADPR) can be separated from the DNA or RNA fraction by hydroxyapatite column chromatography (*25*). The peak fractions from the hydroxyapatite column may each be separated by polyacrylamide gel electrophoresis into two distinct subfractions differing in chain length and terminal structure (*26*). Type-1 oligo(ADPR) has a

complete structure and type-2 oligo(ADPR) has a terminal degraded structure, suggesting the existence of a new phosphodiesterase hydrolyzing poly(ADPR) endonucleolytically, like snake venom phosphodiesterase, or exonucleolytically from both termini. Individual polymers up to at least 33 units can be completely separated according to their chain length by electrophoresis in sodium dodecyl sulfate on polyacrylamide gels (*27*). Nuclei of Ehrlich ascites tumor cells produce a poly(ADPR) pattern distinctly different from that of rat liver nuclei with respect to the chain-length distribution (*27*).

Electrophoretic analysis on polyacrylamide gel has led to the discovery of a branched structure of poly(ADPR) (*28*). From the discrepancy between the size (more than 4.5×10^5) estimated by electrophoresis and the chain length determined by the ratio of total radioactivity to that derived from the terminus, it appears that the polymer has a branched structure (*28*). The trisaccharide 2′-[5-phospho-2(or 3)-(5-phosphoribosyl)ribosyl]adenosine 5′-phosphate, or 5-phosphoribosyl-(1 → 2 or 3)-5-phosphoribosyl-(1 → 2)-5-phosphoribosyl-(1 → 9)-adenine, was obtained by phosphodiesterase hydrolysis of poly(ADPR) (*29*). The existence of such a compound is evidence for a branched structure of poly(ADPR), previously thought to be a linear molecule (Fig. 1). The frequency of branching appeared to be about 1 per 20–30 ADPR residues of high-molecular-weight poly(ADPR). The finding of a branched structure suggests that poly(ADPR) may play an important role in chromatin architecture as well as in covalent modification of nuclear proteins. The natural occurrence as well as the enzymic mechanisms underlying the synthesis of this branched poly(ADPR) is yet unknown. If this branched polymer is also synthesized by poly(ADPR) polymerase, the enzyme catalyzes three different reactions: (*a*) ADPR transfer to acceptor protein (initiation); (*b*) addition of ADPR units on preexisting mono- or oligo(ADPR) (chain elongation); and (*c*) addition of ADPR units onto poly(ADPR) chains (branching).

B. Natural Occurrence of Poly(ADPR)

Poly(ADPR) was first discovered as an *in vitro* reaction product (*1–4*). There are several reports suggesting the natural occurrence of poly(ADPR) (see for review *13–16*).

A specific antibody against purified poly(ADPR) synthesized *in vitro* affords a sensitive, reproducible radioimmunoassay system (*30*) that has produced evidence for the natural occurrence of poly(ADPR) in calf thymus, liver, kidney, brain, pancreas, and spleen (*31*). Naturally occurring poly(ADPR) in calf thymus is composed of molecules of various chain lengths like that synthesized by an *in vitro* system. Calf

thymus was estimated to contain about 0.02 μg of poly(ADPR) per milligram of DNA (*31*).

Naturally occurring antibodies to poly(ADPR) suggest that poly(ADPR) occurs naturally in human beings, although the evidence is indirect and is a result of complex biological events. The binding activities of the sera of patients with systemic lupus erythematosus (SLE) to [^{14}C]poly(ADPR) were higher than those of the sera of normal individuals, and binding activities of the sera of SLE patients to [^{14}C]poly(ADPR) were attributable to immunoglobulins, mainly to IgG, but not to nonspecific interactions between [^{14}C]poly(ADPR) and IgG (*32*). The specificity of the binding was verified by inhibition tests using unlabeled poly(ADPR) and 14 related compounds. During this study, a population of antibodies cross-reacting with poly(A) · poly(U) was found among the antibodies to poly(ADPR), and this population could be completely removed by absorption of the SLE sera with poly(A) · poly(U). Moreover, rabbit antibodies to poly(ADPR) do not cross-react at all with synthetic homopolynucleotides. The mechanism of induction was studied with rabbits immunized with poly(ADPR) or poly(A) · poly(U) (*33*). A rabbit immunized with poly(A) · poly(U) induced antibodies specific to poly(ADPR) as well as antibodies specific to poly(A) · poly(U). In contrast, a rabbit immunized with a complex of poly(ADPR) with MBSA (methylated bovine serum albumin) in Freund's complete adjuvant showed no antibody activity to poly(A) · poly(U), although a sevenfold higher specific activity of antibody to poly(ADPR) was detected compared to that of rabbit immunized with poly(A) · poly(U).

The significance of antibodies to poly(ADPR) in systemic lupus erythematosus has been further studied (*34*). Of 82 human sera tested, antibodies to poly(ADPR) were found only in the SLE and in SLE-like rheumatic diseases (42 sera). Anti-DNA antibodies, on the other hand, were found not only in the SLE and SLE-like diseases, but also in rheumatoid arthritis and chronic active hepatitis. Therefore, estimation of poly(ADPR) binding was more specific for, and more discriminatory of SLE from other diseases than the estimation of double-stranded DNA binding. The results indicate that the estimation of poly(ADPR) binding in serum may be more useful in the diagnosis of SLE than the presently employed estimation of DNA binding using the Amersham kit. DNA · (anti-DNA) immune complexes were detected in some of the SLE sera after DNase I digestion, confirming earlier reports of the existence of circulating DNA · (anti-DNA) complexes in SLE patients. Snake venom phosphodiesterase treatment of some of the SLE sera also resulted in increased poly(ADPR) binding activity, suggesting the existence of poly(ADPR) · [anti-poly(ADPR)]

immune complexes in the circulation of SLE patients. This observation raises the possibility that poly(ADPR) immune complexes may play some part in the pathogenesis of some cases of SLE.

C. Some Direct Biological Functions of Poly(ADPR)

Inhibition of rat liver Ca^{2+}, Mg^{2+}-dependent endonuclease activity by its poly(ADP-ribosylation) is a well known example of a biological function of poly(ADPR) (*35, 36*). Direct inhibition of deoxyribonuclease activity in an extract of rat liver nuclei by poly(ADPR) has been observed (*37*). Only one of the two DNase activities separated by DEAE-cellulose column chromatography was inhibited by poly(ADPR) and poly(A).

The myeloid leukemia cells of mice differentiate into cells with phagocytic activity, Fc receptors, and lysozyme activity on treatment with poly(ADPR) (*38*). Cells with morphological characteristics of mature macrophages and granulocytes also appear on incubation with poly(ADPR). Dextran sulfate and polyvinylsulfate are also effective for the induction of phagocytic cells, but poly(A), poly(U), poly(C), poly(I) · poly(C), and poly(A) · poly(U) were not (*38*).

When a chromatin-bound neutral protease inhibitor isolated from rat peritoneal macrophages was incubated with poly(ADPR) glycohydrolase from calf thymus, the inhibitory potency was markedly decreased (*39*). An authentic poly(ADPR), with a mean chain length of approximately 30 ADPR units, produced significant inhibition of the neutral protease isolated from macrophage chromatin. No such inhibition was produced by DNA, RNA, poly(A), poly(C), or monomeric ADPR. It was concluded that the inhibitor isolated is identical with poly(ADPR) with an average chain length from 4 to 7 ADPR units (*39a*).

III. Poly(ADPR) Polymerase

A. Purification of Poly(ADPR) Polymerase

Poly(ADPR) polymerase is a chromatin-bound nuclear enzyme. The localization of this enzyme in cell nuclei suggests that the enzyme may play an important role in nuclear events. The enzyme activity was measured by the incorporation of labeled ADPR from radioactive NAD into the acid-insoluble fraction. To elucidate the biological function of this enzyme, the analysis of the enzyme reaction is indispensable. The purification of poly(ADPR) polymerase was one important step for this, as well as the analysis of the enzymic reaction products,