

## SPECIFIC INHIBITION OF POLY ADPRIBOSE POLYMERASE BY THYMIDINE AND NICOTINAMIDE IN HeLa CELLS

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Received 1 October 1971

### 1. Introduction

The enzyme forming poly ADPR seems to be related to DNA in a yet unknown manner. The exclusive localisation in the nucleus [1–3], the even distribution over the chromatin [4, 5], the firm association with nuclear proteins [6, 7], its inhibitory action on “endogenous” DNA polymerase activity [8], and the correlation of poly ADPR polymerase activity with the DNA *content* of various malignant and non-malignant tissues [9] point to a close connection with the nuclear DNA. In spite of these findings, however, the true function of the polymer is still obscure, and additional information is needed. We wish to report on a highly specific inhibition of the poly ADPR-forming enzyme from HeLa cells by thymidine and by nicotinamide.

### 2. Materials

Enzymes, coenzymes, analogues of NAD and nicotinamide were obtained from E. Merck (Darmstadt, Germany), from Boehringer u. Soehne (Mannheim, Germany), and from Nutritional Biochemicals (Cleveland, Ohio). 5-Methyl nicotinamide was a generous gift of Dr. J.B. Clark, London. HeLa S3 cells were

#### Abbreviations:

UR = uridine  
UDR = 2'-deoxy uridine  
FUDR = 5-fluoro-deoxy uridine  
BUR = 5-bromo uridine  
BUDR = 5-bromo-2'-deoxy uridine  
JUDR = 5-iodo-2'-deoxy uridine

propagated in modified Joklik medium (Grand Island Biological Co., Grand Island, USA) with 5% calf serum in suspension culture [10].

### 3. Results and discussion

During experiments with synchronized HeLa cell cultures it was observed that thymidine had a pronounced effect on poly ADPR polymerase. As shown in table 1, a dose-dependent inhibition occurs at concentrations used to block DNA synthesis and cell proliferation. This effect of thymidine is highly specific (table 2). From all “natural” bases and nucleosides tested only thymine produced a comparable inhibition. The thymidine analogues BUR, BUDR, and JUDR were also effective, while the uridine analogue FUDR showed only small effects. The increasing efficiency in the series UR–UDR–FUDR–BUR–BUDR again points to the highly specific structural requirements for inhibition of the enzyme by thymidine. It seems important to point out that thymidine nucleotides showed but a weak retardation of enzyme activity. Similar observations could be made with partially purified enzyme from Ehrlich carcinoma and from murine mastocytoma (not shown).

There is another type of inhibitor with a similarly high specificity: nicotinamide. Effective concentrations were in the same range as thymidine. The retarding action of nicotinamide on the poly ADPR forming enzyme has been noted by others [13, 14]. Its type of inhibition is competitive, as we have shown previously [14, cf. also 15]. This is somewhat

Table 1  
Dose-dependent inhibition of poly ADPRibose polymerase by thymidine.

Addition	Poly ADPR polymerase activity	
	(cpm incorporated)	(%)
none	450 ± 65	100
Thymidine 3 × 10 <sup>-4</sup> M	290	64
Thymidine 1 × 10 <sup>-3</sup> M	175	39
Thymidine 3 × 10 <sup>-3</sup> M	80	17
Thymidine 1 × 10 <sup>-2</sup> M	20	4
Thymidine added after incubation 1 × 10 <sup>-2</sup> M	430	95

Samples containing 100 mM Tris-HCl buffer pH 8.4; 2.4 mM MgCl<sub>2</sub>; 1 mM Clelands reagent; 1 mM <sup>3</sup>H-β-NAD (2 × 10<sup>4</sup> cpm), and nuclei corresponding to 15 μg DNA in 150 total volume were incubated at 25° for 45 min. 100 μl were then spotted onto filter papers, dried, and analyzed for acid insoluble radioactivity according to [11]. Nuclei were prepared from HeLa S3 cells propagated in suspension culture [10]. Cells (1.5 × 10<sup>8</sup>) were washed and treated with 5 ml 10 mM Tris buffer pH 7.4–10 mM KCl – 1.5 mM MgCl<sub>2</sub> at 0° for 15 min, and then broken up in a Dounce homogenizer (B. Braun, Melsungen) until less than 1% of unbroken cells were left. Nuclei were washed 3× in isotonic sucrose containing Tris–KCl–MgCl<sub>2</sub> buffer, and finally suspended in 3 ml of this solution. DNA was determined according to [12].

surprising since the inhibition by nicotinamide proved also very specific with the HeLa enzyme (table 3). Even 3-acetyl pyridine was not able to substitute for nicotinamide but to a very limited degree. Only 5-methyl nicotinamide could replace nicotinamide as an inhibitor to some extent. Also, NMN had no affinity at the applied concentration which corresponded to the concentration of the substrate NAD in the test. The high specificity of the enzyme extends to the dinucleotide level. Neither NADP nor 3-acetyl pyridine NAD, which can substitute NAD in all dehydrogenase systems, was able to inhibit significantly the enzyme at equimolar concentrations. There are rare exceptions like α-NAD or thio-NAD which inhibit the enzyme competitively to a somewhat higher degree (not

Table 2  
Specific inhibition of poly ADPR polymerase in HeLa 3 nuclei by thymidine and thymine.

Additions (1 × 10 <sup>-3</sup> M)	Poly ADPR polymerase activity (cpm incorporated)	(% of control)
None	3,470 ± 80	100
Adenine	2,750 ± 140	79
Guanine	2,820 ± 60	81
Uracil	3,000 ± 170	86
Cytosine	3,370 ± 210	96
Hypoxanthine	2,790 ± 200	80
Thymine	870 ± 9	25
Adenosine	3,160 ± 150	91
Guanosine	3,180 ± 150	91
Uridine	3,340 ± 70	96
Cytidine	3,185 ± 50	91
Inosine	3,250 ± 190	93
Deoxy adenosine	3,400 ± 20	97
Deoxy guanosine	2,810 ± 30	81
Deoxy uridine	3,050 ± 60	87
Deoxy cytidine	3,490 ± 50	100
Deoxy thymidine	490 ± 30	14
BUR	610 ± 25	18
BUDR	110 ± 10	3
FUDR	2,980 ± 110	86
Deoxy TMP	2,770 ± 80	80
Deoxy TTP	3,120 ± 60	90

Nuclei corresponding to 60 μg DNA were incubated under the conditions described in the legend to table 1.

shown). Still, it appears that nicotinamide, too, is a unique inhibitor of poly ADPR polymerase of HeLa S3 (and of Ehrlich carcinoma) cells.

The physiological significance of the specific inhibition of poly ADPR polymerase by the two metabolites is not clear. While the concentrations needed for inhibition of the nuclear enzyme are well above the levels found in tissues (equal distribution assumed), the high specificity of the effects may indicate yet unknown correlations. In the case of a thymidine block applied to HeLa cultures (2 mM thymidine) the enzyme should be nearly completely inhibited. Whether this is related to the inhibition of DNA synthesis, observed under these conditions, or to the variations of poly ADPR polymerase during the cell cycle [16–18] remains to be determined.

Nicotinamide at 1 mM and 3 mM concentrations elevated NAD content in HeLa cells, but acted differently on cell proliferation [19]. At 1 mM

Table 3  
Specificity of nicotinamide inhibition.

Additions		Poly ADPR polymerase (relative activity)
None		100
Nicotinamide	$1 \times 10^{-3}$ M	49
	$3 \times 10^{-3}$ M	26
Nicotinate	$3 \times 10^{-3}$ M	94
Pyridine-3-hydroxamate	$1 \times 10^{-3}$ M	102
	$3 \times 10^{-3}$ M	110
Pyridine-3-sulfonate	$1 \times 10^{-3}$ M	99
	$3 \times 10^{-3}$ M	109
3-Acetyl-pyridine	$1 \times 10^{-3}$ M	86
	$3 \times 10^{-3}$ M	99
Nicotinonitrile	$1 \times 10^{-3}$ M	98
	$3 \times 10^{-3}$ M	108
Nicotinate-N-ethylamide	$1 \times 10^{-3}$ M	95
Isonicotinyl hydrazide	$1 \times 10^{-3}$ M	101
6-Amino-nicotinamide	$1 \times 10^{-3}$ M	84
6-Chloro-nicotinamide	$1 \times 10^{-3}$ M	104
5-Methyl nicotinamide	$1 \times 10^{-3}$ M	70
NMN	$1 \times 10^{-3}$ M	87
NADP	$1 \times 10^{-3}$ M	89
3-Acetylpyridine-NAD	$1 \times 10^{-3}$ M	86
Deamino-NAD	$1 \times 10^{-3}$ M	100

Mean values from 2–4 determinations with HeLa nuclei. The controls correspond to  $27.3 \pm 1.1$  cpm/ $\mu$ g DNA incorporated into the acid-insoluble polymer under the conditions described in table 1.

nicotinamide, there was a slight stimulation (to 107–125%), while 3 mM nicotinamide inhibited cell proliferation (by 10–30%). In both cases, however, poly ADPR polymerase activity should be partially inhibited, provided intracellular nicotinamide levels approach outside concentrations. These observations have some bearing on a postulate of Burzio and Koide [8] that the proliferation-inhibiting action of nicotinamide [20] should come

about by an elevation of NAD concentration which in turn would increase poly ADPR formation and thus inhibit DNA synthesis. This postulate did not take into account the inhibitory action of nicotinamide on the poly ADPR-forming enzyme.

### Acknowledgements

We want to thank Miss M. Kittler and Dr. S. Nolde for carrying out some determinations, and Mr. Jarmer for providing the cells. Mit Unterstützung des Ministeriums für Wissenschaft und Forschung.

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