IN VITRO ADP-RIBOSYLATION OF HISTONES BY PURIFIED CALF THYMUS POLYADENOSINE DIPHOSPHATE RIBOSE POLYMERASE

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1. Introduction

Polyadenosine diphosphate ribose (ADPR) polymerase is an ubiquitous nuclear enzyme which exists in a variety of eukaryote cell nuclei [1–3]. This enzyme catalyzes the formation of a homopolymer of ADPR from NAD. Histones are known to be the major acceptor proteins of this homopolymer, although some nonhistone chromosomal proteins were also suggested to be ADP-ribosylated [4–6]. Recent studies showed a tissue-specific modification of nuclear proteins by polyADPR polymerase. In rat liver or mouse liver nuclei, histone H2B was the major acceptor of ADPR [7]. In contrast in HeLa cell nuclei [8], rat brain nuclei or M2 cell nuclei [9], histone H1 showed the highest incorporation of ADPR. On this basis it was suggested that histone or nonhistone nuclear protein ADP-ribosylation may play an important role in cell differentiation or gene expression.

We have developed a new procedure for polyADPR polymerase purification from calf thymus without other detectable protein contamination [10,11]. We have also demonstrated the ADP-ribosylation of calf thymus histone H1 with purified enzyme and NAD as substrate [12]. Here we show that the other histones can be ADP-ribosylated as well as histone H1 by purified polyADPR polymerase as judged by electrophoretic mobilities in a urea–SDS gel system.

2. Materials and methods

Omnifluor and [^{3}H]NAD (3.28 Ci/mmol) were purchased from New England Nuclear. Acrylamide and *N,N'*-methylene bisacrylamide were products of Eastman Kodak Co. Sodium dodecyl sulfate (SDS) was purchased from Roth and Coomassie brilliant blue R from Edward Gurr, Ltd. Triton X-100 and NAD were purchased from Sigma Chemicals Co. All other chemicals were of analytical grade obtained from Merck.

2.1. *Preparation of purified calf thymus polyADPR polymerase*

Purified enzyme was prepared from calf thymus gland as in [10,11]. The enzyme preparation is electrophoretically homogeneous and contains a small amount of DNA. Its activity is DNA independent [13].

2.2. *Preparation of histones*

Histones H1 and H2B were obtained from fractions F_1 and F_{2b}, respectively [14] by preparative electrophoresis on polyacrylamide slab gel according to [15]. Histone H3 was precipitated from the 'ethanol–HCl' extract [14] by addition of 0.7 vol. acetone, and was purified by gel filtration chromatography on a Biogel P10 column equilibrated and eluted with HCl 0.01 N. Histones H4 and H2A were prepared as follows: the 'ethanol–GuCl' extract containing fraction F_{2a} [16], was treated by 1.25 vol. of acetone to precipitate fraction F_{2a2}. Fraction F_{2a1} corresponding to pure histone H4 was recovered from the supernatant by adding 1.75 vol. acetone. Histone H2A was isolated from fraction F_{2a2} by ion-exchange chromatography on Biorex 70 as in [17].

The purity of the histones was assessed by polyacrylamide gel electrophoresis in 2.5 M urea at pH 2.7

[18] using 17% acrylamide. Each histone was electrophoretically homogeneous.

2.3. *ADPR-histone formation by purified polyADPR polymerase*

The method for ADPR-histone formation by purified enzyme is essentially the same as described for histone H1 [12]. Each histone (2 μg) was incubated separately with standard buffer containing 2.3 μM [^{3}H]NAD (~800 000 cpm), 0.1 M Tris–HCl (pH 7.9), 8 mM $MgCl_2$, 0.4 mM dithiothreitol, 20% glycerol (v/v) and 32 pmol purified DNA-independent calf thymus polyADPR polymerase in 125 μl total vol. After incubation for 3 min at 37°C the reaction was stopped by adding 2 ml 20% trichloroacetic acid, stirred for 10 s and allowed to stand overnight at 0°C. The suspension was centrifuged at 4000 × *g* for 15 min and the sediment containing ADPR-proteins was washed once with 20% trichloroacetic acid at 2°C. The precipitate was dissolved in 100 μl 0.1 M NaP_i buffer (pH 7.0), containing 0.1% sodium dodecyl sulfate and 5 M urea.

2.4. *Urea–SDS polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis at pH 7.0 in the presence of SDS and urea was done as in [19]. In order to analyze the distribution of radioactivity, the gel was cut into slices of 2.6 mm thickness. Each gel disk was digested with 0.5 ml 30% H_2O_2 at 54°C overnight and mixed with 5 ml Triton X-100 toluene omnifluor cocktail (5.6 g Omnifluor 1/l 30% Triton X-100 in toluene) and the radioactivity was counted in an 'Intertechnique' scintillation counter. Control gels were run simultaneously with each histone under the same conditions, fixed in 50% trichloroacetic acid overnight at 20°C and stained with 0.25% Coomassie in 50% trichloroacetic acid for 60 min at 37°C. Destaining was carried out with 20% methanol and 7.5% acetic acid at 37°C.

3. Results and discussion

We have shown that our purified DNA-independent calf thymus polyADPR polymerase could catalyze the formation of ADPR-histone H1 in a reconstituted system containing only histone H1 and NAD as substrate [12]. PolyADP-ribosylation of other histones was examined with purified enzyme under the same conditions as for histone H1.

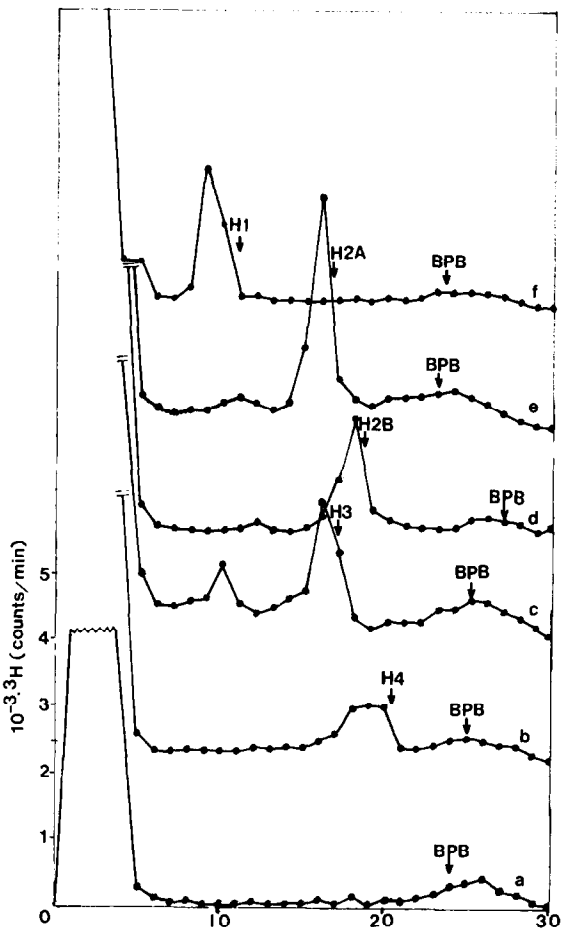

Fig.1. Urea–SDS–polyacrylamide gel electrophoreses of ADP-ribosylated-enzyme and -histones. Incubation conditions and analytical methods were as described in the text. (a) Enzyme alone; (b–f) enzyme + histone H4, H3, H2B, H2A and H1, respectively. Arrows indicate the native histones positions before incubation. The migration was from left to right. BPB, bromophenol blue.

After incubation of histone and NAD with the purified DNA-independent enzyme, ADPR-proteins were precipitated with 20% trichloroacetic acid and analyzed by polyacrylamide gel electrophoresis in the presence of 2.5 M urea and 0.1% SDS. After incubation without histone a single large peak is observed (fig.1a) which is confirmed as ADPR-polyADPR polymerase (ADPR-enzyme) [12,20]. Histone H1-containing incubations always showed two radioactive peaks (fig.1f). The second small peak was identified as ADPR-histone H1 and the first large peak was identified as ADPR-enzyme [12]. This evidence and the localization of native histone stained separately suggest strongly that the second small labelled peaks

obtained from incubation mixture containing individual histone H2A, H2B, H3 and H4 could be the corresponding ADPR-histones. The migration of ADPR-H1 was less rapid than that of non-ADP-ribosylated H1. Similarly, when pure polyADPR polymerase was incubated with H2A, or H2B, or H3, or H4, the second radioactive peak migrated more slowly than the native histone (indicated by the arrow). Since the native histones were the only proteins present in the incubation media in addition to the enzyme we must conclude that the labelled proteins which appeared are the ADP-ribosylated histone fractions. In fact, after mild alkali treatment, we could remove ADPR and recover the corresponding histone fractions.

In our incubation system no exogenous DNA is required, since our enzyme preparation contains ~10% DNA (w/w). It is interesting to note that when the enzyme was further treated by hydroxylapatite column chromatography, exogenous DNA was absolutely required for its activity [13]. Enzyme and H1 ADP-ribosylation could be observed when DNA was added in the incubation medium. However, after removal of DNA our enzyme could hardly ADP-ribosylate histones other than H1, even after addition of exogenous thymus DNA showing the importance of the enzyme bound DNA (not shown).

The preferential ADP-ribosylation of histone H2B in rat liver nuclei and of histone H1 in rat brain nuclei has been demonstrated [7,9]. These findings suggest a tissue specific ADP-ribosylation of histones. Two contradictory results for the localization of polyADPR polymerase activity in chromatin substructure were reported [21,22]. According to [21] in HeLa cell nuclei, polyADPR polymerase activity is associated primarily with template active regions in which histone H1 is localized and the highest radioactivity of ADPR has been detected in histone H1 when nuclei are incubated with radioactive NAD. On the contrary in rat liver nuclei according to [22] this specific localization of polyADPR polymerase activity is not observed and histone H2B shows the highest ADPR incorporation beside histone H1. It appears that the pure enzyme is able to ADP-ribosylate all 5 histone fractions; thus complementary factors should determine in vivo which one among different histone fractions will be ADP-ribosylated. The different results obtained with HeLa cell and rat liver nuclei could be explained by a tissue-specific localization of poly-ADPR polymerase.

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References

[1] Chambon, P., Weill, J. D., Doly, J., Strosser, M. T. and Mandel, P. (1966) Biochem. Biophys. Res. Commun. 25, 638–643.
[2] Nishizuka, Y., Ueda, K., Nakazawa, K. and Hayaishi, O. (1967) J. Biol. Chem. 242, 3164–3174.
[3] Fujimura, S., Hasegawa, S., Shimizu, Y. and Sugimura, T. (1967) Biochim. Biophys. Acta 145, 247–259.
[4] Otake, H., Miwa, M., Fujimura, S. and Sugimura, T. (1969) J. Biochem. 65, 145–146.
[5] Nishizuka, Y., Ueda, K., Yoshihara, K., Yamamura, H., Takeda, M. and Hayaishi, O. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 781–786.
[6] Smith, J. A. and Stocken, L. A. (1973) Biochem. Biophys. Res. Commun. 54, 297–300.
[7] Okayama, H., Ueda, K. and Hayaishi, O. (1978) Proc. Natl. Acad. Sci. USA 75, 1111–1115.
[8] Tanuma, S., Enomoto, T. and Yamada, M. (1977) Biochem. Biophys. Res. Commun. 74, 599–605.
[9] Rickwood, D., MacGillivray, A. J. and Whish, W. J. D. (1977) Eur. J. Biochem. 79, 589–598.
[10] Okazaki, H., Niedergang, C. and Mandel, P. (1977) CR Acad. Sci. Paris sér. D 285, 1545–1548.
[11] Mandel, P., Okazaki, H. and Niedergang, C. (1977) FEBS Lett. 84, 331–336.
[12] Okazaki, H., Niedergang, C. and Mandel, P. (1980) Biochimie, in press.
[13] Niedergang, C., Okazaki, H. and Mandel, P. (1979) Eur. J. Biochem. 102, 43–57.
[14] Johns, E. W. (1964) Biochem. J. 92, 55–59.
[15] Martinage, A., Sautière, P., Kerckaert, J. P. and Biserte, G. (1976) Biochim. Biophys. Acta 420, 37–41.
[16] Johns, E. W. (1967) Biochem. J. 105, 611–614.
[17] Sautière, P., Tyrou, D., Laine, B., Mizon, J., Ruffin, P. and Biserte, G. (1974) Eur. J. Biochem. 41, 563–576.
[18] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346.
[19] Hamana, K. and Iwai, K. (1974) J. Biochem. (Tokyo) 76, 503–512.
[20] Yoshihara, K., Hashida, T., Yoshihara, H., Tanaka, Y. and Ohgushi, H. (1977) Biochem. Biophys. Res. Commun. 78, 1281–1288.
[21] Mullins, D. W., jr, Giri, C. P. and Smulson, M. (1977) Biochemistry 16, 506–513.
[22] Yukioka, M., Okai, Y., Hasuma, T. and Inoue, A. (1978) FEBS Lett. 86, 85–88.