CATALASE DEFICIENCY IN MYELOPEROXIDASE DEFICIENT POLYMORPHONUCLEAR LEUCOCYTES FROM CHICKEN

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Received 29 June 1977

1. Introduction

Hydrogen peroxide is produced by phagocytosing polymorphonuclear leucocytes from mammalian species [1–3]. These cells possess three main mechanisms for H_2O_2 disposal, which involve myeloperoxidase, catalase and glutathione peroxidase, respectively [4]. Chicken polymorphonuclear leucocytes have been reported to be devoid of myeloperoxidase activity [5]. We report here that chicken polymorphonuclear leucocytes also lack catalase activity, and discuss the possible pathways for H_2O_2 disposal in these cells.

2. Materials and methods

2.1. *Cell preparation*

Ubard strains of adult male chicken were injected intraperitoneally with 70 ml 1% sterile sodium caseinate solution in 0.9% NaCl. The exudate was collected 5 h later. The polymorphonuclear leucocyte content of the exudates was usually between 85% and 90%. Longer periods of exudate elicitation resulted in an increased percentage of mononuclear cells in the exudate. Exudates contaminated with red cells were discarded. Cells were harvested by centrifugation at 250 × *g* for 7 min and, after resuspension in Krebs-Ringer–phosphate buffer (KRP) pH 7.4, were counted in a hemocytometric Burker chamber. Suspensions of the desired cell concentration were obtained by dilution with KRP.

2.2. *Assay of catalase activity*

This was done on both the whole cell lysate and the cell-free supernatant. Cells ($4–8 \cdot 10^8$/ml) were lysed with 0.04% (final) Triton X-100 at 37°C for 4 min, and the supernatant was obtained by centrifuging the lysate at 8000 × *g*. Two different methods were used to assay catalase activity:

(i) A polarographic method employing a Clark oxygen electrode for continuous recording of the oxygen liberated from H_2O_2.

(ii) A spectrophotometric method in which the disappearance of H_2O_2 at 230 nm was followed.

In both assays KRP was the basic medium. In the polarographic assay H_2O_2 was generated enzymatically in the electrode chamber with xanthine and xanthine oxidase. After completion of the xanthine–xanthine oxidase reaction the catalase containing sample was added into the electrode chamber. A backward deflection of the recording trace, due to liberation of oxygen, revealed the presence of catalase. The rate of oxygen liberation was quantitated from the initial linear part of the recording trace. The rate of H_2O_2 consumption was calculated from the rate of oxygen liberation based on a stoichiometry of 2 mol. hydrogen peroxide destroyed/1 mol. oxygen liberated.

In the spectrophotometric assay 10 mM (final) H_2O_2 from Merck was used as substrate and the decrease in optical density was recorded with a 165 Perkin Elmer recorder attached to a 124 Hitachi-Perkin Elmer double beam spectrophotometer, using an extinction coefficient of 81 M^{-1} cm^{-1} [2].

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+ Recipient of a fellowship from the Anna Villa Rusconi Foundation, Varese, Italy

North-Holland Publishing Company – Amsterdam

2.3. *Assay of glutathione reductase and glutathione peroxidase*

Both enzyme activities were measured on cell homogenate and on the cell free supernatant. Cells ($1-2 \cdot 10^8$/ml) were frozen and thawed three times in acetone dry-ice and then were centrifuged at 20 000 × *g*. Glutathione reductase was assayed according to the method of Horn [6]. The assay medium contained in final vol. 3 ml, 20 mM KH_2PO_4–Na_2HPO_4 buffer, pH 6.6, 0.1% bovine serum albumin, 0.5 mM GSSG and 0.08 mM NADPH. Glutathione peroxidase was assayed according to the method of Reed [7]. The assay mixture contained in final vol. 3 ml, 20 mM KH_2PO_4–Na_2HPO_4 buffer, pH 6.6, 1 mM GSH, 0.1 mM H_2O_2, 0.08 mM NADPH and 2 μg yeast glutathione reductase (Boehringer, Mannheim, 120 U/mg). (One unit is the amount of enzyme which converts 1 μmol oxidized glutathione to reduced glutathione in 1 min, at 25°C and at pH 7.6.) The equivalent of $0.5-1.5 \cdot 10^7$ cells was used for each assay. Assays were carried out at 37°C and the decrease in absorbance at 340 nm was followed in a 124 Hitachi Perkin Elmer double beam spectrophotometer.

3. Results

Figure 1 shows the polarographic recordings of the oxygen uptake by the xanthine–xanthine oxidase reaction and the subsequent liberation of oxygen, from the H_2O_2 produced, upon addition of either purified catalase or a catalase-containing supernatant from extracts. Addition of 100 μl KRP caused a slight

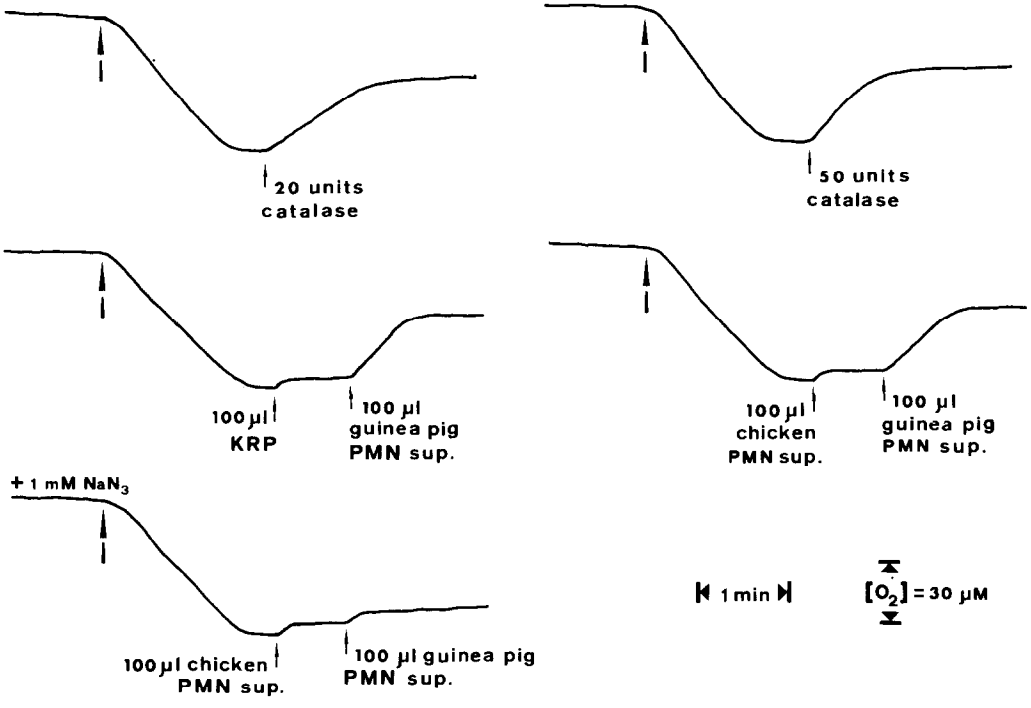

Fig.1. Polarographic assay of catalase activity in guinea pig and chicken polymorphonuclear leucocytes. The recording traces of oxygen uptake by the xanthine–xanthine oxidase reaction and of oxygen release from H_2O_2 after catalase addition are shown. The assay medium contained 100 μM xanthine in 2 ml Krebs-Ringer–phosphate, pH 7.4. At the broken arrows 0.05 U xanthine oxidase (cow milk, Boehringer, Mannheim) were added. (One Unit is the amount of enzyme which converts 1 μmol hypoxanthine to uric acid in 1 min, at 25°C and at pH 7.4.) One unit of catalase (beef liver, Boehringer, Mannheim) is the amount of enzyme which destroys 1 μmol H_2O_2 in 1 min, at 25°C and at pH 6.8, as measured at 240 nm. 100 μl of supernatant is equivalent to $7.5 \cdot 10^7$ chicken polymorphonuclear leucocytes (PMN) and $7.5 \cdot 10^6$ guinea pig PMN, respectively. Temperature 37°C.

backward deflection of the recording trace due to the oxygen dissolved in the KRP. Addition of 100 μl of a concentrated supernatant of chicken polymorphonuclear leucocytes caused a backward deflection equal to that seen with 100 μl KRP. The oxygen released after addition of as much as one-tenth (in terms of cell equiv.) of guinea pig polymorphonuclear supernatant with respect to chicken cells is shown for comparison. Addition of chicken cell supernatant prior to addition of guinea pig cell supernatant did not modify the rate of oxygen release, suggesting that no soluble inhibitors of catalase were present in chicken cells. Azide fully inhibited the oxygen release caused by guinea pig cell supernatant, but had no effect on the slight backward deflection observed after addition of KRP or chicken cell supernatant. This is a further proof that the effect seen after addition of chicken cell supernatant is not due to catalase but to oxygen addition into the electrode chamber.

Table 1 shows the results of the spectrophotometric assay of catalase in guinea pig and chicken cells. With both the whole lysate and the supernatant of chicken cells no catalase activity could be detected. Neither the whole lysate or the supernatant of chicken cells, affected the catalase activity of guinea pig cells, indicating again that chicken leucocytes do not contain inhibitors of catalase.

4. Discussion

Catalase activity could not be demonstrated in chicken polymorphonuclear leucocytes using two different assays. It seems unlikely that these cells contain an inhibitor of catalase, since the catalase assay in guinea pig polymorphonuclear leucocytes (which are rich in catalase) was not affected by addition to the assay medium of a chicken cell extract. One report in the literature indicates that chicken polymorphonuclear leucocytes do not produce hydrogen peroxide during phagocytosis [8]. According to our measurements however, these cells produce during phagocytosis about 35 nmol H_2O_2/2 min/2 · 10^7 cells (paper in preparation). Only one-tenth of this amount of H_2O_2 is released from the cell and is recovered in the incubation medium. This indicates that most of the H_2O_2 produced is dealt with by some intracellular mechanisms. Due to the absence of both myeloperoxidase and catalase, the two main H_2O_2-destroying systems, glutathione peroxidase appears as the only mechanism whereby chicken polymorphonuclear leucocytes can detoxify H_2O_2. This enzyme functions in connection with another enzyme, glutathione reductase, according to the following reactions:

$$H_2O_2 + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2H_2O + GSSG$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+$$

The activities of glutathione peroxidase and glutathione reductase in chicken leucocytes, as measured in our laboratory, are in terms of nmol/NADPH oxidized/min/10^7 cells 26.1 ± 4.35 SEM (6 determinations) and 54.3 ± 3.46 SEM (4 determinations), respectively. It is evident, therefore, that the level of both enzymes is adequate to dispose of the amount of H_2O_2 formed by chicken leucocytes during phagocytosis.

The $NADP^+$ generated by the glutathione cycle

Table 1
Spectrophotometric assay of catalase activity in guinea pig and chicken polymorphonuclear leucocytes

	Micromoles H_2O_2/min/10^6 cells			
	Whole lysate		Supernatant	
Guinea pig	13.5	(2)	10.7	(2)
Guinea pig + 1 mM azide	< 0.008	(2)	< 0.008	(2)
Chicken	< 0.008	(4)	< 0.008	(4)
Guinea pig + chicken	13.1	(2)	9.9	(2)

The assay medium contained 10 mM H_2O_2 in 3 ml Krebs-Ringer–phosphate, pH 7.4. The equivalent of 1.9 · 10^5 cells and 1.9 · 10^6 cells was used in each assay for guinea pig and chicken cells, respectively. Temperature 37°C.

results in a stimulation of the hexose monophosphate shunt. The shunt stimulation in chicken polymorphonuclear leucocytes during phagocytosis as compared to resting leucocytes has been reported to be about 20-fold [8]. This stimulation is much higher than that reported for leucocytes from mammalian species such as man [9] and guinea pig [9,10] which contain both catalase and myeloperoxidase. Furthermore the stimulated HMP activity exceeds the stimulated respiration in chicken leucocytes ([8], our manuscript in preparation) much more than in guinea pig or human cells [9,10]. This peculiarity of chicken polymorphs may well be explained by the channelling of hydrogen peroxide predominantly or exclusively through the glutathione cycle, due to the absence of both myeloperoxidase and catalase. The lack of catalase in chicken PMN could also explain the failure by other authors to detect H_2O_2 in these cells during phagocytosis employing the method of formate oxidation [8]. This method is based on the release of CO_2 from formate in the presence of H_2O_2 due to the peroxidatic activity of catalase. In absence of catalase therefore no appreciable oxidation of formate would be expected even if the cells are normal producers of H_2O_2.

Acknowledgements

Supported by grant No. 75.00695.04 of the National Research Council of Italy.

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