THE EFFECT OF H_2O_2 ON COLONY FORMATION AND ANAEROBIC CO_2 PRODUCTION BY YEAST

by

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INTRODUCTION

The production of H_2O_2 by irradiation of aqueous solutions has been demonstrated by RISSE[1], FRICKE[2, 3], and BONÉT-MAURY AND LEFORT[4]. EVANS[5] observed that some of the deleterious effects of x-radiation on *Arbacia* sperm could be duplicated by solutions of H_2O_2 of the same order of concentration as produced by x-radiation of sea water (100 000 r). On the other hand, BARRON, FLOOD, AND GASVODA[6] were unable to demonstrate the presence of H_2O_2 in sea water irradiated with x-rays (200 000 r). This result, together with the observation that catalase failed to protect sperm from the action of irradiated sea water, led these workers to postulate that inhibition of *Arbacia* sperm respiration is due to the action of stable organic peroxides which are produced by x-radiation.

SHERMAN AND CHASE[7] reported experiments which suggest that the primary effect of x-radiation on anaerobic CO_2 production by yeast is also due to irradiated water products. The production of H_2O_2 by ionizing radiations has been postulated by LOISELEUR, LATARJET, AND CAILLOT[8] and LOISELEUR AND LETARJET[9] as being the primary effect of irradiating aqueous solutions. In the present paper experiments are reported in which suspensions of yeast cells were incubated in aqueous solutions of H_2O_2 at concentrations approximating that of irradiated media (EVANS *et al.*[10], BONÉT-MAURY AND FRILLEY[11]) to determine what, if any, role H_2O_2 plays in the indirect action of x-rays on yeast cells. Evidence is presented which indicates that H_2O_2 at these concentrations inhibits anaerobic CO_2 production of yeast. However, the data on dilution effects suggest that H_2O_2 may not actually be the primary agent involved in x-irradiation of yeast cells suspended in $M/15$ KH_2PO_4. Under the conditions employed H_2O_2 has little or no effect on cell division in this strain of yeast.

PROCEDURE

The yeast used in these experiments was derived from the strain used in the previous experiments (SHERMAN AND CHASE[12]). It was cultured in READER's medium[13] supplemented with glucose and biotin as described previously[12], and in a nonsynthetic medium having the following composition: KH_2PO_4, 2 g; $MgSO_4$, 1 g; peptone, 5 g; 85% lactic acid, 3.8 ml; yeast extract (Difco), 0.1 g; glucose, 20 g; distilled H_2O, 1 liter. The nonsynthetic medium of NICKERSON AND CARROLL[14] was also employed. After a 48-hour

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incubation period at 30° C the yeast was harvested, washed and brought to standard density as previously described[12]. The cell suspension was dispensed into small erlenmeyer flasks, and H_2O or H_2O_2 added. After varying periods of incubation at 30° C, aliquots were removed for the determination of anaerobic CO_2 production and cell counts[12].

RESULTS

A. Anaerobic CO_2 production

Effect of H_2O_2 concentration on anaerobic CO_2 production. A measurable effect of H_2O_2 on anaerobic CO_2 production by yeast is produced by concentrations as low as $2 \cdot 10^{-6}$ g/ml. Concentrations of H_2O_2 of this order of magnitude have been shown to be produced by x-radiation of water[12]. Increasing the concentration to $4.1 \cdot 10^{-6}$ g/ml and $10.3 \cdot 10^{-6}$ g/ml reduced the amount of anaerobic CO_2 produced by yeast as shown in Fig. 1.

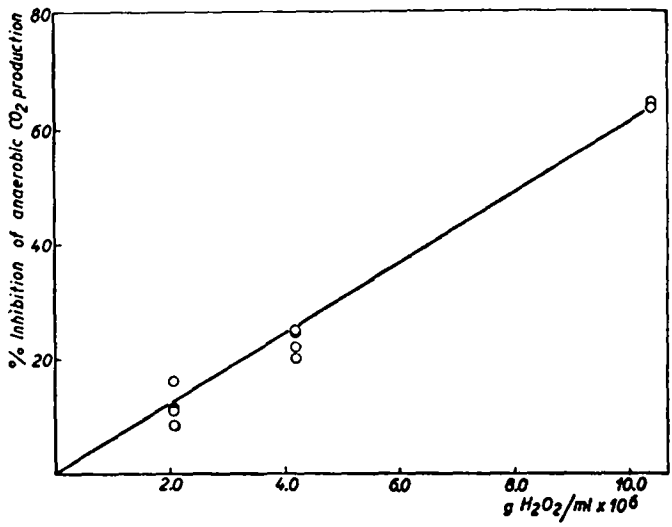

Fig. 1. Effect of H_2O_2 concentration on anaerobic CO_2 production. Incubated 4 hours at 30° C

This distribution suggests that the relationship between the concentration of H_2O_2 and inhibition of anaerobic CO_2 production may be linear.

Inhibition of anaerobic CO_2 production by H_2O_2 as a function of time. Hydrogen peroxide does not inhibit anaerobic CO_2 production in yeast immediately after its addition to the cell suspension. At least thirty minutes must elapse before H_2O_2 has an appreciable effect on anaerobic CO_2 production. Incubation periods of 30 and 60 minutes at 30° C resulted in intermediate effects (Fig. 2). At the end of the incubation period the vessel and contents were flushed with nitrogen while shaking in the 30° bath as previously described[12]. Glucose was tipped into the main compartment and the anaerobic CO_2 production followed for 90 minutes. In every instance the rate of CO_2 production remained constant throughout the observation period.

The maximum inhibitory effect of H_2O_2 appeared to be reached between three and four hours of incubation. These results confirm the observations of EVANS *et al.*[10] and EVANS[5] on the effect of H_2O_2 on *Arbacia* sperm.

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Density of Cell Suspension. Increasing the number of cells per ml appears to have no protective effect against the action of H_2O_2. If H_2O_2 is the primary inactivating agent in aqueous solutions subjected to large doses of x-radiation (LOISELEUR *et al.*[8]) these

Fig. 2. Inhibition of anaerobic CO_2 production by $4.1 \cdot 10^{-6}$ g H_2O_2/ml after various periods of incubation at 30° C. Glucose was added at the end of the incubation period.

results would not be expected in view of our earlier observations on the influence of dilution in irradiated yeast suspension (SHERMAN AND CHASE[7]). The fact that the effect of H_2O_2 on the anaerobic CO_2 production of yeast suspensions appears to be independent of the cell density suggests that H_2O_2 is not the primary agent in inhibiting fermentation in irradiated yeast suspensions.

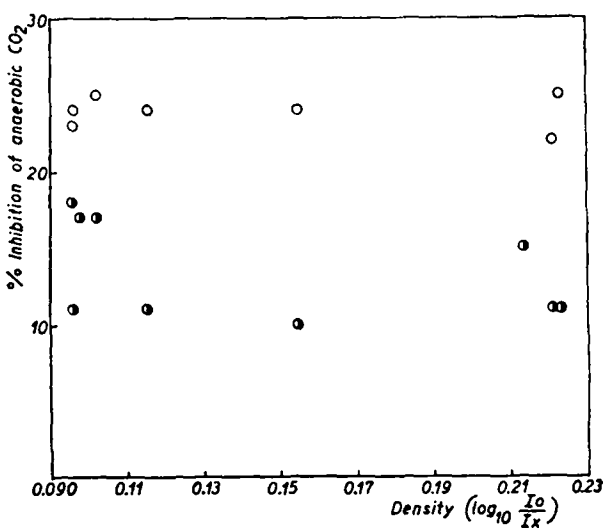

Fig. 3. Effect of cell density on inhibition of anaerobic CO_2 production by H_2O_2. Open circles, $4.1 \cdot 10^{-6}$ g H_2O_2/ml. Half solid circles $2.0 \cdot 10^{-6}$ g H_2O_2/ml

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Composition of the culture medium. In the experiments described above, the yeast cells were grown in supplemented READER's medium[13]. Hydrogen peroxide was found to be without measurable effect on the anaerobic CO_2 production of yeast grown in non-synthetic media containing peptone and yeast extract. These data are summarized in Table I. These results are similar to the earlier observations of SHERMAN AND CHASE[7].

TABLE I

PERCENT INHIBITION OF ANAEROBIC CO_2 BY H_2O_2 ($4.1 \cdot 10^{-6}$ g/ml) OF YEAST GROWN IN VARIOUS MEDIA

Experiment No.	Suppl. READER's	NICKERSON AND CARROLL*	Peptone-Yeast extract medium
1	24	0	5
2	25	2	3

* Medium devised by NICKERSON AND CARROLL[14]

They showed that the anaerobic CO_2 production of yeast grown in supplemented READER's medium was inhibited by the action of x-rays; whereas fermentation of glucose by the same yeast grown in a medium containing peptone and yeast extract was not inhibited by similar doses of x-rays.

B. The effect of H_2O_2 on colony formation.

Cell division by yeast to form new colonies was not markedly inhibited by H_2O_2 at the concentrations employed. The percentage inhibition of colony formation by H_2O_2 at various cell densities is shown in Fig. 4.

This distribution indicates that the effect of H_2O_2 is independent of the number of cells present in the range between $3 \cdot 10^6$ cells/ml and 10^7 cells/ml. The high values obtained at the lowest densities are probably not significant because of the relatively large

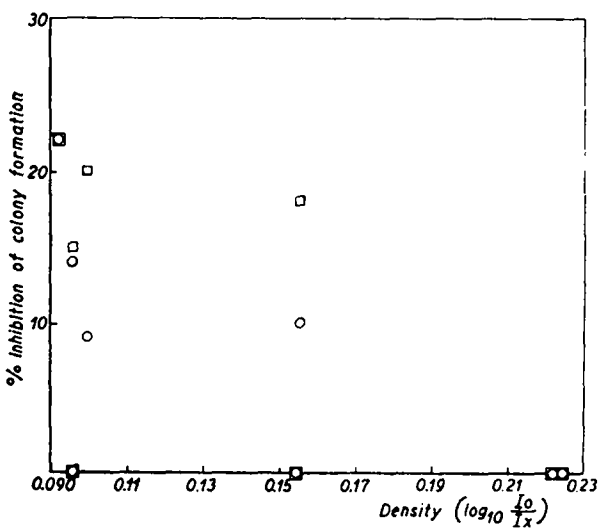

Fig. 4. Effect of cell density on inhibition of colony formation by H_2O_2. Circles, $4.1 \cdot 10^{-6}$ g H_2O_2/ml. Squares $2.0 \cdot 10^{-6}$ g H_2O_2/ml

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error inherent in the counting method. Moreover, the observation that $4.1 \cdot 10^{-6}$ g H_2O_2/ml has no greater effect than $2.0 \cdot 10^{-6}$ g H_2O_2/ml suggests that these concentrations of H_2O_2 have little if any effect on colony formation.

DISCUSSION

Increasing the concentration of H_2O_2 results in a corresponding decrease in the amount of anaerobic CO_2 produced by yeast. However, enlarging the number of sites to be inactivated (*i.e.*, increasing the number of cells per ml) by a factor of two or three apparently has no effect on the level of inhibition of anaerobic CO_2 production. This can be interpreted to mean that the loci at which H_2O_2 may react are, relative to the number of H_2O_2 molecules present, few in number. Comparing the effect of increasing the number of cells in the H_2O_2 experiments with the results obtained by increasing the cell density in the x-radiation experiments[7] suggests that H_2O_2 is not the primary agent in the production of "indirect" effects. This point of view is supported by the experiments of BARRON AND DICKMAN[15] which showed that only a partial reversal of irradiation induced inhibition of purified phosphoglyceraldehyde dehydrogenase could be effected by glutathione. DALE, GRAY, AND MEREDITH[16] have recently reported that purified carboxypeptidase was not inhibited by 0.1 *M* H_2O_2. During the irradiation of aqueous solutions with x-rays, "activated water" products are being produced and destroyed continuously (WEISS[17], [18], DAINTON[19], ALLEN[20]). The concentration at any moment may be small due to the short half life of most of these compounds. It appears probable that some of these products are more reactive than ordinary H_2O_2 for short periods. This relatively greater reactivity would have the effect of increasing the number of compounds to which energy could be transferred. In irradiated yeast suspensions then, increasing the number of sites (increase in cell density) at which reaction with activated water products could take place, would result in a proportionately much greater sumber of opportunities for highly reactive products to be captured.

Growing this yeast in a nonsynthetic medium containing large amounts of peptone and yeast extract appears to change the character of the yeast cell so as to protect the enzymes associated with fermentation from the action of H_2O_2. This is similar to the results obtained with x-radiation (SHERMAN AND CHASE[7], [12]). When this yeast was grown in supplemented READER's medium, yeast cells were produced which were sensitive both to the action of H_2O_2 and x-radiation. SHERMAN AND CHASE[7] have discussed some of the factors which may account for this difference in behaviour.

The small effect of H_2O_2 on colony formation by this yeast (Fig. 4) suggests that cell division mechanisms are not completely dependent upon fermentation processes as their source of energy. Cell division proceeds, apparently normally, in H_2O_2 treated cells in spite of a 20–25% reduction in glucose fermenting capacity.

SUMMARY

Under certain conditions of growth H_2O_2, at concentrations of the order of 2 to $4 \cdot 10^{-6}$ g/ml, inhibits the anaerobic CO_2 production of yeast. This inhibition by H_2O_2 is independent of the cell density. The fact that increasing the cell number does not confer protection against the action of H_2O_2 suggests that H_2O_2 may not be the only active agent in irradiated yeast suspensions. Finally, H_2O_2 at the concentrations employed has only a small effect on colony formation.

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RÉSUMÉ

Dans certaines conditions de culture, H_2O_2 à des concentrations de l'ordre de 2 à $4 \cdot 10^{-6}$ g/ml inhibe la production anaérobique de CO_2 par la levure étudiée. L'inhibition par H_2O_2 est indépendante du nombre de cellules. Le fait que l'augmentation du nombre de cellules ne donne pas de protection contre l'action de H_2O_2 fait penser que H_2O_2 n'est pas le seul agent actif dans les suspensions irradiées de la levure. De plus H_2O_2 à la concentration employée a seulement peu d'effet sur la formation des colonies.

ZUSAMMENFASSUNG

Unter gewissen Wachstumsbedingungen hemmt H_2O_2, in Konzentrationen von der Grössenordnung 2 bis $4 \cdot 10^{-6}$ g/ml. die anaerobe CO_2-Produktion der Hefe. Diese Hemmung durch H_2O_2 ist von der Zelldichte unabhängig. Die Tatsache dass eine vergrösserte Anzahl Zellen keinen Schutz gegen die Wirkung von H_2O_2 gewährt, lässt darauf schliessen, dass H_2O_2 nicht das einzige aktive Agens in bestrahlten Hefesuspensionen sei. Endlich hat H_2O_2 in den verwendeten Konzentrationen nur wenig Einfluss auf die Bildung von Kolonien.

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