ADP-Ribosyl Protein Lyase

PURIFICATION, PROPERTIES, AND IDENTIFICATION OF THE PRODUCT*

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ADP-ribosyl protein lyase, formerly termed ADP-ribosyl histone-splitting enzyme (Okayama, H., Honda, M., and Hayashi, O. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2254–2257), was purified approximately 4,000-fold from rat liver and characterized. The purified enzyme exhibited a single protein band at the position of $M_r = 83,000$ upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme split the bond between ADP-ribose and histone H2B or H1, and also acted on ADP-ribosyl pentapeptide (Pro-(ADP-ribosyl)Glu-Pro-Ala-Lys) of H2B but not its deacylated derivative, phosphoribosyl pentapeptide. The enzyme cleaved the bond between histone and mono(ADP-ribose), but hardly cleaved the bond with oligo- or poly(ADP-ribose).

The enzymatic product was close to, but not identical with, ADP-ribose. The terminal ribose residue, obtained by hydrolysis of the split product by snake venom phosphodiesterase and alkaline phosphatase, was identified as 3-deoxy-β-glycero-pentos-2-ulose by the following gas chromatography-mass spectrometric analyses: 1) the reduced sugar was a mixture of 3-deoxy-threo- and 3-deoxy-erythro-pentitol, and 2) the deuterated reduced sugar was identical with that derived from synthetic 3-deoxy-β-glycero-pentos-2-ulose. This result indicated that the direct product was $5''$-ADP-3''-deoxypent-2''-eno furanose, a dehydrated form of ADP-ribose, and that the enzyme is a lyase and not a hydrolase.

Poly(ADP-ribose) is a homopolymer of ADP-ribose derived from NAD and linked together through $\alpha(1''\rightarrow2'')$ ribose-ribose bonds (1, 2). The biological function of this polymer is not fully understood yet (1, 2). Recently, several lines of evidence have accumulated to suggest its close relationship to the repair of DNA damage induced by chemical carcinogens and ionizing radiation (3–6). Poly(ADP-ribose) synthesis is initiated by a covalent attachment of ADP-ribose monomer to histone or non-histone proteins including poly(ADP-ribose) synthetase itself (7). Recent reports from this and other laboratories (8–11) have elucidated that ADP-ribose is bound through an ester linkage to a γ-carboxyl group of a glutamatic acid residue of histone H1 or H2B, or the a-carboxyl group of a COOH-terminal lysine residue of histone H1.

Two enzymes degrading poly(ADP-ribose) have been found in animal tissues and characterized; one is phosphodiesterase which splits the pyrophosphate bonds in the polymer (12), and the other is poly(ADP-ribose) glycohydrolase which cleaves the ribose-ribose bonds (13, 14). Analysis of in vivo degradation products of the polymer indicated that the latter, glycohydrolase, is mainly responsible for degradation in various tissues (15). In 1978, another enzyme, termed ADP-ribosyl histone-splitting enzyme, was discovered in this laboratory (16). This enzyme split the bond between ADP-ribose and histone, but the product was not exactly identical with ADP-ribose (16, 17). The structure of this product and, thus, the reaction mechanism of this enzyme have remained unclarified (18). In order to solve these problems, we purified the enzyme to apparent homogeneity, and analyzed the reaction product. This paper describes the purification and some properties of the enzyme as well as the identification of an unusual sugar product.

EXPERIMENTAL PROCEDURES1

RESULTS

Purification of ADP-Ribosyl Protein Lyase

Details of the purification procedure are presented in the Miniprint. The overall purification is summarized in Table I. The specific activity of the final preparation assayed at the substrate concentration of 5 μM was 480 nmol/min/mg of protein. The final enzyme preparation (Step 8) was unstable; the half-life in a frozen state at −20 °C was about 4 days. The addition of bovine serum albumin (0.5 mg/ml), glycerol (50%, v/v), or NAD (1 mM) did not stabilize the enzyme activity. By contrast, Peak I enzyme of Step 7, after concentration on a hydroxyapatite column up to >0.2 mg/ml, was stable for at least 3 months when stored at −20 °C without freeze-thaws.

1 Portions of this paper (including "Experimental Procedures," part of "Results," and Figs. 1–3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1523, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
The Peak enzyme was free of poly(ADP-ribose) glycohydrolase and phosphodiesterase activities. Because of this stability and purity, most studies reported in this paper were performed using the concentrated Peak 1 enzyme of Step 7, unless otherwise stated.

Physicochemical Properties of ADP-Ribosyl Protein Lyase

The purified enzyme (Step 8 fraction) exhibited a single protein band at the position of $M_r = 83,000$ upon Na dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). The Stokes radius was estimated as 5.2 nm with Bio-Gel P-300 filtration (Fig. 2), and the sedimentation coefficient as 3.4 S with glycerol gradient centrifugation (Fig. 3). Assuming the partial specific volume as 0.73, the molecular weight of the enzyme was calculated as 75,000 (38). These results suggest that the enzyme is composed of a single and asymmetrical polypeptide of $M_r \approx 80,000$ with a fractional ratio of 1.8 (38).

Enzymological Properties of ADP-Ribosyl Protein Lyase

Optimum pH—The enzyme was most active around pH 7.0 (Fig. 5A). The standard assay was, however, performed at pH 6.0, because nonenzymatic splitting was less at this pH than pH 7.0, especially upon longer incubations.

Sulfhydryl Compound Requirement—A sulfhydryl-reducing agent such as dithiothreitol (21 mM) or 2-mercaptoethanol (22 mM) was required for full activity (Fig. 5B).

Substrate Specificity of ADP-Ribosyl Protein Lyase—Purified enzyme cleaved the bonds between ADP-ribose and histone H2B as well as its NH$_2$-terminal pentapeptide (Table II). The $K_0$ values for ADP-ribosyl histone H2B and ADP-ribosyl pentapeptide were estimated as 8.5 and 8.8 $\mu M$, respectively. The enzyme did not act on a deacetylated derivative of the latter, phosphoribosyl pentapeptide. The enzyme acted also on ADP-ribosyl histone H1, whereas it did not split a reduced Schiff base in nonenzymatic ADP-ribose-histone H1 adduct. Furthermore, the enzyme cleaved ADP-ribosyl nonhistone proteins (a mixture) (17), but not the ADP-ribosyl nicotinamide bond in NAD or the carboxyl ester bond in $p$-nitrophenyl acetate.

The effect of varying chain lengths of ADP-ribose on the reactivity was investigated using a mixture of mono- and oligo(ADP-ribose) histone H1. When the mixture (49 $\mu M$ as ADP-ribose residues as the chain length distribution shown in Table III) was incubated with varying amounts (0.1-0.4 unit) of the enzyme for 10 min, maximally 39% of total $^{14}$C was released. The released products were analyzed, and compared with the products released nonenzymatically (Table III). Only ADP-ribose monomer was detected in a significant amount as the enzymatic product; the amounts of dimer, trimer, and so on were much less and almost identical in enzymatic and nonenzymatic reactions. Furthermore, a longer incubation (up to 120 min) with the enzyme (0.1 unit) did not promote a net enzymatic release of more than 32% of total $^{14}$C in spite of a gradual increase in nonenzymatic cleavage. These results suggest that ADP-ribose protein lyase cleaves almost exclusively mono(ADP-ribose) histone under these conditions.

Effects of Various Compounds on ADP-Ribosyl Protein Lyase—The enzyme activity was strongly inhibited by ADP-ribose. The inhibition was competitive with substrate; the $K_i$ value, estimated by Dixon's plot (39), was 0.5 mM. $\beta$-NAD also inhibited the activity competitively, albeit to a lesser degree ($K_i = 3.5$ mM). Neither ribose 5-phosphate nor ribose, the portion through which ADP-ribose links to histone, was inhibitory up to 10 mM. AMP or ADP did not inhibit the activity up to 10 mM. These results, taken together with the substrate specificity described above, suggest that this enzyme recognizes ADP-ribose as a whole and not by fragments. $\alpha$-NAD and NADP were not inhibitory up to 10 mM. Various inhibitors of related enzymes such as 3',5'-cyclic AMP, an
products were obtained from the same substrate by a 10-min incubation at 37 °C with or without the enzyme (0.32 unit) in 50 pl of 100 mM Na cacodylate (A, △). K phosphate (●, ○) or Tris/HCl (■, □) of various pH values as indicated. Enzyme activity (x—x) was the difference between the reactions with (closed symbols, ——) and without (open symbols, ——) the enzyme (0.010 unit). B, the Step 7 enzyme (1 ml) was dialyzed against 500 ml of 20 mM K phosphate (pH 6.0) containing 1 M KCl and 20% (v/v) glycerol with three changes. The reaction was carried out under the standard conditions except that dithiothreitol (C—C) or 2-mercaptoethanol (●—●) of specified concentrations was used.

Chain length distribution of these lyophilized materials was determined by the addition of 160 μl of 15% C14COOH. The supernatant fraction (8,160 cpm) obtained by centrifugation (20,000 g, 20 min) was washed with diethyl ether and applied to an AG 1-X2 column (formate form, 0.7 x 14 cm), together with unlabeled ADP-ribose (ADPR, 0.3 μmol) and AMP (0.2 μmol). The column was eluted with a linear gradient of 0 to 6 m formic acid (total volume, 100 ml). Fractions (1 ml) were collected, and examined for A260 (●—●). Inhibitor of poly(ADP-ribose) glycohydrolase (13, 14), ATP, an inhibitor of rat liver phosphodiesterase (40), and nicotinamide, thymidine, and 3-aminobenzamide, inhibitors of poly(ADP-ribose) synthetase (41), did not inhibit the activity of ADP-ribosyl protein lyase up to 10 mM. Divalent cations (Mg2+ or Ca2+) or EDTA had no effect on the activity up to 10 mM.

TABLE II
Enzymatic reaction was carried out by incubating specified substrates (5 μm as ADP-ribose or ribose-5-phosphate residues) with the enzyme (0.011 unit) as described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Initial velocity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ade-14C]ADP-ribosyl histone H2B</td>
<td>350</td>
</tr>
<tr>
<td>[Ade-14C]ADP-ribosyl pentapeptide</td>
<td>600a</td>
</tr>
<tr>
<td>Phospho[14C]ribosyl pentapeptide</td>
<td>0b</td>
</tr>
<tr>
<td>[Ade-14C]ADP-ribosyl histone H1</td>
<td>186</td>
</tr>
<tr>
<td>[Ade-14C]ADP-ribosyl-histone H1 adduct</td>
<td>0</td>
</tr>
</tbody>
</table>

a The reaction was terminated by the addition of 0.2 ml of 0.8 M formic acid. The mixture was applied directly to an AG 1-X2 column (formate form, 0.5 x 1 cm). The column was washed with 1.8 ml of 0.8 M formic acid to elute the remaining ADP-ribosyl pentapeptide. The column was then eluted with 1.8 ml of 6 M formic acid containing 1 M ammonium formate, and 14C in this eluate was determined.

b The standard mixture was scaled up to 100 μl. The reaction was terminated by the addition of 40 μl of 100% C14COOH. The whole mixture was washed with diethyl ether, and subjected to paper chromatography (solvent system II).

TABLE III
Chain length distribution of ADP-ribosyl histone H1 and its split products

<table>
<thead>
<tr>
<th>ADP-ribose units</th>
<th>Substrate</th>
<th>Split products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+Enzyme</td>
<td>—Enzyme</td>
</tr>
<tr>
<td>Monomer</td>
<td>5840 (44)</td>
<td>4540 252</td>
</tr>
<tr>
<td>Dimer</td>
<td>2760 (21)</td>
<td>155 119</td>
</tr>
<tr>
<td>Trimer</td>
<td>2260 (17)</td>
<td>125 99</td>
</tr>
<tr>
<td>Tetramer</td>
<td>1240 (9)</td>
<td>70 49</td>
</tr>
<tr>
<td>Pentamer</td>
<td>614 (5)</td>
<td>25 26</td>
</tr>
<tr>
<td>Hexamer</td>
<td>470 (4)</td>
<td>6 0</td>
</tr>
</tbody>
</table>

The reaction was terminated by the addition of 0.2 ml of 0.8 M formic acid. The mixture was applied directly to an AG 1-X2 column (formate form, 0.5 x 1 cm). The column was washed with 1.8 ml of 0.8 M formic acid to elute the remaining ADP-ribosyl pentapeptide. The column was then eluted with 1.8 ml of 6 M formic acid containing 1 M ammonium formate, and 14C in this eluate was determined.

Inhibitor of poly(ADP-ribose) glycohydrolase (13, 14), ATP, an inhibitor of rat liver phosphodiesterase (40), and nicotinamide, thymidine, and 3-aminobenzamide, inhibitors of poly(ADP-ribose) synthetase (41), did not inhibit the activity of ADP-ribosyl protein lyase up to 10 mM. Divalent cations (Mg2+ or Ca2+) or EDTA had no effect on the activity up to 10 mM.

Analysis of Split Product
Production of ADP-X and Compound X—As reported previously with a crude enzyme preparation (16), the split product by ADP-ribosyl protein lyase did not co-elute with authentic ADP-ribose from an AG 1-X2 column (Fig. 6). An
APPARENTLY IDENTICAL PRODUCT WAS OBTAINED WITH ENZYME IN K
PHOSPHATE AND TRIS/HCl BUFFER AT pH 6.0 AS WELL AS pH 7.0.
ON THE OTHER HAND, WHEN ADP-RIBOSYLATED HISTONE H2B WAS SPLIT
NONENZYMICALLY BY TREATMENT (10 MIN AT 25 °C) WITH 0.1 M
NAOH OR 2 M NH2OH (pH 7.0), THE SPLIT PRODUCT CO-ELUTED
WITH ADP-RIBOSE IN THE SAME COLUMN CHROMATOGRAPHY (DATA
NOT SHOWN).

WHEN THE ENZYMATIC SPLIT PRODUCT WAS DIGESTED WITH
PHOSPHODIESTERASE AND ANALYZED BY AG 1-X2 COLUMN
CHROMATOGRAPHY, TWO PRODUCTS WERE RECOVERED: ONE, DERIVED FROM
THE PHOSPHORIBOSE PORTION AS MONITORED BY ADP-[14C]RIBOSYL
HISTONE H2B, ELUTED SLIGHTLY AFTER AUTHENTIC RIBOSE 5-PHOSPHATE,
WHILE THE OTHER, AMP PORTION AS MONITORED BY [ADE-14C]ADP-
RIBOSYL HISTONE H2B, CO-ELUTED WITH AUTHENTIC 5'-AMP (DATA
NOT SHOWN). THE FORMER PRODUCT WAS THEN TREATED WITH ALKA-
LINE PHOSPHATASE. THIS TREATMENT GAVE A COMPACT, DESIG-
NATED AS X, WHICH ORIGINATED FROM THE RIBOSE PORTION BUT
MIGRATED DIFFERENTLY FROM RIBOSE OR OTHER PENTOSES IN PAPER
CHROMATOGRAPHY (Fig. 7A; Table IV). IN CONTRAST, THE RIBOSE
PORTION, RECOVERED BY PHOSPHODIESTERASE AND ALKALINE PHOS-
PHATASE TREATMENTS OF ADP-[14C]RIBOSYLATED HISTONE H2B, ELUTED FROM HISTONE
H2B BY 0.1 M NH2OH OR NEUTRAL NH2OH, COINCIDED WITH AUTHENTIC
RIBOSE IN ALL SOLVENT SYSTEMS TESTED. A COMPOUND X-LIKE
MATERIAL WAS DETECTED AS A MINOR PEAK IN PAPER CHROMATOGRAPHY
OF A PHOSPHODIESTERASE/ALKALINE PHOSPHATASE DIGEST OF THE
SPLIT PRODUCT OBTAINED BY TREATMENT (15 H, 37 °C) OF ADP-
[14C]RIBOSYLATED HISTONE H2B WITH 100 mM TRIS/HCl (pH 7.0)
(Fig. 7B). BECAUSE INCUBATION OF ADP-[14C]RIBOSYLATED HISTONE
H2B WITH ADP-RIBOSYL PROTEIN L YASE DID NOT PRODUCE ADP-X, NOR
DI S I M I L A R ENZYMATIC DIGESTION OF THE INCUBATED MATERIAL GIVE
COMPOUND X, ADP-X DID NOT APPEAR TO BE FORMED FROM LIBERATED ADP-
RIBOSE BY AN ENZYMATIC ACTION, BUT APPEARED TO BE PRODUCED FROM
HISTONE-BOUND ADP-RIBOSE BY SOME ALTERATION OR MODIFICATION
OF THE TERMINAL RIBOSE UPON THE ENZYMATIC CLEAVAGE OF ADP-
RIBOSE.

TABLE IV

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent A</th>
<th>Solvent B</th>
<th>Solvent C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxyribose</td>
<td>0.55</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Anhydroribitol</td>
<td>0.54</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>1-Deoxyribose</td>
<td>0.55</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>Lyxose</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Ribulose</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>2-Deoxyribose</td>
<td>0.52</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Ribonolactone</td>
<td>0.48</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>3-Deoxyribose</td>
<td>0.55</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>3-Deoxyribitol</td>
<td>0.55</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Anhydroarabinose</td>
<td>0.54</td>
<td>0.54</td>
<td>0.54</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.53</td>
<td>0.78</td>
<td>0.78</td>
</tr>
</tbody>
</table>

FIG. 7. Paper chromatography of phosphodiesterase and alka-
line phosphatase digests of split products obtained with (A)
or without the enzyme (B). The reaction was carried out in the
mixture (36 µL) containing 100 mM Tris/HCl (pH 7.0), 1 mM dihy-
diothreitol, ADP-[14C]ribosyl histone H2B (53,000 cpm), and (A) ADP-
ribosyl protein lyase (2.68 units) for 20 min at 37 °C and (B) WITH
NO ADDITION FOR 15 H AT 37 °C. THE REACTION WAS TERMINATED BY THE
ADDITION OF 40 µL OF 100 mM CH3COOH. THE SUPERNATANT ISOLATE
(A, 35,000 cpm, or B, 35,000 cpm) OBTAINED BY CENTRIFUGATION (20,000 ×
g, 10 MIN) WAS WASHED WITH DIETHYL ETHER, AND DIGESTED WITH PHOS-
PHODIESTERASE (0.2 UNIT) AND ALKALINE PHOSPHATASE (0.76 UNIT) FOR 2 H AT
37 °C. AN ALIQUOT (4,000 cpm) OF EACH MIXTURE WAS SUBMITTED TO PAPER
CHROMATOGRAPHY (SOLVENT SYSTEM I), TOGETHER WITH RIBOSE (RIB) AND
ADENOSINE (ADO) AS MARKERS.

FIG. 8. Paper chromatography of compound X treated with
phenylhydrazine (A) or NaBH4 (B). Compound X was obtained
as described under "Experimental Procedures." A, compound X (4,000
cpm) was treated with (- - -) or without (---) 10 mM Phenylhydral-
azine in 10 µL of 100 mM Na acetate (pH 4.5) for 90 min at 37 °C. AFTER
INCUBATION, THE MIXTURE WAS DIRECTLY TREATED AND CHROMATO-
GRAPHED IN SOLVENT SYSTEM I, TOGETHER WITH RIBOSE (RIB) AND
ADENOSINE (ADO) AS MARKERS. B, compound X (58,000 cpm) WAS TREATED
WITH (- - -) OR WITHOUT (---) 83 mM NaBH4 IN 18 µL OF 170 mM K
PHOSPHATE (pH 6.5) AT ROOM TEMPERATURE FOR 60 MIN. THE REACTION
WAS TERMINATED BY THE ADDITION OF 6 µL OF 1 M ACETIC ACID. THE MIXTURE
WAS SUCCESSIVELY PASSED THROUGH COLUMNS OF AG 50W-X2 (H+ FORM,
0.5 X 1 CM) AND AG 1-X2 (ACETATE FORM, 0.5 X 1 CM), AND LYOPHILIZED.
BORIC ACID WAS REMOVED AS METHYL ETHER BY THE ADDITION OF METHANOL
AND EVAPORATION THREE TIMES. AN ALIQUOT (4,800 cpm) OF EACH SAMPLE
(19,000 cpm with NaBH4 or 22,000 cpm without NaBH4) WAS SUB-
JECTED TO PAPER CHROMATOGRAPHY AS ABOVE.
ribosyl histone H2B bond. Compound X retained its original five carbon atoms of ribose, as revealed by identical yields of 

\[ ^{14}C \text{-products from ADP-}[1''\text{''-}^{14}C]\text{ribosyl} \text{ and ADP-}[5''\text{''-}^{14}C] \text{ribosyl histone H2Bs.} \]

**Chemical Properties of Compound X**—Treatment of compound X with phenylhydrazine at pH 4.5 at 37 °C produced a distinct product (Fig. 8A). This apparent hydrazone formation distinguished clearly compound X from ribose which did not react with phenylhydrazine under these conditions, and suggested the presence of reactive carbonyl group(s) other than the one participating in hemiacetal formation in compound X. When compound X was treated with NaBH₄ under the conditions which reduced ribose to ribitol, a new product was obtained (Fig. 8B). The reduced substance (reduced-X), which retained both C-1 and C-5 carbon atoms of ribose, was no longer reactive with phenylhydrazine (data not shown). Reduced-X co-migrated with 3-deoxyribitol in paper chromatography (solvent system I) apart from various other pentitols such as ribitol, arabinitol, xylitol, 1- or 2-deoxyribitol, and anhydroribitol (Table IV).

**Identification of Compound X**—For further characterization of compound X, reduced-X and various deoxypentitols were acetylated, and their GC-MS² patterns were compared. Upon gas chromatography, reduced-X acetate split into two peaks (Fig. 9); their retention times (7.2 and 7.4 min) and mass fragment patterns³ (Fig. 10, A and B) coincided with those of 3-deoxyarabinitol acetate (Fig. 10C) and 3-deoxyribitol acetate (Fig. 10D), indicating that reduced-X was a mixture of these two deoxypentitols.

Further information about the structure of compound X was obtained by GC-MS analysis of deuterated reduced-X (reduced-X-d₂) acetate. The mass spectra of this derivative exhibited doublets at \( m/z = 70 \) and 71, 130 and 131, and 232 and 233 of approximately the same heights (27). These values,

²The abbreviation used is: GC-MS, gas chromatography-mass spectrometry.
³Some fragments partly retained deuterium used in preliminary NMR measurements in D₂O.

![Fig. 9. Gas chromatography of reduced-X acetate.](image)

![Fig. 10. Mass spectra of the earlier peak of Fig. 9 (A), the later peak of Fig. 9 (B), 3-deoxyarabinitol acetate (C), and 3-deoxyribitol acetate (D).](image)
Since the discovery of ADP-ribosyl protein lyase (formerly termed ADP-ribosyl histone-splitting enzyme), the structure of the split product which behaves close to, but not exactly as, ADP-ribose remained a mystery, because no addition or disintegration appeared to take place on the ADP-ribose moiety upon liberation from acceptor proteins, and because possible modification(s) of substrate or product by contami-

**DISCUSSION**

**FIG. 11.** Gas chromatography of 3-deoxypentitols-1,2-d$_2$ silylate (A), 3-deoxypentitols-1,4-d$_2$ silylate (B), and reduced-X-d$_2$ silylate (C). The retention times of Peaks 1 and 2 were the same as those of 3-deoxyribitol and 3-deoxyarabinitol silylates (7.8 and 8.3 min, respectively) as determined by separate experiments. The peak with the retention time of 9.2 min (in C) was due to a C$_3$-aliphatic alcohol contaminating the sample, as identified by mass spectrometry.

These results clearly indicated that deuterium was located at positions 1 and 2 in reduced-X-d$_2$, and thus that the original compound X was 3-deoxypentos-2-ulose (Fig. 13).

**FIG. 12.** Mass spectra of Peak 1 of Fig. 11A (A), Peak 2 of Fig. 11A (B), Peak 1 of Fig. 11B (C), Peak 2 of Fig. 11B (D), Peak 1 of Fig. 11C (E), and Peak 2 of Fig. 11C (F). The chemical structures show possible assignments for the larger fragment peaks. TMS, trimethylsilyl.
Two mechanisms of ADP-3′′-deoxypent-2′′-enose formation by the enzyme appear possible (Fig. 14): 1) the glutamic acid residue is attached to the terminal ribose at the 3′′-OH group. When the proton at C-2′′ is somehow subtracted by the enzyme, double-bond formation between C-2′′ and C-3′′ may induce elimination of the glutamic acid residue, resulting in liberation of ADP-3′′-deoxypent-2′′-enose. 2) The glutamic acid residue is linked to 1′′-OH (hemiacetal) group with α-configuration. A similar proton subtraction from C-2′′ as supposed above may induce a nucleophilic attack of 3′′-OH on the carbonyl carbon of the glutamic acid residue, forming a six-membered intermediate (ortho-ester type) to ADP-3′′-deoxypent-2′′-enose.

Fig. 13. Structures of reduced-X-d2 (A) and compound X (B).

Fig. 14. Possible mechanisms of action of ADP-riboseyl protein lyase. Two kinds of substrate are postulated; 1) the glutamic acid residue (R) is linked to 3′′-OH group of ADP-ribose, or 2) to 1′′-OH group in α-configuration.

nating enzyme(s) appeared unlikely with an apparently homogeneous preparation of the lyase (18). In the present study, we prepared the split product of high purity in sufficient amounts to allow chemical analyses, and identified the terminal ribose-derived sugar X as 3-deoxy-D-glycero-pentos-2-ulose. The possibility of its being an epimeric stereoisomer, 3-deoxy-β-glycero-pentos-2-ulose, was excluded from the viewpoint that the configuration of a single chiral center, C-4, in compound X remains the same as in D-ribose of ADP-riboseyl histone H2B. 3-Deoxypentos-2-ulose is a tautomeric form of 3-deoxypent-2-enofuranose, and the direct split product appears to be 5′-ADP-3′′-deoxypent-2′′-enofuranose. ADP-ribose has been shown to bind to the glutamic acid residue 2 of histone H2B through an ester linkage (9, 10). The occurrence of ADP-3′′-deoxypent-2′′-enose is an indication that the enzyme is a lyase catalyzing an elimination reaction of the glutamyl-oxy or hydroxyl group at C-3′′ of the original ribose.

γ-Ray radiolysis of D-ribose has been known to yield 3-deoxypentos-2-ulose (42); OH radicals attack C-2, and the formed ribosyl radical undergoes elimination of the 3-OH group followed by disproportionation.

J. Oka and K. Ueda, unpublished results.
ADP-Ribosyl protein lyase is highly specific for the mono-(ADP-ribosyl) moiety of the ADP-ribosyl protein. The degradation of the poly(ADP-ribosyl) protein, therefore, appears to be carried out by cooperative actions of poly(ADP-ribose) glycohydrolase and this enzyme (ADP-ribosyl protein lyase); the former enzyme shortens the polymer chain exoglycosidically up to a monomer (14), which is then liberated from the acceptor protein by the latter enzyme. In this sense, ADP-ribosyl protein lyase plays a principal role in ADP-ribosylation systems in mammalian cells. This idea is supported by a recent work of Wielckens et al. (48), who showed that a removal of primary ADP-ribosyl groups from acceptor proteins was the rate-limiting step in the overall turnover of poly(ADP-ribosyl) groups in cells treated with a DNA-fragmenting agent.

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REFERENCES
**EXPERIMENTAL PROCEDURES**

**Preparation of ADP-Ribosyl Protein Lyase**—Adenine nucleotide translocase (ADP-ribosylprotein lyase, ADPRP-lyase) was prepared by the method of Ogata et al. (35). The lyase activity of this enzyme is increased by 100-fold (64) by the addition of 500 μM MgCl₂, which is the natural cofactor. The reaction was carried out as follows: 1) The reaction mixture contained 50 μl of 100 mM Tris-HCl buffer (pH 7.4), 2) The reaction was incubated at 37°C for 10 min, and 3) The reaction was stopped by the addition of 20 μl of 10% trichloroacetic acid. The reaction was repeated in the presence of 50 mM MgCl₂.

**Preparation of ADP-Ribosyl and Phosphorylthiolated Proteins**—ADP-ribosyl phosphate (ADP-ribose) was prepared by the method of Ogata et al. (35). The procedure was carried out as follows: 1) The reaction mixture contained 50 μl of 100 mM Tris-HCl buffer (pH 7.4), 2) The reaction was incubated at 37°C for 10 min, and 3) The reaction was stopped by the addition of 20 μl of 10% trichloroacetic acid. The reaction was repeated in the presence of 50 mM MgCl₂.

**Preparation of ADP-Ribosyl Protein Lyase Activity**—The standard reaction mixture contained 50 μl of 100 mM Tris-HCl buffer (pH 7.4), 200 μl of 10 mM MgCl₂, and 200 μl of 10% trichloroacetic acid. The reaction was carried out as follows: 1) The reaction mixture was incubated at 37°C for 10 min, and 2) The reaction was stopped by the addition of 20 μl of 10% trichloroacetic acid. The reaction was repeated in the presence of 50 mM MgCl₂.

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ADP-Ribosyl Protein Lyase

Fig. 1. ADP-ribosyl histone H2B splitting time course (A) and effect of amount of the purified enzyme (B). A, the splitting reaction was carried out under the standard conditions with (---), or without (---) the enzyme (0.045 unit). B, the reaction was carried out under the standard conditions with varying amounts of the enzyme.

Fig. 2. Chromatography of ADP-ribosyl protein lyase on Bio-Gel P-300. Three ml fractions were collected. Enzyme activity (---) was measured with 1-ml aliquots under the standard conditions with a lower concentration (1.25 M) of ADP-ribose histone H2B. 0.5 ml aliquots were assayed with each fraction. Areas show the fractions purified. Areas show the sedimentation patterns of blue dextran (V), ferritin (Fer) (spherical radius 7.9 nm), catalase (Cat) (5.2 nm), yeast alcohol dehydrogenase (ADH) (4.6 nm), bovine serum albumin (BSA) (3.5 nm), and cytochrome C (Cytochrome C) (1.7 nm).

Fig. 3. Glycerol gradient centrifugation of ADP-ribosyl protein lyase. Four drops per fraction were collected. Enzyme activity (---) was measured with 0.1-ml aliquots as described for Fig. 2. 0.5 ml aliquots were assayed with each fraction. Areas show the sedimentation position of catalase (Cat) (11.6 nm), yeast alcohol dehydrogenase (ADH) (7.0 nm), and cytochrome C (Cytochrome C) (1.7 nm).
ADP-ribosyl protein lyase. Purification, properties, and identification of the product.
J Oka, K Ueda, O Hayaishi, H Komura and K Nakanishi


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