Biochem. J. (1980) **185**, 779–782
Printed in Great Britain

Poly(ADP-Ribose) Polymerase from Ehrlich Ascites-Tumour Cells

AMINO ACID COMPOSITION, *N*-TERMINAL ANALYSIS AND CHEMICAL CLEAVAGE OF THE PURIFIED PROTEIN

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(*Received 19 November 1979*)

Poly(ADP-ribose) polymerase was purified from Ehrlich ascites-tumour cells by two novel methods. Analysis for amino acid composition revealed a high percentage of acidic amino acids or their amides, and of basic amino acids. *N*-Terminal analysis with dansyl chloride revealed no terminal amino acid, indicating a blocked *N*-terminal amino group. Analysis by gel electrophoresis of protein treated with 3-bromo-3-methyl-2-[(2-nitrophenylthio)-3*H*-indole, under conditions where selective cleavage of the polypeptide chain at tryptophan residues is obtained, showed six major peptide bands.

ADP-ribosylation is a post-translational modification of nuclear proteins in which the ADP-ribose moiety of NAD^+ is linked covalently to the proteins, either as single residues or as the polymer poly(ADP-ribose). This formation of protein-linked ADP-ribose and poly(ADP-ribose) is supposed to take place in all eukaryotic cells. The polymer has been reported to be involved in the control of cell differentiation, DNA synthesis and DNA repair, but details on its mode of action are not yet known. Reviews on the structure, synthesis and mode of action of poly(ADP-ribose) have been published by Hilz & Stone (1976) and Hayaishi & Ueda (1977).

Poly(ADP-ribose) polymerase, the enzyme catalysing the formation of poly(ADP-ribose) from NAD, has been prepared with a high purity (90–99%) from various sources by different groups. The calf thymus polymerase has mol.wt. 120000–130000 (Mandel *et al.*, 1977; Yoshihara *et al.*, 1977; Ito *et al.*, 1979), with an isoelectric point of 6.5 reported by Mandel *et al.* (1977) and 9.8 by Ito *et al.* (1979). Pig thymus polymerase has mol.wt. 63500 and an isoelectric point of 8.4 (Tsopanakis *et al.*, 1978), whereas the corresponding values for polymerase from Ehrlich ascites-tumour cells are 130000 and 9.4 (Kristensen & Holtlund, 1978). It is not known if these different values reflect real differences in the structure of polymerases from various sources, or arise during the different purification steps involved.

In the present paper we will give more details of the molecular properties of the polymerase from Ehrlich ascites-tumour cells, data which allow a more detailed comparison of polymerases from different sources.

Experimental

Purification of poly(ADP-ribose) polymerase

Nuclei were prepared, and the polymerase was extracted and partially purified by chromatography on DNA–agarose, as described previously (Kristensen & Holtlund, 1976). The polymerase was then purified further by two novel procedures.

(*a*) *By chromatography on Blue Sepharose.* The partially purified preparation was diluted with 2vol. of 10mM-Tris/HCl, pH 8.0, containing 1mM-dithiothreitol, 0.1mM-EDTA, 10% (w/v) glycerol and 0.02% NaN_3 (buffer A), and applied to a 10ml column of Blue Sepharose CL-6B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) equilibrated with buffer A containing 0.25M-NaCl. The column was then washed with 30ml of the equilibration buffer, and the polymerase eluted with 25ml of 0.4M-NaCl in buffer A.

(*b*) *By preparative polyacrylamide-gel electrophoresis.* The protein in the partially purified preparation was precipitated with 0.5M-$HClO_4$. The precipitate was washed with 70% (v/v) ethanol and dissolved in 400μl of 0.9M-acetic acid containing 5M-urea and 50mM-dithiothreitol. The final fractionation was done by electrophoresis in a 12cm-long polyacrylamide gel (5% acrylamide, 0.2% bisacrylamide), diameter 1.2cm, after pre-electrophoresis of the gel. The electrophoresis was performed at 60V for 12h with water cooling;

0306-3275/80/030779-04 $1.50/1

0.9M-acetic acid was used as electrode solution. The protein band corresponding to the polymerase was clearly visible during the electrophoresis as a schlieren zone; this allowed us to excise the polymerase-containing part of the gel after electrophoresis without fixing and staining the gel. The gel piece was placed in a Sartorius dialysis bag (Sartorius Membranfilter G.m.b.H., Göttingen, Germany) containing 0.9M-acetic acid, and the enzyme was run out of the gel into the bottom of the bag by electrophoresis at 250V for 2h.

Analytical polyacrylamide-gel electrophoresis

The purity of different polymerase preparations was analysed by electrophoresis in polyacrylamide gels (5% acrylamide, 0.2% bisacrylamide) containing 0.9M-acetic acid and 4.5M-urea, as described previously (Kristensen & Holtlund, 1978). Chemically cleaved polymerase was analysed by electrophoresis in 15% polyacrylamide gels containing 0.9M-acetic acid and 2.5M-urea (Panyim & Chalkley, 1969).

Protein assay

Protein was determined by the Lowry *et al.* (1951) method, after precipitation of the protein with trichloroacetic acid in the presence of deoxycholate (Bensadoun & Weinstein, 1976), with bovine serum albumin as reference protein.

Amino acid analysis

A portion (150μg) of polymerase was hydrolysed under vacuum with 6M-HCl at 108–110°C for 24h, and the hydrolysate analysed on a BioCal BC 200 automatic amino acid analyser. No corrections were made for destruction of serine and threonine.

N-Terminal analysis

Poly(ADP-ribose) polymerase was treated with dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride at pH9.8 by standard procedures (Gray, 1972). Two samples (0.2–0.3mg) of protein were hydrolysed with 6M-HCl at 110°C, for 6 and 16h respectively. The hydrolysates were extracted with ether (Gros & Labouesse, 1969) and the ether and water phases analysed by t.l.c. (Gray, 1972; Deyl & Rosmus, 1965).

Cleavage with 3-bromo-3-methyl-2-[(2-nitrophenyl)-thio]-3H-indole (BNPS-skatole)

A sample (50μg) of polymerase was reduced with dithiothreitol and alkylated with iodoacetate (Konigsberg, 1972). The reduced and alkylated protein was then treated with BNPS-skatole in the presence of phenol (Fontana *et al.*, 1973; Houghten & Li, 1978), and the cleavage product analysed by electrophoresis in 15% polyacrylamide gels containing acetic acid and urea as described above.

Results and Discussion

Purification of the polymerase

We have previously published a purification procedure for poly(ADP-ribose) polymerase from Ehrlich ascites-tumour cells which included isoelectric focusing as a last step, and which gave a 80–90%-pure preparation (Kristensen & Holtlund, 1978). To avoid possible difficulties with amino acid analysis owing to the presence of Ampholines in the preparation, we have developed two alternative purification methods where the use of electrofocusing is avoided. Purification by chromatography on Blue Sepharose gave a preparation with a specific activity of 3600units/mg of protein as defined previously (Kristensen & Holtlund, 1976), with a 35% yield relative to whole nuclei. Analysis of this preparation by polyacrylamide-gel electrophoresis (Fig. 1)

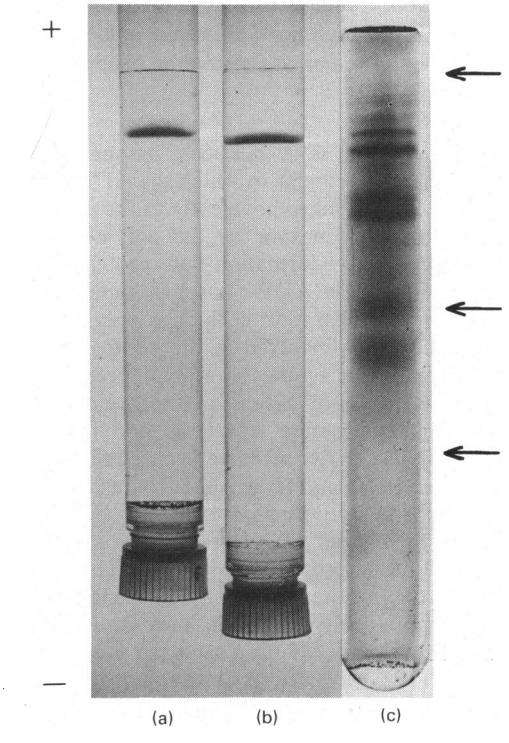

Fig. 1. *Analysis by polyacrylamide-gel electrophoresis of poly(ADP-ribose) polymerase*

(*a*) Purified by chromatography on Blue Sepharose (5μg of protein). (*b*) Purified by preparative polyacrylamide-gel electrophoresis (5μg of protein). (*c*) Chemically cleaved polymerase (50μg of protein). The arrows indicate the migration of intact polymerase (top), of intact cytochrome *c* (middle) and of the cleavage product obtained by BNPS-skatole treatment of cytochrome *c* (bottom). Pyronine Y, which was used as a marker, was run to the end of the gels.

revealed the presence of two minor protein bands in addition to the polymerase band; scanning of the gel indicated that these two bands contained less than 5% of the total protein in the gel.

Purification of the polymerase by preparative polyacrylamide-gel electrophoresis gave an enzymically inactive preparation in a 50% yield relative to nuclei. No proteins other than the polymerase were detected by analytical polyacrylamide-gel electrophoresis (Fig. 1). The detection limit for this electrophoresis system is approx. 10ng of protein in a sharp band.

Amino acid composition of the polymerase

Although poly(ADP-ribose) polymerase from various sources has been purified to homogeneity, until recently no data on the amino acid composition have been available. Table 1 shows the amino acid composition obtained by us for the Ehrlich-ascites-tumour-cell enzyme. The most remarkable feature of the composition is the high percentage, about 40%, of residues that are either acidic or basic. The high isoelectric point of the polymerase indicates that a large fraction of the acidic residues must be amidated in the intact polymerase.

The composition is in reasonable agreement with the amino acid composition of calf thymus polymerase reported by Ito *et al.* (1979). High contents of acidic amino acids or their amides, and of basic amino acids, have also been reported for other chromatin proteins, such as the high-mobility-group chromatin proteins (HMG proteins) (Goodwin & Johns, 1977), and a non-histone protein from hen oviduct (Teng *et al.*, 1978). It is somewhat surprising that the relatively small HMG proteins (mol.wts. ranging from 8500 to 30000) and the substantially larger hen oviduct protein (mol.wt. 95000) and poly(ADP-ribose) polymerase (mol.wt. 130000) have this structural aspect in common, and it will be interesting to see if this predominance of charged residues will be found in other purified chromatin proteins, including poly(ADP-ribose) polymerase from other sources.

Table 1. *Amino acid composition of poly(ADP-ribose) polymerase purified by two different methods*

Left column: polymerase purified by chromatography on Blue Sepharose. Right column: polymerase purified by preparative polyacrylamide-gel electrophoresis.

Amino acid	Content (mol %)	
Pro	4.7	5.0
Asp	9.4	9.0
Thr	3.9	3.5
Ser	9.5	8.2
Glu	11.9	12.3
Gly	8.6	7.4
Ala	7.5	7.8
Val	5.8	5.4
Met	2.1	2.1
Ile	2.9	3.2
Leu	9.1	9.2
Tyr	2.7	2.9
Phe	2.6	2.9
His	1.8	1.9
Lys	12.0	12.6
Arg	3.3	3.7
Cys (½)*	0.9	1.8
Trp†	1.1	1.2

* Estimated as cysteine after hydrolysis under vacuum.

† Determined spectrophotometrically by the method of Edelhoch (1967).

N-Terminal analysis

No *N*-terminal amino acid was found when fractions of dansylated and hydrolysed poly(ADP-ribose) polymerase corresponding to 0.15–0.3nmol were analysed by t.l.c. Tsopanakis *et al.* (1978), investigating the pig thymus polymerase, were also unable to detect a *N*-terminal amino acid after dansylation, and have suggested that the *N*-terminal may be blocked. The same conclusion was reached by Ito *et al.* (1979) for the calf thymus polymerase. Our results, although not conclusive, substantiate their findings and indicate that even the Ehrlich-ascites-tumour-cell polymerase may be blocked *N*-terminally.

Cleavage of the polymerase with BNPS-skatole

The pattern obtained by polyacrylamide-gel electrophoresis of polymerase cleaved with BNPS-skatole is shown in Fig. 1(*c*). Six distinct peptide bands were resolved on the gel, in addition to a number of fainter bands. The low number of prominent bands suggest that the cleavage procedure used here may be of value when one wishes to compare polymerases from different sources, in addition to a comparison of molecular weights, isoelectric points, amino acid compositions and kinetic properties.

Under the conditions used, BNPS-skatole is reported to cleave the polypeptide chain solely at tryptophan residues, with an efficiency, at least with respect to the single cleavage point in cytochrome *c*, of about 50% (Fontana *et al.*, 1973). From the tryptophan content of the polymerase given in Table 1 one would expect about 15 primary peptides, in addition to a large number of partially cleaved products. It is thus likely that some of the distinct bands seen on the gel contain more than one peptide, although the possibility that some tryptophan residues may be more easily attacked by BNPS-skatole than the rest also may contribute to give a relatively low number of distinct bands on the gel.

Several aspects of the function of poly(ADP-ribose) still remain obscure. For instance, do the diverse properties of the several nuclear proteins known to be potential acceptors of poly(ADP-ribose) imply the presence of several distinct polymerases in the cell? Does the high rate of poly(ADP-ribose) synthesis observed in transformed cells by Burzio *et al.* (1975) reflect the presence of an enzyme with changed molecular and catalytic properties? These and other questions may be answered by a detailed comparison between polymerases from different species and tissues. We hope that the observations reported here may contribute to simplify such a comparison.

The support of the Norwegian Research Council for Science and the Humanities is acknowledged.

References

Bensadoun, A. & Weinstein, D. (1976) *Anal. Biochem.* **70**, 241–250

Burzio, L., Reich, L. & Koide, S. S. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 933–938

Deyl, Z. & Rosmus, J. (1965) *J. Chromatogr.* **20**, 514–520

Edelhoch, H. (1967) *Biochemistry* **6**, 1948–1954

Fontana, A., Vita, C. & Toniolo, C. (1973) *FEBS Lett.* **32**, 139–142

Goodwin, G. H. & Johns, E. W. (1977) *Methods Cell Biol.* **16**, 257–267

Gray, W. R. (1972) *Methods Enzymol.* **25**, 121–138

Gros, C. & Labouesse, B. (1969) *Eur. J. Biochem.* **7**, 463–470

Hayaishi, O. & Ueda, K. (1977) *Annu. Rev. Biochem.* **46**, 95–116

Hilz, H. & Stone, P. (1976) *Rev. Physiol. Biochem. Pharmacol.* **76**, 1–58

Houghten, R. A. & Li, C. H. (1978) *Int. J. Pept. Protein Res.* **11**, 49–58

Ito, S., Shizuta, Y. & Hayaishi, O. (1979) *J. Biol. Chem.* **254**, 3647–3651

Konigsberg, W. (1972) *Methods Enzymol.* **25**, 185–188

Kristensen, T. & Holtlund, J. (1976) *Eur. J. Biochem.* **70**, 441–446

Kristensen, T. & Holtlund, J. (1978) *Eur. J. Biochem.* **88**, 495–501

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mandel, P., Okazaki, H. & Niedergang, C. (1977) *FEBS Lett.* **84**, 331–336

Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130**, 337–346

Teng, C. S., Gallagher, K. & Teng, C. T. (1978) *Biochem. J.* **176**, 1003–1006

Tsopanakis, C., Leeson, E., Tsopanakis, A. & Shall, S. (1978) *Eur. J. Biochem.* **90**, 337–345

Yoshihara, K., Hashida, T., Yoshihara, H., Tanaka, Y. & Ohgushi, H. (1977) *Biochem. Biophys. Res. Commun.* **78**, 1281–1288