# INHIBITION OF POLY(ADP-RIBOSE) POLYMERASE BY METHYLATED XANTHINES AND CYTOKININS

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## 1. Introduction

The chromosomal enzyme poly(ADP-ribose) polymerase catalyzes the synthesis of poly(ADP-ribose) from the ADP-ribosyl moiety of NAD [1,2]. The biological role(s) of this polymer are unknown. The effect of several pyridine derivatives and thymidine on poly-(ADP-ribose) polymerase activity has been reported [3–6]. The most effective known inhibitors of the polymerase are nicotinamide and thymidine. We have examined two additional pyridine derivatives and seven purine derivatives for their effect on poly-(ADP-ribose) polymerase activity in isolated 3T3 cell nuclei. We report here a potent inhibition of the polymerase by methylated xanthines and $N^6$-substituted derivatives of adenine (cytokinins). Both of these classes of compounds have been shown to inhibit cAMP phosphodiesterase [7–12].

## 2. Materials and methods

### 2.1. *Commercial materials*

Kinetin, kinetin riboside, isopentenyl adenine, isopentenyl adenosine, quinolinate, nicotinic acid, nicotine, nicotinamide, thymidine, NAD and Trizma base were purchased from Sigma Chemical Co., St Louis, MO. Theophylline and caffeine were obtained from Calbiochem, Los Angeles, CA. Aquasol and [adenine-$^3$H]NAD (3.4 mCi/$\mu$mol) were from New England Nuclear, Boston, MA. Isobutylmethylxanthine was purchased from Aldrich Chem. Corp. Inc., Milwaukee, WI. Balb/c 3T3 mouse embryo fibroblasts were obtained from Dr G. Todaro.

### 2.2. *Tissue culture*

3T3 cells were grown as in [13]. Logarithmically growing cells at $1 \times 10^5$ cells/cm$^2$ were removed from dishes by treatment with 0.05% trypsin–EDTA in phosphate-buffered saline (PBS) (0.01 M $Na_2HPO_4$–$NaH_2PO_4$, pH 7.2, 0.15 M NaCl) and nuclei were isolated.

### 2.3. *Nuclei isolation*

Nuclei were isolated according to a modification of the procedure in [14]. Approx. $1 \times 10^7$ cells were collected by centrifugation and washed with PBS. The cell pellet was suspended in 2 ml 3 mM Tris–HCl, pH 7.0, 0.5 mM $MgCl_2$, 3 mM NaCl. Cells were allowed to swell for 15 min and 1 ml 0.5% (v/v) Triton X-100 solution was added to effect lysis. After 10 min, the suspension was centrifuged at $800 \times g$ for 10 min. The crude nuclei pellet was resuspended in 2 ml 10 mM Tris–HCl, pH 7.0, 1.5 mM $MgCl_2$, 10 mM NaCl. After 4 min, 0.3 ml detergent solution containing 6% (v/v) Tween 80 and 3% (w/v) sodium deoxycholate was added and mixed with a vortex mixer for 4 s. After centrifugation at $800 \times g$ for 10 min, the supernatant was removed and the nuclei pellet was washed twice with 10 mM Tris–HCl, pH 7.0, 1.5 mM $MgCl_2$, 10 mM NaCl. At this stage, the nuclei were free of microscopically-visible cytoplasmic debris. DNA was determined as in [15] except that the reagent contained 3% (w/v) diphenylamine and 0.01% (v/v) paraldehyde in glacial acetic acid and the test solution was combined with the reagent at a 1:1 ratio. Percent recovery of DNA was $72 \pm 13$ ($n$=6) and the nuclei had an average protein/

DNA mass ratio of 4.1 and an RNA/DNA ratio of 0.53. Freshly isolated nuclei were used to determine poly(ADP-ribose) polymerase activity.

2.4. *Poly(ADP-ribose) polymerase assay*

The incubation mixtures (0.1 ml) contained 100 mM Tris–HCl, pH 8.0, 2 mM $MgCl_2$, 8.2 mM NaCl, 1 mM 2-mercaptoethanol, 0.2 mM EDTA, 1 mM [adenine-$^3$H]NAD (0.8 $\mu$Ci) and nuclei containing 17.6 $\mu$g DNA. Inhibitors were present at 1 mM. Isobutylmethylxanthine and the cytokinins were dissolved in dimethylsulfoxide (DMSO). DMSO in the incubation mixture was final conc. 9% (v/v) which did not affect the control incubation. Incubations were carried out at 25°C with occasional agitation and portions were removed and added to equal vol. 40% (w/v) ice-cold trichloroacetic acid (TCA). Bovine serum albumin (100 $\mu$g) was used as coprecipitant. The precipitates were washed 3 times with 1 ml 20% (w/v) TCA and were solubilized in 0.1 ml 88% formic acid. Radioactivity of the samples was determined by scintillation counting in 2 ml Aquasol. The rate of formation of poly(ADP-ribose) was proportional to the amount of nuclei added to the incubation. Incorporation was linear for 10 min and reached a maximum at approx. 30 min.

## 3. Results and discussion

The effect of pyridine and purine derivatives on poly(ADP-ribose) polymerase activity is listed in table 1. Each compound tested was present at equimolar concentration to NAD (1 mM). Polymerase activity is shown for a 10 min incubation. Similar results were observed for 20 min incubations.

Three of the compounds tested have been examined. Our results are very similar to the previous findings in that 1 mM nicotinamide and thymidine inhibited poly(ADP-ribose) polymerase by 55% and 60%, respectively, while nicotinic acid had no effect. Inhibition of the reaction by nicotinamide has been observed in nuclei from rat liver [3,4], HeLa [5] and Ehrlich ascites [6] cells. This inhibition was shown to be competitive with NAD [4,6]. Nicotinic acid at equimolar concentration to NAD did not cause inhibition [5]. Inhibition of the polymerase by thymidine has been observed in nuclei from rat liver [16], HeLa [5] and cultured mouse [17] cells. The effect of thymidine is also competitive [16,17]. We have examined two additional pyridine derivatives, nicotine and quinolinic acid. Neither of these caused inhibition.

Table 1
Effect of pyridine and purine derivatives on poly(ADP-ribose) polymerase activity in 3T3 nuclei

| Additions | Poly(ADP-ribose) polymerase activity (% control) |
|---|---|
| None | 100 |
| Thymidine | 45 |
| Nicotinamide | 41 |
| Nicotine | 104 |
| Nicotinic Acid | 102 |
| Quinolinate | 93 |
| Theophylline | 26 |
| Caffeine | 67 |
| 3-Isobutyl-1-methylxanthine | 87 |
| Kinetin (6-furfurylamino purine) | 87 |
| Kinetin riboside (6-furfurylamino purine riboside) | 70 |
| $N^6$-[$\Delta^2$-isopentenyl]-adenine | 55 |
| $N^6$-[$\Delta^2$-isopentenyl]-adenosine | 75 |

Nuclei containing 17.6 $\mu$g DNA were incubated as in section 2. The poly(ADP-ribose) polymerase activity is shown for a 10 min incubation. The control incubation had 3070 cpm. All inhibitors were present at 1 mM. Duplicate analyses agreed within 5%

The methylated xanthines theophylline, caffeine and isobutylmethylxanthine were examined. These compounds have previously been shown to be potent inhibitors of cAMP phosphodiesterase [7,11,12]. Theophylline was the most effective inhibitor of poly(ADP-ribose) polymerase activity while caffeine inhibited to a lesser extent. Isobutylmethylxanthine which is the most potent inhibitor of cAMP phosphodiesterase [12] was only slightly inhibitory.

Isopentenyladenine was the most effective inhibitor of the polymerase among the four cytokinins examined, although kinetin riboside and isopentenyl adenosine also inhibited. Kinetin was only slightly inhibitory. Cytokinins have been shown to inhibit cAMP phosphodiesterase in cell homogenates [8]. In plants, they constitute a group of growth promoting hormones [18,19]. When added to cultured animal cells, they generally have pronounced inhibitory effects on cell growth [20–23].

The effects of methylated xanthines and cytokinins on whole cells have generally been interpreted in terms of effects on cyclic nucleotide metabolism. Although isopentenyl adenine and theophylline show very similar effects on cAMP phosphodiesterase, theophylline causes an elevation of the intracellular levels of cAMP several fold whereas isopentenyladenine does not [8]. In view of the results presented here, the effect of these compounds on both cAMP and NAD metabolism will have to be considered. It should also be noted that studies of the mode of action of cholera toxin have established a direct relationship between NAD and cAMP metabolism [24,25].

The availability of inhibitors of poly(ADP-ribose) polymerase may be useful in studying the biological role(s) of poly(ADP-ribose). The possible involvement of poly(ADP-ribose) in DNA repair has been postulated [26–28]. It is interesting that both caffeine [29] and theophylline [30] have been to inhibit DNA repair.

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## References

[1] Hayaishi, O. and Ueda, K. (1977) Ann. Rev. Biochem. 46, 95–116.
[2] Hilz, H. and Stone, P. (1976) Rev. Physiol. Biochem. Pharmacol. 76, 1–58.
[3] Fujimura, S., Hasegawa, S., Shimuzu, Y. and Sugimura, T. (1967) Biochim. Biophys. Acta 145, 247–259.
[4] Clark, J. B., Ferris, G. M. and Pinder, S. (1971) Biochim. Biophys. Acta 238, 82–85.
[5] Preiss, J., Schlaeger, R. and Hilz, H. (1971) FEBS Lett. 19, 244–246.
[6] Römer, V., Lambrecht, J., Kittler, M. and Hilz, H. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 109–112.
[7] Bucher, R. W. and Sutherland, E. W. (1962) J. Biol. Chem. 237, 1244–1250.
[8] Johnson, G. S., D'Armiento, M. and Carchman, R. A. (1974) Exp. Cell Res. 85, 47–56.
[9] Hecht, S. M., Faulkner, R. D. and Hawrelak, S. D. (1974) Proc. Natl. Acad. Sci. USA 71, 4670–4674.
[10] Wood, H. N., Lin, M. C. and Braun, A. C. (1972) Proc. Natl. Acad. Sci. USA 69, 403–406.
[11] Kakiuchi, S., Yamazaki, R., Teshima, Y., Uenishi, K. and Miyamoto, E. (1975) Biochem. J. 146, 109–120.
[12] Wells, J. N., Wu, Y. J., Baird, C. E. and Hardman, J. G. (1975) Mol. Pharmacol. 11, 775–783.
[13] Jacobson, E. L. and Jacobson, M. K. (1976) Arch. Biochem. Biophys. 175, 627–634.
[14] Penman, S. (1969) in: Fundamental Techniques in Virology (Habel, K. and Salzman, N. P. eds) pp. 36–37, Academic Press, New York, London.
[15] Richards, G. M. (1976) Anal. Biochem. 57, 369–376.
[16] Ueda, K., Miyakawa, N. and Hayaishi, O. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 844–845.
[17] Stone, P. R., Whish, W. J. D. and Shall, S. (1973) Biochem. Soc. Trans. 1, 692–693.
[18] Skoog, F. and Armstrong, D. J. (1970) Annu. Rev. Plant. Physiol. 21, 359–384.
[19] Suk, D., Simpson, C. L. and Mihich, E. (1970) Cancer Res. 30, 1429–1436.
[20] Gallo, R. C., Hecht, S. M., Whang-Peng, J. and O'Hopp, S. (1972) Biochim. Biophys. Acta 281, 488–500.
[21] Hampton, A., Biesele, J. J., Moore, A. E. and Brown, G. B. (1956) J. Am. Chem. Soc. 78, 5695.
[22] Fleysher, M. H., Hakala, M. T., Block, A. and Hall, R. H. (1968) J. Med. Chem. 11, 717–720.
[23] Fleysher, M. H., Bloch, A., Hakala, M. T. and Nichol, C. A. (1969) J. Med. Chem. 12, 1056–1061.
[24] Gill, M. D. (1975) Proc. Natl. Acad. Sci. USA 72, 2064–2068.
[25] Moss, J., Manganiello, V. C. and Vaughan, M. (1976) Proc. Natl. Acad. Sci. USA 73, 4424–4427.
[26] Smulson, M. E., Schein, P., Mullins, D. W. and Sudhakar, S. (1977) Cancer Res. 37, 3006–3012.
[27] Miller, E. G. (1975) Biochem. Biophys. Res. Commun. 66, 280–286.
[28] Miller, E. G. (1975) Biochim. Biophys. Acta 395, 191–200.
[29] Cleaver, J. E. and Thomas, G. H. (1969) Biochem. Biophys. Res. Commun. 36, 203–208.
[30] Lehman, A. R. and Kirk-Bell, S. (1972) Eur. J. Biochem. 31, 438–445.