Vol. 105, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1982 Pages 404-411

INVOLVEMENT OF POLY(ADP-RIBOSE) METABOLISM IN INDUCTION OF DIFFERENTIATION OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS

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Received January 29, 1982

SUMMARY: Poly(ADP-ribose) metabolism during differentiation of HL-60 human promyelocytic leukemia cells induced by dimethylsulfoxide was studied. Endogenous poly(ADP-ribose) decreased to 15% of the uninduced level at 14 hours after addition of dimethylsulfoxide, when the morphology, phagocytic activity and DNA synthesis of the cells had not yet changed and myeloperoxidase activity had decreased only slightly. Endogenous poly(ADP-ribose) remained at a low level until day 3, but increased sharply to 800% of the uninduced level on day 5 when most of the differentiated phenotypes became obvious. This is the first report of measurement of the endogenous level of poly(ADP-ribose) during cellular differentiation.

INTRODUCTION

Poly(ADP-Rib)[1] is a biopolymer with α(1→2) ribose-ribose glycosidic bonds and pyrophosphate bonds that is synthesized from NAD^+ in eukaryotes (1-4). It has been suggested that poly(ADP-Rib) synthesis is related to cell differentiation (5-9), DNA repair (10-15), sister chromatid exchanges (16), DNA synthesis (17) and cell transformation (18).

Yamada et al. (6) demonstrated that mouse myeloid leukemia cells differentiated into mature macrophages and granulocytes on treatment with purified poly(ADP-Rib). Morioka et al. (8) and Terada et al. (9) reported that nicotinamide and related compounds with inhibitory effects on poly-(ADP-Rib) polymerase induced erythroid differentiation of murine

Abbreviations : poly(ADP-Rib), poly(adenosine diphosphate ribose); Ado-Rib, dephosphorylated form of Ado(P)-Rib-P, O-α-D-ribofuranosyl(1"→2')-adenosine; Ado-Rib*, Ado-Rib pentalcohol which was synthesized by oxidation of Ado-Rib with $NaIO_4$ followed by reduction with KBH_4; Ado-[^{3}H]Rib*, Ado-[2,3-$^3H_2$]Rib*; Ado(P)-Rib-P, 2'-ribosyl adenosine-5',5"-bis(phosphate) equal to O-α-D-ribofuranosyl-(1"→2')-adenosine-5',5"-bis(phosphate) or 2'-(5"-phosphoribosyl)-5'AMP; HPLC, high performance liquid chromatography; DMSO, dimethylsulfoxide.

0006-291X/82/060404-08$01.00/0
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erythroleukemia cells. However no report is available on the endogenous level of poly(ADP-Rib) during differentiation.

HL-60 cells, human promyelocytic leukemic cells (19), differentiate into myelocytes, metamyelocytes, and banded and segmented neutrophils on addition of DMSO (20). In this work we examined the endogenous poly-(ADP-Rib) level during differentiation of HL-60 cells induced by DMSO. For quantitation of endogenous poly(ADP-Rib), we adopted a new highly sensitive and rapid method for determination of the monomer unit of poly-(ADP-Rib)(21).

MATERIALS AND METHODS

Cells and cell culture: The HL-60 cells used in these experiments were provided by Dr. R. C. Gallo (National Cancer Institute, Bethesda, Md.). They were grown in Roswell Park Memorial Institute Tissue Culture Medium (RPMI)1640 as previously reported (19). For induction of differentiation, the cell density of HL-60 cells in the logarithmic growth phase was adjusted at 5 x 10^5 cells/ml in a tissue culture flask and the cells were incubated with or without 1.25% DMSO (Merck).

DNA synthesis: Cells were incubated at a concentration of 2.5 x 10^5 cells per ml for 30 min at 37°C in the presence of [^{3}H]thymidine (specific activity 45 Ci/mmol) at 10 μCi/ml. The 5 ml samples of cell suspension were mixed with 10% trichloroacetic acid. The precipitate was collected on a glass filter and its radioactivity was determined in toluene-PPO-POPOP scintillation fluid in a liquid scintillation counter.

Observation of morphological changes: Aliquots of cell suspension (3 x 10^4 cells) were collected on a glass slide using a centrifugation apparatus (Cytospin, Shandon). Cell smears were stained with Wright-Giemsa solution (Merk) and observed under a light microscope.

Assay of phagocytosis: Phagocytic activity was assayed by incubating the cells for 30 min at 37°C with polystyrene latex particles (1 μm in diameter, Dow Diagnostics) coated with human immunoglobulin (22).

Assay of myeloperoxidase: Myeloperoxidase activity was assayed essentially by the method of Chance & Maehly using guaiacol (23). One unit of myeloperoxidase was defined as the amount of enzyme causing an increase of 1 unit in absorbance at 470 nm in 1 min at room temperature.

Determination of endogenous poly(ADP-Rib) content: Details of the procedure will be reported elsewhere. Briefly, [Ado-^{14}C(U)]poly(ADP-Rib) (1.0 x 10^4 cpm, 542 mCi/mmol) was added as a tracer to a suspension of 5 x 10^7 cells followed by addition of 0.1 volume of 100% trichloroacetic acid and 0.1 volume of 20 mM EDTA. Then the precipitate was collected by centrifugation, and suspended in 10 ml of 0.1 M Tris-HCl buffer (pH 8.0). Pronase E was added at a concentration of 2 mg/ml and the mixture was incubated overnight at 37°C in the presence of 0.02% NaN_3. The mixture was extracted with phenol, and the aqueous layer was isolated and washed with ether to remove a trace amount of phenol. The aqueous layer was heated for 2 min at 100°C, rapidly cooled to denature the nucleic acids, and incubated for 2 hrs at 50°C with nuclease P_1 at a concentration of 10 μg/ml in 30 mM veronal acetate buffer (pH 5.3). Snake venom 5'-nucleotidase and phosphodiesterase were each added to the extract at a final concentration of 0.01 unit/ml and the mixture was incubated for 2 hrs at 37°C in 10 mM Tris-HCl buffer (pH 8.0) in the presence of 10 mM $MgCl_2$ and 0.02% NaN_3. The Ado(P)-Rib-P fraction was obtained by DE-52

column chromatography (24) and treated with alkaline phosphomonoesterase of E. coli and the resulting Ado-Rib fraction was purified by high performance liquid chromatography (HPLC). The fractions in the peak of [^{14}C]Ado-Rib were mixed with $NaIO_4$ at a final concentration of 1.8 mM, incubated for 2 hrs in the dark at room temperature and cooled to 0°C. Then 1 M potassium phosphate buffer (pH 6.8) and 0.1 M KB[^{3}H]H_4 (3 Ci/mmol) (25) in 0.1 N KOH were added to final concentrations of 20 mM and 5 mM, respectively, and the mixture was incubated for 2 hrs in the dark at room temperature. The mixture was subjected to HPLC on an octadecylsilicone column (μ-Bondapak C-18, 4 mm x 30 cm, Waters) with authentic Ado-Rib* (unlabeled Ado-Rib pentalcohol), which had been prepared from Ado-Rib by oxidation with $NaIO_4$ and reduction with unlabeled KBH_4. The column was developed with methanol: 0.5% monoethanolamine phosphate (pH 3.0)(3:97, v/v). Fractions in the peak of Ado-Rib* on HPLC were pooled and subjected to HPLC twice more. [^{14}C]Ado-Rib, derived from [^{14}C]poly(ADP-Rib) as a tracer, and endogenous Ado-Rib were labeled with KB[^{3}H]H_4 (21) to form [^{14}C]Ado-[^{3}H]Rib* and Ado-[^{3}H]Rib*, respectively, and they were processed in an Autosample Oxidizer (Packard 306) as [^{3}H]H_2O and [^{14}C]CO_2 and their radioactivities were counted in a liquid scintillation spectrometer.

Assay of poly(ADP-Rib) polymerase activity: Poly(ADP-Rib) polymerase was assayed in permeabilized cells and with or without treatment of DNase I. Cells were permeabilized with 0.1% Kyro EOB in 0.25 M sucrose for 20 min at 0°C. Kyro EOB was kindly provided by Dr. D. H. Hughes, Procter and Gamble Co.. In DNase I treatment the cell suspension in 0.25 M sucrose was incubated with 100 μg/ml of DNase I by the procedure of Berger et al. (26). The reaction mixture contained in 125 μl, 150 mM Tris-HCl (pH 8.0), 37 mM $MgCl_2$, 1.5 mM dithiothreitol and 1.5 mM [^{14}C]NAD^+ (3.3 x 10^6 cpm/μmol NAD^+). The reaction was allowed to proceed for 10 min at 25°C. Aliquot of 50 μl of the reaction mixture was applied to a filter paper disc and the acid-insoluble radioactivity was determined (27).

RESULTS

Phenotypic changes of HL-60 cells treated by dimethylsulfoxide

Growth of HL-60 cells in the presence of 1.25% DMSO was the same at that of control cells for 2 days, but then reached a plateau at a cell density of 1.2 x 10^6 cells/ml and remained at this density for a further 5 days. Without DMSO, growth of HL-60 cells reached a maximal cell density of 1.8-2.1 x 10^6 cells/ml on day 3 or 4 and then the viable cell density gradually decreased to 1.3 x 10^6 cells/ml, or 60% of the maximum, on day 7. After induction for 3 days, DNA synthesis, as measured by incorporation of [^{3}H]thymidine into acid-insoluble material, decreased greatly and remained low for 4 days (Table I).

After treatment with 1.25% DMSO there were no characteristic phenotypic changes within 14 hrs. However, on day 3, 45% of the cells showed the differentiated morphology of myelocytes, metamyelocytes and mature granulocytes, and on day 5 to 7 about 80% of the cells had differentiated

Table I. Effect of DMSO induction on DNA synthesis of HL-60 cells

Treatment	[^{3}H]Thymidine incorporation cpm/10^6 viable cell
None	14,900
DMSO (1.25%)	
14 hours	11,800
3 days	3,100
5 days	1,100
7 days	700

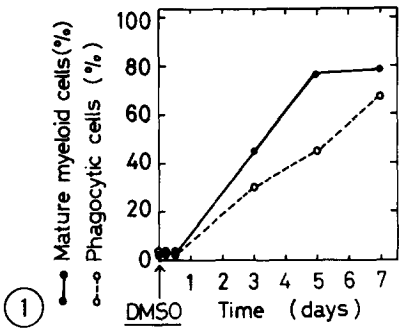
(Fig. 1). After incubation for 7 days with 1.25% DMSO, 70% of the cells phagocytized latex particles, whereas only about 2% of untreated HL-60 cells were phagocytic throughout the cultivation period (Fig. 1). These findings are consistent with those by Collins et al. (20).

The myeloperoxidase activity of uninduced HL-60 cells was 39.6 units/10^8 cells. On treatment with DMSO the myeloperoxidase activity of HL-60 cells decreased to 78% of the uninduced level after 14 hrs and to 24% after 3 days and then remained at this level for 7 days.

Changes of endogenous poly(ADP-Rib) levels during induction of differentiation

The level of endogenous poly(ADP-Rib) in HL-60 cells was determined by a newly developed method (21). As shown in Fig. 2, on DMSO treatment for 14 hrs it decreased remarkably to 15% of the uninduced level. This low level persisted until day 3 when the endogenous level of poly(ADP-Rib) was 18% of the uninduced level, and then it increased to 800% of the uninduced level on day 5, when most of the differentiated phenotypes were clearly seen.

In contrast to induced cells, uninduced cells exhibited a constant level of about 50 ng of poly(ADP-Rib)/mg DNA, irrespective of whether the cell density was as low as 0.5 x 10^6 cells/ml or as high as 2 x 10^6 cells/ml.



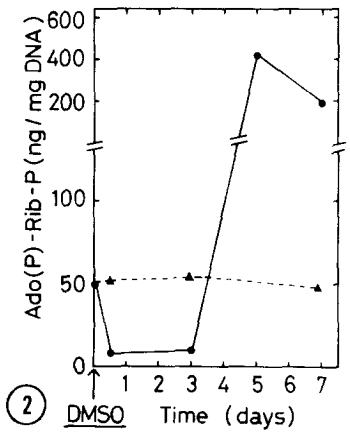


Fig. 1. Appearance of phenotypes of differentiation. Percentage of mature myeloid cells among HL-60 cells treated with 1.25% DMSO (●—●). Percentage of phagocytic cells among HL-60 cells treated with 1.25% DMSO (o--o). Cells that took up 5 or more latex particles were regarded as phagocytic.

Fig. 2. Endogenous poly(ADP-Rib) level, as shown by the amount of its monomer unit, Ado(P)-Rib-P, during differentiation of HL-60 cells. Poly-(ADP-Rib) level in HL-60 cells treated with 1.25% DMSO(●). Poly(ADP-Rib) level in untreated HL-60 cells (▲). The value was the mean of two independent experiments.

Poly(ADP-Rib) polymerase activity and poly(ADP-Rib) hydrolyzing enzyme activity during induction of differentiation

Changes in poly(ADP-Rib) polymerase activity of permeabilized HL-60 cells with or without DNase I cultured with 1.25% DMSO are shown in Fig. 3. The poly(ADP-Rib) polymerase activity of permeabilized cells without DNase I is thought to reflect the activity of poly(ADP-Rib) polymerase in native chromatin (26). After 14 hrs it had decreased to 40% of the uninduced level and it remained at this level for 7 days. The full activity of poly(ADP-Rib) polymerase free from regulation by the chromatin structure was determined after DNase I treatment (26). Poly(ADP-Rib) polymerase activity after DNase I treatment of the permeabilized cells decreased only to 70% of the initial level in the first 14 hrs, but then continued to decrease for 7 days. The poly(ADP-Rib) hydrolyzing activity of HL-60 cells treated with 1.25% DMSO remained the same for 5 days as that of untreated HL-60 cells.

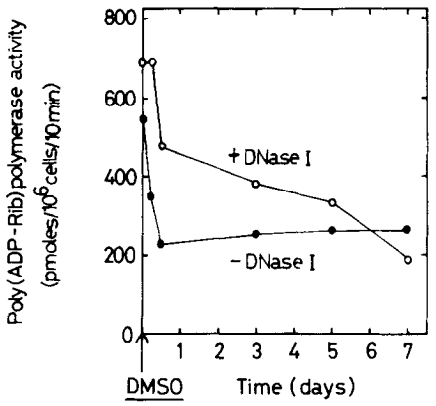


Fig. 3. Activity of poly(ADP-Rib) polymerase during differentiation of permeabilized HL-60 cells. Poly(ADP-Rib) polymerase was assayed in the absence (●) and presence (○) of DNase I.

DISCUSSION

The present results indicated that there is a marked change in the endogenous poly(ADP-Rib) level during differentiation of HL-60 cells. The transient reduction in poly(ADP-Rib) in HL-60 cells occurred before the appearance of the morphological and biochemical changes, i.e., phagocytosis and inhibition of DNA synthesis.

This great reduction of the poly(ADP-Rib) level could be explained partly by decrease in poly(ADP-Rib) polymerase activity because poly(ADP-Rib) hydrolyzing enzyme activity (28), which includes poly(ADP-Rib) glycohydrolase (29) and phosphodiesterase (30), did not change for 5 days after addition of DMSO. However, the marked increase of the poly(ADP-Rib) level, on day 5 after addition of DMSO to 8-fold that in uninduced cells could not be explained by poly(ADP-Rib) polymerase activity because poly(ADP-Rib) polymerase activity remained at a low level of 40%-50% of that of uninduced cells until day 7 after addition of DMSO. This example shows that the poly(ADP-Rib) polymerase activity does not necessarily reflect the endogenous level of poly(ADP-Rib).

Rastle and Swetly reported that the poly(ADP-Rib) polymerase activity in murine erythroleukemia cells increases when the cells are induced to differentiation (7), whereas Morioka et al. (8) and Terada et al. (9)

reported that it decreases. Pekala et al. (31) found that the poly-(ADP-Rib) polymerase activity first decreases and then increases during differentiation of mouse preadipocytes. However, endogenous poly(ADP-Rib) has not been determined in any of the various systems of cellular differentiation. Using our new method for direct chemical determination of poly(ADP-Rib)(21), we demonstrated for the first time in this work that endogenous poly(ADP-Rib) rapidly decreases during the early stage of differentiation and then increases very greatly with expression of differentiated phenotypes. Changes in poly(ADP-Rib) polymerase activity and poly(ADP-Rib) degrading enzyme activity do not explain these remarkable changes of the endogenous level of poly(ADP-Rib) in the last stage of differentiation. The above findings were made possible by development of the new sensitive procedure for determining poly(ADP-Rib), which is present in only small amount in vivo.

Measurement of the endogenous level of poly(ADP-Rib) should provide information on the biological functions poly(ADP-Rib) in cell differentiation, DNA repair and other events.

ACKNOWLEDGMENTS

We are grateful to Dr. Robert C. Gallo for kindly providing the HL-60 cell line. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, from the Ministry of Health and Welfare, and from the Society for Promotion of Cancer Research of Japan.

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