Note

Antimicrobial and Respiration-inhibitory Activities of Oosporein

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In the course of our screening for bioactive metabolites, a filamentous fungus isolated from a soil sample collected at the campus of our university was found to produce large amounts of a red pigment having antimicrobial activity. The fungus was identified as *Chaetomium aureum* Chivers, and the pigment, formed approximately at 1 mg per 1 ml of the culture broth under the optimum conditions, was identical with oosporein (3,3',6,6'-tetrahydroxy-5,5'-dimethyl-2,2'-bi-*p*-benzoquinone). Oosporein was first isolated in 1944 from the culture broth of *Oospora colorans* van Beyma by Kögl and van Wessem.¹⁾ Since then, the compound has been reported to be produced by various fungi,²⁾ but its biological activities are still unknown.^{3,4)} In this paper, the correlation between the antimicrobial activity and the respiration-inhibitory activity of oosporein was investigated.

Antibacterial activity was determined by the broth dilution method using peptone medium after incubation at

37°C for 2 days, and antifungal activity using malt wort after incubation at 25°C for 2 days.⁵⁾ Oosporein exhibited a moderate antimicrobial activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus lysodeikticus*; the range of minimum inhibitory concentration (MIC) was 12.5~50 µg/ml (41~163 µM). Gram-negative bacteria, yeasts and molds were almost or totally insensitive. Interestingly, the growth of some yeasts and molds in malt wort adjusted to acidic pH tended to be inhibited by oosporein. As shown in Table I, the inhibition of fungi by oosporein at various pH values differed according to species. Among the yeasts tested, *Candida utilis* and *Rhodotorula rubra*, which were susceptible to oosporein at acidic pH, are known to assimilate maltose oxidatively but not to ferment it.⁶⁾ On the other hand, other insensitive yeasts, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Hansenula anomala*, are known to have both abilities to assimilate and ferment maltose. It is, therefore, of interest to investigate the effect of oosporein on the functions of oxidative metabolism and respiration in yeasts.

As shown in Fig. 1, when cell suspensions of *Candida utilis* were incubated with 1.31 mM oosporein at pH 3.3, the respiratory activity rapidly decreased; incubation periods resulting in 50 and 100% inhibition were 15 and 120 min, respectively. No inhibition by oosporein was observed at pH 7.0 during incubation within 120 min. This may indicate that the antifungal activity of oosporein at acidic pH occurs through the inhibitory action on mitochondria. Similar respiratory inhibition by oosporein was also observed in *Staphylococcus aureus* at pH 7.0 (data not shown).

To understand the biochemical aspects involved, the

TABLE I. EFFECT OF pH OF THE MEDIUM ON ANTIFUNGAL ACTIVITY OF OOSPOREIN

Microorganisms tested	MIC (µg/ml) ^a			
	pH ^b			
	6.5	4.5	3.6	2.8
<i>Schizosaccharomyces pombe</i> IFO 0342	>100	>100	>100	>100
<i>Saccharomyces cerevisiae</i> IFO 0203	>100	>100	100	100
<i>Hansenula anomala</i> IFO 0136	>100	>100	>100	100
<i>Candida utilis</i> ATCC 42402	>100	50	12.5	3.13
<i>Rhodotorula rubra</i> IFO 0001	>100	100	25	6.25
<i>Mucor mucedo</i> IFO 7684	>100	100	100	50
<i>Rhizopus chinensis</i> IFO 4745	>100	100	50	6.25
<i>Sclerotinia libertiana</i> Fuckel	>100	50	25	6.25
<i>Aspergillus niger</i> ATCC 6275	>100	>100	>100	>100
<i>Penicillium crustosum</i> Thom	>100	>100	>100	>100

^a Defined as the lowest concentration of oosporein at which there was no visible growth, and determined by a two-fold serial broth dilution method.⁵⁾

^b Adjusted with solutions of HCl and NaOH.

FIG. 1. Effect of Oosporein on Maintenance of Respiratory Activity in *Candida utilis* during Incubation at pH 3.3 and 7.0.

Candida utilis ATCC 42402 was cultured in 4% malt wort with shaking at 25°C for 20 hr. The cells were harvested and washed with distilled water by centrifugation. Then cells equivalent to 20 mg dry weight were suspended in 2 ml of 100 mM citrate- Na_2HPO_4 buffer (pH 3.3 or 7.0) containing 28 mM glucose, 10 mM KCl, 0.1 mM EDTA and 1.31 mM oosporein, and incubated with shaking at 25°C. At the intervals indicated on the abscissa, 0.2 ml of each incubation mixture was taken and poured into 1.8 ml of 100 mM citrate- Na_2HPO_4 buffer (pH 7.0) containing 10 mM KCl and 0.1 mM EDTA. Oxygen consumption in this mixture was measured at 25°C polarographically with a Yanagimoto PO-100A oxygen electrode.

○, without oosporein; ●, 1.31 mM oosporein; —, pH 3.3; ---, pH 7.0.

TABLE II. EFFECTS OF OOSPOREIN ON SOME ENERGY-LINKED FUNCTIONS IN MITOCHONDRIA ISOLATED FROM RAT LIVER

Rat liver mitochondria were prepared according to the method of Schneider and Hogeboom.⁷⁾ The reaction mixture of mitochondria contained 250 mM sucrose, 10 mM K_2HPO_4 , 10 mM Tris-HCl, 10 mM KCl, 2 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2 mM EDTA, 0.16~4.10 mM oosporein, mitochondria (equivalent to 4 mg mitochondrial protein) and 2 mM succinate as a respiratory substrate in a total volume of 2 ml at pH 7.4. During the course of the reaction, 0.2 μmol ADP was added to the mixture. Oxygen consumption was measured at 25°C polarographically with a Yanagimoto PO-100A oxygen electrode equipped with a YR-101P chart recorder. The measurement was repeated five times. Oxidative phosphorylation activity was calculated from the oxygen electrode tracing⁸⁾ and expressed as a respiratory control ratio and an ADP/O ratio. The phosphorylation rate was calculated as the ADP/O ratio $\times$ the rate of respiration in state 3.

Oosporein (mM)	Respiratory rate (nanoatoms O/mg protein/min)			Respiratory control ratio	ADP/O	Phosphorylation rate ^b
	State					
	3	4	4U ^a			
0	45.0±1.8 ^c	11.7±0.3 ^c	50.4±2.1 ^c	3.85	1.66±0.09 ^c	74.7
0.16	43.7±2.3	11.7±0.2	46.2±2.9	3.74	1.58±0.11	69.0
0.41	27.5±2.8	11.2±0.4	30.8±3.5	2.46	1.69±0.13	46.5
1.63	12.0±1.6	8.9±0.7	13.6±1.0	1.35	1.60±0.14	19.2
4.10	0	0	0	—	—	—

^a In the presence of 2,4-dinitrophenol at 50 μM .

^b Expressed as nanomoles ATP synthesized/mg protein/min.

^c Mean $\pm$ S.E.

effects of oosporein on some energy-linked functions in isolated mitochondria were investigated. Instead of yeast cells, rat liver was used a mitochondrial source because its mitochondria were relatively stable during the isolation procedures and possessed a higher activity of oxidative phosphorylation. As shown in Table II, oosporein at concentrations of 0.41 and 1.63 mM lowered the respiratory control ratio and phosphorylation rate but did not affect the ADP/O ratio. It significantly depressed the rate of respiration in state 3 and in 2,4-dinitrophenol uncoupled mitochondria (state 4U), though it hardly affected that in state 4. Thus, the compound seems to act neither as an uncoupler nor as a phosphorylation inhibitor, because the former stimulates the respiratory rate in states 3 and 4, while the latter inhibits only the respiratory rate in state 3 but shows no effect in state 4 and in state 4U. Oosporein seems to have an action on the respiratory chain enzymes, although detailed studies on the site of action are needed.

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