Biochem. J. (1982) **203**, 511–513
Printed in Great Britain

The inhibitory effect of Zn^{2+} on poly(ADP-ribose) polymerase activity and its reversal

Anne G. LARSEN, Anne C. ØSTVOLD, Jostein HOLTLUND, Tom KRISTENSEN and Søren G. LALAND
Department of Biochemistry, University of Oslo, P.O. Box 1041, Blindern, Oslo 3, Norway

(*Received 10 December 1981/Accepted 10 February 1982*)

Zn^{2+} inhibits purified poly(ADP-ribose) polymerase (50% inhibition at 10μM). Furthermore poly(ADP-ribose) polymerase present in nuclei and metaphase chromosome clusters is also inhibited by Zn^{2+}. The inactivated enzyme could be re-activated by dithiothreitol. The concentration of Zn^{2+} needed to affect the enzyme activity in the organelles is sufficiently low for it to have a possible role in controlling the activity of this chromatin-bound enzyme.

It has been reported, but without any supporting data, that Zn^{2+} is highly inhibitory towards the chromatin-bound enzyme poly(ADP-ribose) polymerase from calf thymus (Ito *et al.*, 1979). Furthermore Zn^{2+} is known to be taken up specifically by nuclei (Weser & Bischoff, 1970), to form complexes with polynucleotides (Shin & Eichhorn, 1968) and to be an integral part of DNA polymerase and RNA polymerase (Prasad, 1979). On the basis of the content of Zn^{2+} in nuclei (Wester, 1965), and assuming an even distribution, the concentration would be of the order of 0.5mM. Hence it seemed possible that Zn^{2+} could be one factor controlling the activity of the enzyme in the cell. Fluctuation in the concentration of Zn^{2+} at the location of the enzyme could result from competition between various nuclear components for the metal ion.

In this paper we have examined the inhibitory effect of Zn^{2+} on poly(ADP-ribose) polymerase in HeLa-S3 cell nuclei and metaphase chromosome clusters and the purified enzyme from pig thymus. Furthermore, to support the possibility that Zn^{2+} is a controlling factor of enzyme activity, we attempted to reverse the inhibitory effect by the addition of Zn^{2+}-binding agents.

Materials and methods

Materials

Percoll was obtained from Pharmacia, Uppsala, Sweden, and [U-^{14}C]NAD was from The Radiochemical Centre, Amersham, Bucks., U.K. Phenylmethanesulphonyl fluoride and dithiothreitol were purchased from Sigma.

Preparation of pig thymus poly(ADP-ribose) polymerase

The enzyme was purified by a method described previously (Holtlund *et al.*, 1980*b*) and was about 80% pure.

Preparation of organelles

(*a*) *Interphase HeLa-S3 cell nuclei.* Cells were grown in suspension culture at 37°C in Eagle's minimum essential medium supplemented with 10% foetal calf serum, Hepes buffer (pH 7.3, final concn. 15mM), 1ml of a 0.1mM solution of non-essential amino acids to 100ml, benzylpenicillin (100μg/ml) and streptomycin (100μg/ml). HeLa-cell nuclei were prepared by the method of Whitlock & Simpson (1976).

(*b*) *Metaphase-chromosome clusters from HeLa S3 cells.* Cells were grown as described previously (Holtlund *et al.*, 1980*b*). Metaphase cells were harvested after being arrested for 16h with 0.05μg of colcemid/ml. About 95% of the cells were in metaphase. Chromosome clusters were prepared essentially by a method developed by Paulson (1980). All steps were carried out at 0°C. Cells were washed with 0.9% NaCl, suspended in a solution containing 10mM-Hepes, pH 7.2, 10mM-NaCl, 10mM-$MgCl_2$, 0.5mM-$CaCl_2$ (ISB buffer), 0.5M-sucrose, 0.1% Nonidet P40 and 0.5mM-phenylmethanesulphonyl fluoride (10^7 cells/ml) and broken in a Dounce homogenizer. The homogenate was centrifuged at 1000g for 5min to remove most of the

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonate.

0306-3275/82/050511-03$01.50/1 © 1982 The Biochemical Society

cytoplasmic material. The pellet was resuspended in the above solution and the metaphase-chromosome clusters were purified by layering the suspension on top of a preformed Percoll gradient and centrifuging for 1h in a HS4 rotor in the Sorvall centrifuge at 3800rev./min. The Percoll gradient was prepared by centrifuging a solution containing 60% (v/v) Percoll, 10mM-Hepes, pH7.2, 10mM-NaCl, 10mM-$MgCl_2$, 0.5mM-$CaCl_2$, 0.5M-sucrose and 0.1% Nonidet P40 in a SS34 rotor in a Sorvall centrifuge at 20000rev./min for 45min. The fraction of metaphase-chromosome clusters obtained, as judged by phase-contrast microscopy, was free from cytoplasm, unbroken metaphase and interphase cells as well as interphase nuclei. The fraction containing metaphase-chromosome clusters was diluted with ISB buffer, pH8.0, containing 0.5M-sucrose, pelleted (1000rev./min), washed once in the same buffer and finally resuspended in 0.25M-sucrose.

Measurement of poly(ADP-ribose) polymerase activity

In this study Hepes buffer, which is known to show little affinity for the Zn^{2+} ion (Elliot & Brewer, 1980), has been used.

(*a*) *After inactivation of enzyme.* The enzyme was inactivated at 25°C for 5min in the presence of 10mM-$MgCl_2$, 40mM-Hepes buffer, pH8.0, and 0.25M-sucrose with additions of $ZnCl_2$ as indicated. For the subsequent measurement of enzyme activity 50μM-[U-^{14}C]NAD$^+$ (0.1μCi/0.1ml) was added and the mixture incubated for a further 10min at 25°C. Acid-insoluble radioactivity was determined as described previously (Holtlund *et al.*, 1980*a*).

(*b*) *After re-activation of inactivated enzyme.* When the enzyme had been inactivated with $ZnCl_2$, as described above, dithiothreitol was added and the mixture incubated for another 5min at 25°C before addition of 50μM-[U-^{14}C]NAD$^+$ (0.1μCi/0.1ml) and measurement of enzyme activity as above.

Results and discussion

Fig. 1 shows that 10μM-Zn^{2+} decreases the activity of poly(ADP-ribose) polymerase to about 50%. At 100μM the activity is about 10%. Dithiothreitol was chosen as an agent to re-activate the enzyme, since it is known to remove enzyme-bound Zn^{2+} (Gracy & Noltman, 1968). Table 1 shows that the inactivated enzyme could be re-activated by the

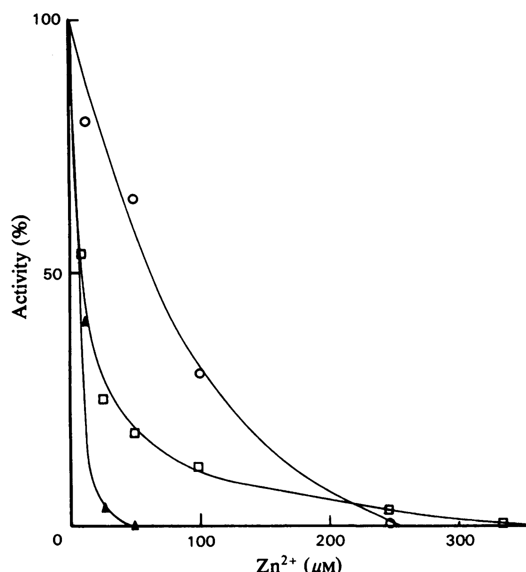

Fig. 1. *Effect of Zn^{2+} on poly(ADP-ribose) polymerase activity in nuclei, metaphase-chromosome clusters and on purified polymerase from pig thymus*

The incubation mixtures contained, in a volume of 0.1ml, nuclei (○) corresponding to 60μg of DNA, metaphase-chromosome clusters (▲) corresponding to 19μg of DNA, or 0.7μg of pig thymus enzyme (□) and 5μg of DNA respectively. For further details see the Materials and methods section.

Table 1. *Re-activation of poly(ADP-ribose) polymerase with dithiothreitol after treatment with $ZnCl_2$*

The amounts of nuclei, metaphase-chromosome clusters and pig thymus poly(ADP-ribose) polymerase used were as described in Fig. 1. Otherwise additions were as indicated. For further details see the Materials and methods section.

Source	Additions	Radioactivity in acid-insoluble material (c.p.m.)
Interphase nuclei	None	5181
	0.5mM-$ZnCl_2$	0
	0.5mM-$ZnCl_2$ + 6mM-dithiothreitol	6651
Metaphase-chromosome clusters	None	1020
	0.5mM-$ZnCl_2$	0
	0.5mM-$ZnCl_2$ + 3mM-dithiothreitol	1360
Pig thymus enzyme	None	3710
	0.1mM-$ZnCl_2$	381
	0.1mM-$ZnCl_2$ + 6mM-dithiothreitol	5111

addition of dithiothreitol. It is known that poly-(ADP-ribose) polymerase is inactivated by thiol-specific reagents such as *p*-chloromercuribenzoic acid (Chambon *et al.*, 1966) and hence that a thiol group is required for activity. Thus it seems likely that the inactivating effect of Zn^{2+} may be caused by its binding to a thiol group.

The inactivating effect of Zn^{2+} on poly(ADP-ribose) polymerase in nuclei and metaphase-chromosome clusters and its re-activation is shown in Fig. 1 and Table 1 respectively. In nuclei the concentration of Zn^{2+} needed to obtain a significant decrease in activity was about 50 μM, and at 100 μM the activity was less than 30%. Table 1 demonstrates that the inactivated enzyme in nuclei could be re-activated by the addition of dithiothreitol. The concentration of Zn^{2+} needed to obtain significant decrease in the activity of the enzyme in nuclei is thus considerably less than the overall concentration of Zn^{2+} in nuclei (Wester, 1965). Hence Zn^{2+} could well play a regulatory role in determining the activity of the poly(ADP-ribose) polymerase.

In metaphase-chromosome clusters 10 μM-Zn^{2+} decreases the activity of the enzyme to less than 50%, and at 25 μM-Zn^{2+} the activity is significant (Fig. 1). Again the inactivated enzyme could be re-activated with dithiothreitol (Table 1). When isolating metaphase chromosomes, the solution of Maio & Schildkraut (1967), which contains $ZnCl_2$ to stabilize the chromosomes, is often used.

The present experiment shows that if it is desired to re-activate the enzyme in metaphase chromosomes after using the buffer of Maio & Schildkraut (1967), this can be achieved by the addition of dithiothreitol.

The finding that poly(ADP-ribose) polymerase can be reversibly inactivated with Zn^{2+} could be useful in experiments where inactivation of the enzyme followed by re-activation is required. With other known inhibitors of this enzyme, such as nicotinamide, thymidine and benzamide (Shall, 1975), this cannot easily be achieved. Inhibition with Zn^{2+} may complement the use of other inhibitors in resolving the role of poly(ADP-ribose) or the polymerase in cellular processes such as DNA repair (Durkacz *et al.*, 1980).

References

Chambon, P., Weill, J. D., Doly, J., Strosser, M. T. & Mandel, P. (1966) *Biochem. Biophys. Res. Commun.* **25**, 638–643

Durkacz, B. W., Nduka, N., Omidiji, O., Shall, S. & Zia'ee, A. H. (1980) in *Novel ADP-Ribosylation of Regulatory Enzymes and Proteins* (Smulson, M. & Sugimura, T., eds.), pp. 207–217, Elsevier/North-Holland, Amsterdam and New York

Elliot, J. I. & Brewer, J. M. (1980) *J. Inorg. Biochem.* **12**, 323–334

Gracy, R. W. & Noltman, E. A. (1968) *J. Biol. Chem.* **243**, 4109–4116

Holtlund, J., Kristensen, T. & Sletten, K. (1980*a*) *Biochem. J.* **185**, 779–782

Holtlund, J., Kristensen, T., Østvold, A. C. & Laland, S. G. (1980*b*) *FEBS Lett.* **116**, 11–13

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

Maio, J. J. & Schildkraut, C. L. (1967) *J. Mol. Biol.* **24**, 29–39

Paulson, J. (1980) *Eur. J. Biochem.* **111**, 189–197

Prasad, A. (1979) *Annu. Rev. Pharmacol. Toxicol.* **20**, 393–426

Shall, S. (1975) *J. Biochem. (Tokyo)* **77**, 2p

Shin, Y. A. & Eichhorn, G. L. (1968) *Biochemistry* **7**, 1026–1032

Weser, U. & Bischoff, E. (1970) *Eur. J. Biochem.* **12**, 571–575

Wester, P. O. (1965) *Biochim. Biophys. Acta* **109**, 268–283

Whitlock, J. P. & Simpson, R. T. (1976) *Nucleic Acids Res.* **3**, 2255–2266