THE EFFECT OF FATTY ACID FRACTION FROM THE LIVER OF X-RAY IRRADIATED RABBITS ON THE RESPIRATION OF MICROORGANISM

A study on the respiration of Saccharomyces carlsbergensis

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X線照射家兎肝より抽出せる脂肪酸分画の Saccharomyces carlsbergensis の呼吸に及ぼす影響

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X線照射家兎肝より抽出した脂肪酸分画（OX）は Saccharomyces carlsbergensis の好気的並びに嫌気的呼吸において，glucose 存在下及び glucose-lecithin 存在下でその呼吸を阻害する．これらの作用を native な脂肪酸 linoleic acid と比較して実験を行つた．

Introduction

In the previous paper the auther reported[1] the effect of fatty acid fraction extracted from X-ray irradiated rabbits liver (OX)[2,3] on the proliferation of Saccharomyces carlsbergensis with the purpose to elucidate its biological action. The proliferation of the Saccharomyces is inhibited by addition of OX in the Sabouraud's medium containing glucose under the aerobic condition and is more strongly inhibited by OX in the medium containing glucose and lipid under the anaerobic condition. The lipid containing the double bond is required for the anaerobic proliferation of the Saccharomyces[1,4], but this proliferation is inhibited by addition of OX having some unsatuated fatty acids. This finding suggests that the OX acts as an inhibitor of the lipid metabolism of the Saccharomyces.

The effect of OX on the aerobic and anaerobic respirations of the Saccharomyces was further investigated and the results are described in this paper.

Materials and Methods

In order to obtain large amounts of the Saccharomyces, the cells were grown in 50 ml of Sabouraud's glucose broth in 100 ml Erlenmyer flask at 37° C. After one week culture the grown cells, in the state at the end of log-phase, were harvested by centrifugation.

Then, the cells were washed twice with distilled water and suspended in 0.05 *M* phosphate buffer (pH 7.4) for aerobiosis or washed with physiological saline solution and suspended in same solution for anaerobiosis. About 6—8 x 10^7 cells per ml were used for manometric examinations.

The aerobic and anaerobic respiration of the Saccharomyces were measured by the routine method with Warburg's apparatus, by using the following medium. For aerabic respiration, each vessel contained one ml of cell suspension in main chamber, 0.8 ml of 0.05 *M* phosphate buffer (pH 7.4), 0.3 ml of 1—0.05% glucose (replaced with distilled water in control system) in side arm, 0.3 ml of 1—0.05% fatty acids and 0.3 ml of 1—0.05% OX (all substances were replaced with distilled water in control system) in side arm, 0.3 ml of 20% potassium hydroxide in the center well. Total volume was 3.0 ml and incubated for 30 to 60 minutes at 37° C with air environment. The added substrates and OX were indicated in tables as final concentration. For anaerobic respiration, each vessel contained 1.2 ml of the cell suspension and 0. 6 ml of phosphate buffer (pH 7.4) in main chamber, 0.3 ml of 1—0.05% glucose, 0.3 ml of 1—0.05% fatty acid and 0.3 ml of 1—0.05% OX (all substances were replaced with physiological saline solution in control system) in side arm a, 0.1 ml of 5 *N* sulfuric acid in side arm b, 0.2 ml of distilled water in center well. Total volume was 3.0 ml and incubated for 60 minutes at 38° C with nitrogen gas environment. All of these reagents were prepared with physiological saline solution. OX (OXIII No. 11228) used in this experiment was donated by Toshiba Seiyaku Company.

Results

I. *The oxygen consumption of the Saccharomyces*:

Oxygen consumption of the Saccharomyces ($6\text{-}8\times10^7$ cells) was increased proportionally to the increment of glucose concentration till 0.05% as shown in Table 1. Thus the 0.05% glucose was used in each experiment as a standard respiratory substrate.

The effect of lecithin: Oxygen consumption of the cells in the presence of glucose is more than that in the presence of lecithin, even though the oxygen uptake of the cells is increased by addition of lecithin comparing with endogenous respiration. In this case, the increment of oxygen consumption of the cells is proportional to the amount of added lecithin, but the consumed oxygen for 60 minutes in the presence of 0.05% lecithin is about half of that in the presence of 0.05% glucose (Table 2). The oxygen consumption in the presence of 0.05% glucose is more increased by addition of lecithin.

Table 1. Oxygen uptake of Saccharomyces carlsbergensis in the presence of glucose

Substrate	Oxygen uptake (μl/30 min.)
None	21.5
0.005% glucose	40.8
0.01% glucose	64.5
0.05% glucose	90.1
0.1% glucose	91.8

Table 2. Oxygen uptake of Saccharomyces carlsbergensis in the presence of lecithin and glucose

Substrate	Oxygen uptake (μl/60 min.)
None	29.0
0.005% lecithin	32.4
0.01% lecithin	33.1
0.05% lecithin	39.9
0.1% lecithin	46.5
0.05% glucose	88.6
0.05% glucose + 0.005% lecithin	87.4
0.05% glucose + 0.01% lecithin	95.0
0.05% glucose + 0.05% lecithin	95.4
0.05% glucose + 0.1% lecithin	103.8

The effect of OX on the endogenous and glucose substrate respiration: The respiration of the cells is accelerated by addition of OX in the concentration of 0.005–0.05% but no difference is observed in the concentration of 0.1% OX. In the presence of glucose the respiration of the cells is increased in low concentration of OX (0.005%) but is inhibited remarkably in the high concentration (0.01–0.1%). In this case the inhibition is proportional to the concentration of OX (Table 3).

Table 3. The effect of OX on the endogenous and glucose substrate respiration of Saccharomyces carlsbergensis. Concentration of glucose is 0.05% and incubation time is 60 minutes.

Substrate	Oxygen uptake (μl)
None	12.2
0.005% OX	24.1
0.01% OX	25.1
0.05% OX	25.9
0.1% OX	13.3
glucose	61.0
glucose + 0.005% OX	71.0
glucose + 0.01% OX	52.3
glucose + 0.05% OX	41.0
glucose + 0.1% OX	29.2

The effect of OX on the respiration of the cells in the presence of lecithin and glucose: In the presence of 0.05% lecithin the respiration of the cells is accelerated by low concentration of OX (0.005–0.05%), but rather inhibited by high concentration (0.1%). In the presence of 0.05% lecithin and 0.05% glucose the respiration of the cells is inhibited by adding OX of 0.05% and 0.1% (Table 4).

The rate of oxygen consumption in the presence of various substrate or OX: The ratio of oxygen consumption was calculated by the percentage of consumed oxygen in the presence of various substrates or OX in same concentration against to that in the presence of glucose and results are shown in Table 5. The ratio is larger at the presence of substrate in the following order of glucose, glucose-lecithin, glucose-lecithin-OX, glucose-OX, lecithin and OX.

Table 4. The effect of OX on the oxygen uptake of Saccharomyces carlsbergensis in the presence of glucose and lecithin as substrate. Concentrations of glucose and lecithin are 0.05% respectively and incubation time is 60 minutes.

Substrate	Oxygen uptake (μl)
None	17.1
Lecithin	23.2
Lecithin + 0.005% OX	32.0
Lecithin + 0.01% OX	33.2
Lecithin + 0.05% OX	26.9
Lecithin + 0.1% OX	18.2
Glucose	95.2
Glucose + lecithin + 0.005% OX	112.0
Glucose + lecithin + 0.01% OX	113.8
Glucose + lecithin + 0.05% OX	70.9
Glucose + lecithin + 0.1% OX	66.3

Table 5. The rate of oxygen uptake of the respiration of Saccharomyces carlsbergensis in the presence of OX, glucose and lecithin in the same concentration. Concentration of each substance is 0.05% and incubation time is 60 minutes.

Substrate	Oxygen uptake (μl)	Ratio of oxygen uptake
None	35.3	28
Glucose	124.2	100
Lecithin	52.3	42
OX	44.8	36
Glucose + lecithin	123.1	99
Glucose + OX	76.2	61
Lecithin + OX	58.8	47
Glucose + lecithin +OX	92.2	80

Namely, the oxygen consumption was increased slightly (8%) by the addition of OX, 14% by lecithin and 19% by lecithin-OX more than that of endogenous respiration. On the other hand when the glucose was present the oxygen uptake was decreased 39% by addition of OX, unchanged by lecithin and decreased 20% by lecithin and OX together as compared with the respiration of the cells in the presence of glucose only.

The effect of linoleic acid: Linoleic acid, one of the components of OX, acts a stimulating substance for the proliferation of the cells as well as lecithin indicating in this paper (discussion) and the endogenous respiration of the cells is also increased by linoleic acid in the concentration of 0.05%. The increased respiration of the cells by linoleic acid can be seen in the presence of lecithin as well as that by OX, but the decreased respirations by linoleic acid in the presence of glucose and glucose-lecithin were slight compared to that by OX (Table 6).

II. *The carbon dioxide production of the Saccharomyces* :

The effect of OX: The endogenous carbon dioxide production oft he cells is very small in quantity. This carbon dioxide production is inhibited completely by addition of OX but accelerated by lecithin. As in the case of aerobic respiration, the carbon dioxide production of the cells is strongly accelerated in quantity by the addition of glucose. This increased

Table 6. The effect of linoleic acid on the oxygen uptake of Saccharomyces carlsbergensis. Concentration of each substance is 0.05% and incubation time is 60 minutes.

Substrate	Oxygen uptake (μl)	Ratio of oxygen uptake
None	25.4	29
Glucose	88.0	100
Lecithin	35.2	40
Linoleic acid	31.1	35
Glucose + lecithin	94.0	107
Glucose + linoleic acid	86.0	97
Lecithin + linoleic acid	36.1	41
Glucose + lecithin + linoleic acid	92.0	105

Table 7. The effect of OX on the carbon dioxide production of Saccharomyces carlsbergensis. Concentration of glucose is 0.02 *M* and that of lecithin and OX are 0.05% respectively. Incubated for 60 minutes and details are described in text.

Substrate	Carbon dioxide production (μl)	Ratio of carbon dioxide production
None	3.2	1
Glucose	225	100
Lecithin	18.3	8
OX	0	0
Glucose + lecithin	222	99
Glucose + OX	116	52
Lecithin + OX	4.0	2
Glucose + lecithin + OX	124	55

Table 8. The effect of linoleic acid on the carbon dioxide production of Saccharomyces carlsbergensis. Concentration of glucose is 0.02 *M* and that of lecithin and linoleic acid are 0.05% respectively. Incubated for 60 minutes and details are described in text.

Substrate	Carbon dioxide production (μl)	Ratio of carbon dioxide production
None	9	6
Glucose	143	100
Lecithin	15	11
Linoleic acid	0	0
Glucose + lecithin	144	100
Glucose + linoleic acid	112	79
Lecithin + linoleic acid	14	9
Glucose + lecithin + linoleic acid	129	97

carbon dioxide production is inhibited to the value of 48% by the added OX but is not changed by the added lecithin. The increased carbon dioxide productions of the cells in the presence of lecithin and glucose-lecithin are inhibited more than half by addition of OX (Table 7).

The effect of linoleic acid: To compare the action of OX with that of native fatty acid,

Table 9. The effects of OX (OXIII No. 11228) and linoleic acid on the proliferation of Saccharomyces carlsbergensis in anaerobic condition in the presence of glucose and lecithin. Concentration of glucose is 2% and that of lecithin, linoleic acid and OX are 0.05% respectively in final. After one week of incubation at 37°C optical density was measured by the method described in the previous paper[1].

Substrate	Optical density at 490 mμ	Ratio of proliferation
Control (glucose in aerobiosis)	0.337	
Glucose	0.071	100
Glucose + lecithin	0.194	290
Glucose + linoleic acid	0.167	250
Glucose + OX	0.108	160
Glucose + lecithin + linoleic acid	0.208	310
Glucose + lecithin + OX	0.167	250

the effect of linoleic acid on the carbon dioxide production of the cells was examined. The endogenous carbon dioxide productions is completely inhibited by the addition of linoleic acid, but the increased carbon dioxide production in the presence of glucose, lecithin and glucose-lecithin are slightly inhibited compared to that of the presence of OX (Table 8).

These biological actions of OX and linoleic acid on the carbon dioxide production are completely similar to that of aerobic respiration of the Saccharomyces.

Discussion

On the anaerobic nutrition of yeast, Andreasen and Steir[5] have made the observation relating to the biosynthesis of unsaturated fatty acids, and have shown that under strict anaerobic conditions, yeast becomes dependent on two types of lipieds as growth factors. One of them is ergosterol or a related sterol, and the other is an unsaturated fatty acid such as linoleate. Such tendency is observed in this laboratory as shown in previous paper[1] and in Table 9 of this paper. Namely, Saccharomyces carlsbergensis, in the case of the strain which was used in these experiments, does not grow in the mere glucose medium, but proliferates enough in the medium added lecithin or linoleic acid in anaerobiosis. OX (OXIII No. 11228), fatty acid extracted from the irradiated rabbits liver, is little utilized as substrate in anaerobiosis, and inhibits the proliferation even under the presence of lecithin. On the other hand linoleic acid accelerates more the lecithin induced proliferation. Therefore OX has different effect from that of lecithin and of linoleic acid on the proliferation of the Saccharomyces in anaerobiosis.

In Saccharomyces carlsbergensis, reported by Bloomfield and Bloch[4,6] molecular oxygen is the obligatory electron accepter in several intermediate steps of lipid biosynthesis[4], and both molecular oxygen and NADPH are essential for the desaturation of long chain fatty acids[6]. For these reasons to study the oxygen consumption of the Saccharomyces is interesting to cralify the mechanism of inhibitory property of OX. As a result, in the presence of glucose and of glucose-lecithin, the oxygen uptake and carbon dioxide production are

inhibited by the addition of OX (Tables 5 & 7), but no inhibition is observed by the addition of linoleic acid as shown in Tables 6 & 8. This finding is coincide with the result obtained by the observation of the cell proliferation affected by OX.

Recently the auther reported[7] that the fatty acid fraction of X-ray irradiated rabbits liver has more unsaturated fatty acid and has less saturated fatty acid compared with the fatty acid fraction of non-irradiated one, and that the former one has a more potent uncoupling action than the latter. Inaba[8] has noted that an OX uncouples the oxidative phosphorylation of cancer cells. And fatty acid contained carbon chain more than ten has a character as an uncoupling agent to oxidative phosphorylation[9),10,11,12]. Futhermore Inaba[13] has found out an uncoupling factor of the oxidative phosphorylation with X-ray irradiation in the lipid fraction of ascites fluid of Ehrlich ascites tumor bearing mouse. These reports may suggest that the increased oxygen uptake observed in addition of OX is respiratory release for uncoupling of the endogenous respiration. The decreased oxygen uptake affected by OX was observed in the presence of glucose, but did not in the presence of lecithin under aerobic condition. However OX inhibits the oxygen uptake in the presence of glucose-lecithin. From these results, it is suggested that the action of OX is correlated to glucose metabolism with regard to electron transfer. In this experiment under anaerobic condition, the carbon dioxide production in the presence of lecithin is low and is accelerated by the addition of glucose. With respect to this result the accelerated proliferation is observed in the presence of glucose and lecithin, but anaerobiosis is not found in the presence of mere lecithin, then this also suggests that the metabolism of lecithin is related with the glucose metabolism in anaerobiosis of this Saccharomyces. On the other hand under anaerobic condition, in the presence of glucose the carbon dioxide production was observed, as it may be due to the existence of soluble oxygen or the presence of endogenous lipids. In this case, OX inhibits the respiration and futher inhibits the carbon dioxide production in the presence of lecithin and of lecithin-glucose.

In any event the properties of OX are differ from the native fatty acid and it is clear that OX inhibits the lipid and glucose metabolism of the Saccharomyces. F. Windish[14,15,16] has observed that Saccharomyces carlsbergensis can proliferate under anaerobic condition in the medium containing a cancer extract probably based unsaturated fatty acid. This extract may contain the sterol substances supporting the hypothesis of Andreasen[5], but in this experiment the sterol or related substance is not included in OX substance

Thus the result obtained by this experiment suggests that the OX substance is different from the native fatty acid with regard to the action on growth or respiration of the Saccharomyces. OX substance inhibits the glucose metabolism which is correlated to lipid metabolism requiring the molecular oxygen and NADPH for the desaturation of long chain fatty acid, though the mechanism of the relation between the glucose and lipid metabolism is obscure.

Sammary

1. The effects of fatty acid fraction (OX) extracted from the liver of X-ray irradiated

rabbits, lecithin, linoleic acid and glucose on the aerobic and the anaerobic respirations of Saccharomyces carlsbergensis were examined, and the findings were as follows.

2. On aerobiosis, OX, lecithin, linoleic acid and glucose were utilized as substrate of respiration of the cells, and the utilization of glucose was the most effective.

3. On anaerobiosis, glucose and lecithin were utilized as substrate, and carbon dioxide production was observed by the cells, though in the case of OX and linoleic acid it was not observed.

4. The respirations of the cells in the presence of glucose as subatrate on aerobiosis and anaerobiosis were increased by addition of lecithin and linoleic acid, but were inhibited by addition of OX.

5. On aerobiosis and anaerobiosis OX had the inhibitory action on the respiration of the cells in the presence of glucose and lecithin as substrates, while no inhibitory action was observed by linoleic acid.

6. From these results it is clear that OX has the different action from linoleic acid on the metabolism of the Saccharomyces. Also it is shown that for the proliferation and the respiration of the Saccharomyces glucose and fatty acid have very close correlation.

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