ASSOCIATION OF POLY ADP-RIBOSE GLYCOHYDROLASE WITH LIVER CHROMATIN*

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SUMMARY: Poly ADP-ribose degrading activity of rat liver chromatin appears to be due to glycohydrolase which splits the ribose-ribose linkage, since the products are ADP-ribose and its oligomers. The activity is highly sensitive to ionic strength; $(NH_4)_2SO_4$ and NaCl inhibit degradation by 47 and 38%, respectively, at 0.1 M. The activity is also depressed markedly by 5 mM adenosine 3',5'-cyclic monophosphate and 5 mM ADP-ribose. These properties are in common with those observed with poly ADP-ribose glycohydrolase found in the nuclear soluble fraction, suggesting that glycohydrolase exists in both free and bound forms in nuclei. This conclusion is reinforced by solubility and gel filtration experiments which indicate that the degrading activity of chromatin is dissociated at physiological or higher ionic strength.

Poly ADP-ribose is a macromolecule composed of repeating ADP-ribose units bound to a nuclear protein at its ribose terminus (1-7). The polymer is synthesized by successive transfer of the ADP-ribose moieties of NAD molecules by an enzyme tightly bound to chromatin (8, 9). As for degradation of this polymer, two distinct enzymes have been separated so far: one is phosphodiesterase which splits the pyrophosphate bond (10) and the other is poly ADP-ribose glycohydrolase which cleaves the ribosyl(1→2)ribose linkage (11, 12). The latter enzyme has been partially purified from the nuclear soluble fraction of rat liver (12). The present communication reports that an apparently identical poly ADP-ribose glycohydrolase is associated with chromatin. Further, evidence is presented that

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the enzyme is dissociated from DNA at a relatively low concentration of salt.

MATERIALS AND METHODS

Rat liver nuclei were isolated with 2.2 M sucrose (13). Preparation of chromatin and the nuclear soluble fraction was as described previously (9, 12). The chromatin preparation was completely free from phosphodiesterase activity.

^{14}C(Adenosine)-NAD was prepared from ^{14}C-ATP as described earlier (14). ^{14}C-Poly ADP-ribose was prepared from ^{14}C-NAD with chromatin and cyclic AMP[1/] (12). The polymer used in this study was almost totally linked to protein.

Poly ADP-ribose degrading activity was assayed by measuring the decrease in acid-insoluble ^{14}C-ADP-ribose. The standard reaction mixture contained 30 µmoles of potassium phosphate buffer (pH 7.0), ^{14}C-poly ADP-ribose (2,200 cpm, 17.4 pmoles as ADP-ribose) and enzyme in a total volume of 0.3 ml. Incubation was carried out for 20 min at 37° and terminated by addition of 5% CCl_3COOH. The mixture was passed through a Millipore filter (HA type) and acid-insoluble radioactivity was determined as described previously (12).

The assay of ADP-ribose polymerase was essentially identical to a procedure described previously (9) except that 0.5 mM dithiothreitol was added.

Protein was measured according to the method of Lowry et al. (15).

RESULTS AND DISCUSSION

Polymerization of ADP-ribose by chromatin is accompanied by rapid degradation of the polymer in situ (9). When ^{14}C-poly ADP-ribose was treated with chromatin, ADP-ribose monomer and oligomers

1/ Abbreviations used are: cyclic AMP, adenosine 3',5'-cyclic monophosphate; cyclic GMP, guanosine 3',5'-cyclic monophosphate.

were produced. Fig. 1 is the result of such an analysis of degradation products on a Dowex 1 (formate) column. The main product

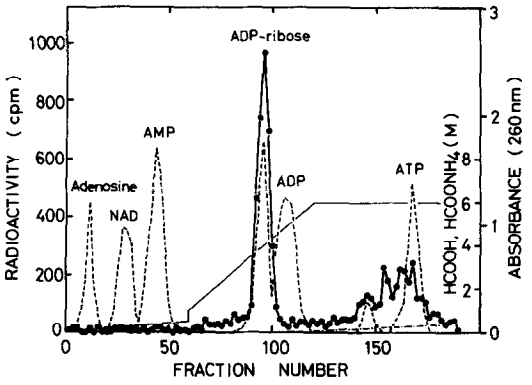

Fig. 1. Analysis of degradation products of poly ADP-ribose. ^{14}C-Poly ADP-ribose (17,000 cpm) was treated with chromatin (3 mg of protein) for 20 min at 37° in the presence of 0.1 M potassium phosphate buffer, pH 7.0 (total volume 2 ml). The mixture was directly applied on to a Dowex 1 (X2, 200-400 mesh) formate column (0.8 X 25 cm) together with markers (adenosine, AMP, ADP-ribose, ADP and ATP). The column was eluted with gradients of HCOOH (————) and, where indicated, with HCOOH + $HCOONH_4$ (—·—). Six milliliter-fractions were collected and assayed for radioactivity (●——●) and absorbance at 260 nm (------).

appearing with an authentic ADP-ribose marker was identified as such by paper chromatography with two different solvent systems (isobutyric acid-1 M NH_4OH-0.1 M EDTA Na_2 (100:60:1.6) and 1 M ammonium acetate (pH 3.8)-ethanol (3:7)). Minor radioactive peaks eluted around the ATP marker appeared to be ADP-ribose oligomers, since the combined material from these peaks gave phosphoribosyl AMP and AMP following treatment with venom phosphodiesterase. These results indicate that the degrading activity of chromatin can be attributed to a glycohydrolase which splits the ribose-ribose linkage (12).

The chromatin glycohydrolase was highly sensitive to alteration in ionic strength. Table I (Experiment I) shows the effect of $(NH_4)_2SO_4$ and NaCl on this enzyme. At 0.1 M these salts brought

Table I

Inhibition of poly ADP-ribose glycohydrolase of chromatin by salts, nucleotides and related compounds

Experiment	Addition		Poly ADP-ribose degraded	Inhibition
			cpm	%
I.	None		1,008	--
	$(NH_4)_2SO_4$	0.1 M	535	46.9
	$(NH_4)_2SO_4$	0.5 M	257	74.5
	NaCl	0.1 M	625	38.0
	NaCl	0.5 M	343	66.0
II.	None		1,132	--
	ADP-ribose	5 mM	346	69.4
	Cyclic AMP	5 mM	595	47.4
	Cyclic GMP	5 mM	1,129	0.3
	AMP	5 mM	997	11.9
	GMP	5 mM	1,108	2.1
	Nicotinamide	5 mM	1,078	4.8
	Thymidine	5 mM	1,005	11.2

about 47 and 38% inhibition of degradation, respectively. A higher concentration of salt (0.5 M) was more inhibitory in each case. This result is in accord with the earlier observation that $(NH_4)_2SO_4$ inhibits the disappearance of newly synthesized poly ADP-ribose in chromatin (9), although glycohydrolase inhibition in the present instance was not as complete as in the case of degradation of the newly formed polymer.

Table I also shows the effect of various nucleotides and related compounds on chromatin glycohydrolase (Experiment II). ADP-ribose and cyclic AMP at 5 mM depressed the degradation by 69 and 47%, respectively. Cyclic GMP, AMP, GMP or many other related com-

pounds tested gave no significant effect, suggesting that the effects of ADP-ribose and cyclic AMP are rather specific. Nicotinamide and thymidine, which are inhibitors of ADP-ribose polymerase (16, 17), were essentially ineffective in the inhibition of glycohydrolase.

All of these properties have now been found to be shared by both glycohydrolases associated with nuclear-soluble (12) and chromatin fractions, suggesting that an identical poly ADP-ribose glycohydrolase exists in both soluble and chromatin-bound forms in nuclei. This view was supported further by the following studies on dissociability of glycohydrolase from chromatin.

The solubility profile of chromatin glycohydrolase at various concentrations of NaCl is shown in Fig. 2. ADP-ribose polymerase was also examined as a reference enzyme that is tightly bound to chromatin. Approximately half of the degradative activity was dissociated from chromatin and made soluble at around 0.15 M NaCl, while the polymerase remained bound to DNA under these conditions. Half maximal solubilization of the latter enzyme was observed with approximately 1 M NaCl.

A dissociation of glycohydrolase from chromatin was also observed on gel filtration of chromatin in the presence of salt. In Fig. 3 is displayed elution profiles of poly ADP-ribose glycohydrolase and DNA (absorbance at 260 nm) from a Sephadex G-200 column in the presence of 1 M NaCl. Apparently the glycohydrolase activity was eluted in several peaks apart from DNA[2/]. When a similar experiment was carried out in the absence of NaCl, the activity of poly ADP-ribose glycohydrolase was almost totally recovered in the void volume along with the DNA.

2/ At a physiological concentration of NaCl, chromatin appeared to aggregate and neither DNA nor glycohydrolase was eluted from the column.

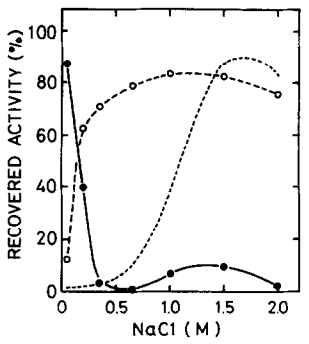

Fig. 2.

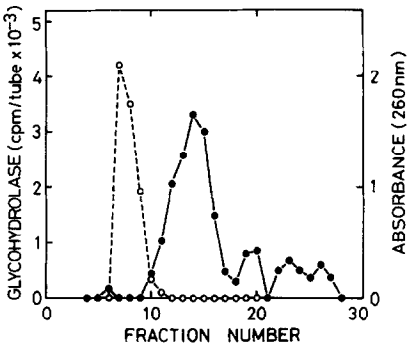

Fig. 3.

Fig. 2. Solubility of chromatin-associated poly ADP-ribose glycohydrolase and polymerase at various concentrations of NaCl. Chromatin was stirred for 60 min at 0° with a specified concentration of NaCl (1 mg of protein/ml) and the mixture was centrifuged for 60 min at 105,000 X g. Supernatant solutions were dialyzed overnight at 0° against a large volume of 5 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM dithiothreitol. Precipitates were dissolved (or suspended) in the same buffer and dialyzed as above. Activities recovered in the supernatant and precipitate were expressed in terms of percent of original chromatin. o----o and ●——●, glycohydrolase in the supernatant and precipitate; ------, polymerase in the supernatant.

Fig. 3. Gel filtration of poly ADP-ribose glycohydrolase associated with chromatin in the presence of 1 M NaCl. Chromatin (4 mg of protein) was stirred for 60 min at 0° in a solution (2 ml) containing 0.001 M Tris-HCl (pH 7.5), 0.5 mM dithiothreitol and 1 M NaCl. The mixture was applied on to a Sephadex G-200 column (1.5 X 40 cm), and the column was eluted with the same buffer-salt solution. Each fraction (4 ml) was dialyzed against 1 mM Tris-HCl (pH 7.5) supplemented with 0.5 mM dithiothreitol and subjected to determinations of absorbance at 260 nm (o----o) and poly ADP-ribose glycohydrolase (●——●).

These observations suggest that glycohydrolase is associated with chromatin but is easily dissociable from the latter by a relatively low ionic strength. Dissociated glycohydrolase is thus far indistinguishable from the enzyme recovered in the nuclear soluble fraction with respect to various properties, e.g., effect of salt, inhibition by nucleotides, pH optimum and substrate specificity.

Since inhibitors of poly ADP-ribose glycohydrolase are capable

of inhibiting the disappearance of polymerized ADP-ribose not only in nuclei (12) but also in chromatin (unpublished data), it seems plausible that glycohydrolase plays a principal role in polymer degradation. Chromatin, therefore, appears to be the site for both biosynthesis and degradation of poly ADP-ribose.

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