Communication

Poly(ADP-Ribose) Synthetase, a Main Acceptor of Poly(ADP-Ribose) in Isolated Nuclei*

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Poly(ADP-ribose) synthetase was identified as the main acceptor of this polymer produced in isolated nuclei of rat liver. When the nuclei were incubated with \(^{32}\)P]NAD at a limited concentration (2.4 \(\mu\)M) and for a brief period (10 s), a protein with \(M_r = 110,000\) was predominantly poly(ADP-ribosyl)ated, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The modification of this protein increased upon longer incubations or at higher NAD concentrations, and induced a marked increase in the apparent molecular weight. A comparison with poly(ADP-ribose) synthetase \((M_r = 110,000)\) of rat liver under various conditions suggested that the increase in the molecular weight of the acceptor resembled that of the synthetase undergoing multiple auto-poly(ADP-ribosylation). This interpretation was further supported by the following observations: 1) \(^{32}\)Ppoly(ADP-ribose) attached to the acceptor co-eluted with the synthetase activity from a hydroxyapatite column; 2) the \(^{32}\)Ppoly(ADP-ribose)-acceptor complex isolated on the column was converted to a very large complex by further incubation with NAD; and 3) a group of large poly(ADP-ribose)-acceptor complexes were reduced to a single molecular species with \(M_r = 110,000\) by extensive digestion with poly(ADP-ribose) glycohydrolase. These findings altogether suggested that poly(ADP-ribose) synthesized in isolated nuclei was principally bound to the synthetase itself.

Poly(ADP-ribose) is a macromolecule synthesized from NAD on various protein acceptors in nuclei (1-4). Its biological function has not yet been fully elucidated, but several lines of evidence have suggested its participation in DNA replication (5), DNA repair (6), cell differentiation (7, 8), or neoplastic transformation (9). Whether or not these possible functions are correlated to modification of specific acceptor(s) has not been extensively studied. So far, histones (mainly H1 and H2B) have been reported as the main acceptors in \textit{in vitro} (9-14) and \textit{in vivo} (15). It should be noted, however, that this view holds true only for the material extractable with dilute acid; more than a half of poly(ADP-ribose) synthesized in nuclei and other systems remains uncharacterized in the residue. We recently reported that a protein with \(M_r = 110,000\) was mainly modified in lymphocytes before as well as after treatment with a DNA-damaging reagent, N-methyl-N'-nitro-N-nitrosoguanidine, and that this protein behaved closely to the synthetase of this polymer (16). In the present study, we extended the analysis to rat liver nuclei, and obtained evidence that the main acceptor in the nuclear system was not histone but poly(ADP-ribose) synthetase.

EXPERIMENTAL PROCEDURES

Materials—\([\text{AMP}-^{32}\text{P}]\text{NAD}\) (34 Ci/mmol) and \([\text{Adenine-}^{32}\text{C}]\text{NAD}\) \((266 \text{ Ci/mmol})\) were obtained from New England Nuclear and the Radiochemical Centre, Amersham, respectively. Molecular weight calibration kits, radioactive and nonradioactive, were the products of the Radioactive Centre, Amersham, and Pharmacia, respectively. Calf thymus DNA (Type I) was purchased from Sigma, proteinase K from Merck, and 3-aminobenzamide from Tokyo Kasei. Nuclei and poly(ADP-ribose) synthetase were prepared from rat liver as previously described (13, 17). Poly(ADP-ribose) glycohydrolase was partially purified from calf thymus by a modification of the method described by Miwa et al. (18), the specific activity was about 3000 units/mg.

Poly(ADP-ribose)ylation of Nuclei or Synthetase—Nuclei \((2 \times 10^9)\) or poly(ADP-ribose) synthetase \((60 \text{ ng})\) was incubated with \([\text{AMP}-^{32}\text{P}]\text{NAD}\) at \(15^\circ\text{C}\) in a solution \((100 \mu\text{l})\) containing 100 mm Tris/HCl \((\text{pH} 8.0), 10 \text{ mm MgCl}_2, 1.25 \text{ mm dithiothreitol},\) and, for the case of synthetase, \(20 \mu\text{g/ml}\) of DNA. The concentration of NAD and the incubation period were specified in each experiment. Incubation was terminated by the addition of \(20 \mu\text{l}\) of \(100\%\) ethanol. An aliquot \((10 \mu\text{l})\) was removed from the mixture and assayed for \(28\%\) COOH-insoluble radioactivity.

Analysis of Poly(ADP-ribose)ylated Products with Gel Electrophoresis—Another aliquot \((10 \mu\text{l})\) from the above incubation mixture was centrifuged at \(17,000 \times g\) for 10 min, and the precipitate, rinsed with ethanol, was suspended in a solubilizing buffer containing \(1\%\) sodium dodecyl sulfate \((16)\). After standing at \(25^\circ\text{C}\) for 8 h, a portion was electrophoresed in a \(0.1\%\) sodium dodecyl sulfate/12.5% polyacrylamide slab gel as previously described (16). After electrophoresis, the gel was stained, dried, and autoradiographed \((16)\), and, when specified, the autoradiogram was analyzed by scanning with a densitometer (Jokko, Tokyo). Molecular weights were estimated by comparison with reference proteins, radioactive or nonradioactive, obtained from commercial sources and electrophoresed side by side.

RESULTS AND DISCUSSION

Incubation of isolated nuclei with 2.4 \(\mu\)M \([\text{AMP}-^{32}\text{P}]\text{NAD}\) for 10 s resulted in incorporation of ADP-ribose on to several proteins as shown by polyacrylamide gel electrophoresis (Fig. 1). The most prominent band was located at the position of \(M_r = 110,000\) and minor bands at the positions of \(M_r = 76,000, 67,000, 52,000, 44,000, 34,000\) (histone H1)\(^1\), \(32,000\) (histone H1), and \(18,000\) (histone H2B). The acceptor with \(M_r = 110,000\) is referred to as the “110K acceptor” in this paper. All these bands disappeared almost completely when the incubation was carried out in the presence of 3 mm 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase (20). The protein nature of all these acceptors was indicated by the disappearance of the bands upon incubation \((37^\circ\text{C}, 10\text{ min})\) of nuclei with proteinase K \((5 \mu\text{g}/10^6\text{ nuclei}; \text{pH} 8.0)\). Upon longer incubations up to 10 min or at higher NAD concentrations up to \(500 \mu\text{M}\), the incorporation of ADP-ribose into 20% COOH-insoluble material increased almost linearly (data not shown). There were parallel increases in incorporation into the several protein acceptors (Fig. 1). Under these conditions, migration patterns of histones in sodium dodecyl sulfate gels have been found to be anomalous with respect to their molecular weights \((19)\).
ditions of increasing incorporation, the main band spread from the position of \( M_r = 110,000 \) to the top of the gel (Fig. 1), whereas other minor bands remained at same positions with no change in breadth. Densitometric analysis of the autoradiograms revealed that about one-third (34 ± 2%) of the total radioactivity was associated with the 110K acceptor under all these conditions, while 7% was with histone H1, and less than 4% each with others. When the nuclei incubated with \([\text{\textsuperscript{32}}\text{P}]\)NAD were treated with poly(ADP-ribose) glycohydrolase before electrophoresis, a group of bands originally resolved in the region between \( M_r = 110,000 \) and the gel top almost totally disappeared, and a distinct band emerged at the position of \( M_r = 110,000 \) (Fig. 1C), indicating that the diversity of the electrophoretic mobility was bestowed by poly(ADP-ribose)lation to varying extents of a single type acceptor. The product located at the top of the gel appeared to resist the glycohydrolase digestion, probably due to formation of aggregates too large to be solubilized. The same treatment with poly(ADP-ribose) glycohydrolase did not reduce the amounts nor the electrophoretic mobilities of other minor bands, an indication that the ADP-ribose residues attached to these acceptors existed mostly in monomeric or oligomeric forms.

The molecular weight, 110,000, of the main acceptor was the same as that of poly(ADP-ribose) synthetase of rat liver (17). This coincidence, together with the fact that the synthetase catalyzes automodification (17, 21-24), suggested the possibility that the 110K acceptor might be the enzyme. We investigated this possibility by comparing the electrophoretic behaviors of the products synthesized under various conditions. The autoradiograms of automated poly(ADP-ribose) synthetase showed a molecular weight change that closely resembled that of the 110K acceptor described above (Fig. 2).

The view that the 110K acceptor was probably poly(ADP-ribose) synthetase itself was further supported by the following experiment. When a KCl extract was made from nuclei incubated with \([\text{\textsuperscript{32}}\text{P}]\)NAD and chromatographed on a hydroxyapatite column, the main peak of ADP-ribose protein co-eluted with poly(ADP-ribose) synthetase activity (Fig. 3).

The peak fractions, as were isolated, gave a distinct band at the position of \( M_r = 110,000 \), while this band moved to the top of the gel after additional incubation with nonradioactive NAD (Fig. 3, insets). These results were in accord with the view that the synthetase activity was associated with the 110K acceptor.

So far, the major acceptors of poly(ADP-ribose) in nuclei
have been reported as histones with no reference to the fraction unextractable with dilute acid (9–14). This was due, partly, to the apparent “insolubility” of poly(ADP-ribose) in the residual fraction upon electrophoresis, and, partly, to its marked heterogeneity in various chromatographic systems. In the light of our present results and our previous observation that as many as 15 molecules of the polymer composed of up to about 80 ADP-ribose units were attached to a single poly(ADP-ribose) synthetase molecule (24), these properties appear to be ascribable to auto-poly(ADP-ribose)ylation to varying extents of poly(ADP-ribose) synthetase.

Jump et al. (25) reported that the major acceptors in isolated HeLa cell nuclei were a 125,000-dalton protein and histone H3. They assigned the former protein to the synthetase on the basis of molecular weight and co-purification through some steps. We recently demonstrated that the main acceptor in permeabilized lymphocytes was a protein, probably the synthetase, of M_r = 110,000 prior to as well as subsequent to DNA damage (16). The present work showed that automodification of the synthetase took place also in isolated liver nuclei, and that it represented a main portion of poly(ADP-ribose) synthetase in this system.

Recently, Benjamin and Gill (26) distinguished two classes of poly(ADP-ribose)ylation in cultured cells subjected to DNA breakage: one was dependent upon the introduction of strand breaks into DNA and the product was a polymer, while the other was independent of DNA breakage and the product was a monomer or short oligomers attached to a variety of proteins. Our present results suggesting the presence of two kinds of ADP-ribosylation in isolated nuclei, one on poly(ADP-ribose) synthetase and polymeric, and the other on histones or other proteins and mostly monomeric or oligomeric, are consistent with their observation, except that the polymer was not free but bound to the synthetase in our experiment.

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