Experiments on the Metabolism of the Myxomycete Amoeba

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The metabolism of the plasmodium derived from the zygote of a Myxomycete, *Physarum polycephalum*, was reported by Allen & Price[1] and Ohta.[2,3] However, the metabolism of the myxamoeba, the gamete of this organism, has not been investigated. Culture methods for the myxamoebae were recently reported by Dee,[5,7] we have used a slight modification of this method to culture the amoebae in large quantity. The present paper reports some information on the metabolism of these myxamoebae.

Material and Methods

Material

The myxamoebae used in this work were from the strain (strain J) which emerged from a single spore and has been cultured in our laboratory since 1969.

Stock cultures of the myxamoebae were routinly maintained in two-membered culture with a pure strain of bacteria according to the method of Dee[4,5,6] except for the following points. A pure strain of *Aerobacter aerogenes* was used as food for the amoebae in place of *Escherichia coli*. The basic medium for the two-membered culture therefore was not SAL (Dee's agar medium), but an agar medium containing 20 g agar, 5.00 g glucose, 0.50 g yeast extract, 5.00 g bacto-peptone, 2.25 g KH_2PO_4, 1.50 g $K_2H_2PO_4$ and 0.50 g $MgSO_4 \cdot 7H_2O$ in 1 *l* of distilled water. After the amoebae were transplanted onto the bacteria grown on the agar in petri dishes, the cultures were incubated at 24°C under a humid and dark condition.

The myxamoebae from a 6–7 day-old culture were collected by scraping with glass rods, and freed from bacteria by repeated (four times) centrifugation with salt solution containing 10 mM KCl, 10 mM NaCl and 3 mM $CaCl_2$.

Measurements of O_2 consumption and CO_2 production

The isolated myxamoebae were diluted with a suitable amount of the salt solution described above. This suspension was then transferred into the manometer vessels. In general, these suspensions in the vessels were buffered at pH 6.0 with 1/10 M to 1/30 M phosphate buffer. O_2 consumption and CO_2 production of the myxamoebae were measured by a Warburg manometer. The temperature was maintained at 25.00 ± 0.05°C during these experiments. O_2 uptake was measured by using 15% KOH, but in the presence of cyanide, Krebs' KCN-KOH mixture was used. In order to set up the anaerobic conditions, the gas phase was replaced by 100% N_2 or 95% N_2+5% CO_2. After these measurements, the suspensions in the vessels were transferred into weighing bottles and lyophilized, and the dry weights of the myxamoebae were determined.

Absorption spectra

The isolated myxamoebae were homogenized with a glass-homogenizer or sonicated. Cytochromes of the myxamoebae were detected by using the spectrophotometer (Hitachi 124) on these samples.

Results

1. Preliminary experiments

To investigate whether or not the amount of *Aerobacter aerogenes* included in the myxamoebae suspension had any influence on the respiratory rate of the myxamoebae, the suspensions were centrifuged 1–5 times. Microscopic observation after each centrifugation showed that the amount of bacteria diminished markedly after the second centrifugation. The effect on O_2 consumption of added glucose was investigated with the same samples. The results in Table 1 show that small amounts of the bacteria had no influence on the respiratory rate of the suspension. As the myxamoebae were used in this work after being centrifuged four times, *Aerobacter aerogenes* would have no influence on the measurable respiratory rate of the suspension.

Table 1. Effect of centrifugation.

	Times of centrifugation				
	1	2	3	4	5
Exp. I	319.1%	139.2	140.4	134.6	134.9
Exp. II	288.2	174.5	165.5	177.6	163.4

O_2 uptake of myxamoebae, as percentage of the control rate before addition of glucose ($3.3 \times 10^{1-3}$ M).

The myxamoebae become encysted under certain conditions such as dryness. The number of encysted myxamoebae in the isolated myxamoebae culture varied with the period of incubation; before 1 week of incubation very few encysted amoebae were found, but thereafter their number increased gradually. To investigate the influence of the encysted myxamoebae on the respiratory rate of the amoebae suspension, the Qo_2 value of myxamoebae cultures which included various proportions of encysted amoebae was measured, as shown in Fig. 1. The fact that the Qo_2 value decreased with increase in the proportion of encysted myxamoebae indicates that the Qo_2 value of the encysted amoebae is very low. In these experiments, therefore, the 6–7 day cultures including very small amounts of encysted myxamoebae were used, and it was assumed that the Qo_2 value of these cultures is approximately equal to the Qo_2 value of the motile myxamoebae.

2. General features of the respiration of the myxamoebae

1) Qo_2 value

The Qo_2 value, measured on the cultures before 6–7 days of incubation, was found to be about 1.00–1.10 (Fig. 1). The respiratory rate of the myxamoebae was almost constant during 4-hour measurements.

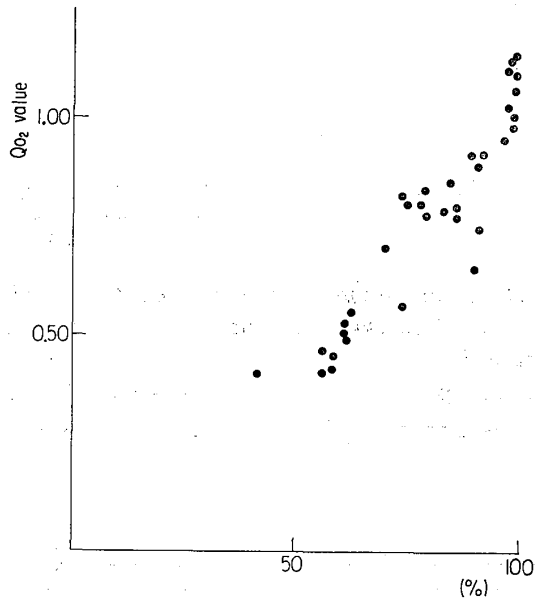

Fig. 1. Relation between the Qo_2 value and the proportion of encysted myxamoebae. Abscissa: percentage of non-encysted myxamoebae. Ordinate: Qo_2 value.

2) R.Q. value

The R.Q. value was measured at pH 5.0, maintained with 1/30 M citrate phosphate buffer, because CO_2 is apt to stay in the solution at pH's above 5.0. The R.Q. value was almost constant regardless of the

amount of encysted myxamoebae. The average R.Q. value at pH 5.0 was 0.99.

3. Effects of pH

The respiratory rate of the myxamoebae showed a maximum at pH 6.0 with either 1/30 M phosphate buffer or 1/30 M citrate phosphate buffer (Fig. 2). It was almost constant over the pH range from 4.0–8.0, and at pH 3.0 was about one-half that at pH 6.0. The respiratory rate of the myxamoebae in citrate phosphate buffer was a little higher than that in phosphate buffer. At pH 6.0, the concentration of the buffer had no effect on the respiratory rate (Table 2).

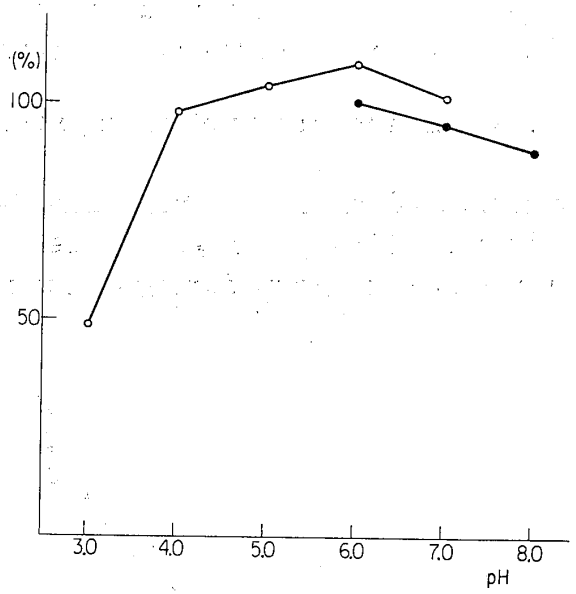

Fig. 2. Relation between the pH of the medium and the respiratory rate. Abscissa: pH of the medium. Ordinate: the respiratory rate in per cent of the control rate at pH 6.0 in a medium of 1/30 M phosphate buffer. ○——○, 1/30 M citrate phosphate buffer and ●——●, 1/30 M phosphate buffer.

Table 2. Effect of buffer concentration (pH 6.0).

	Concentration of buffer				
	A	B	C	D	E
Phosphate buffer	112.5%	110.0	108.5	100.0	118.0
Citrate phosphate buffer	——	——	107.0	100.0	90.7

The respiratory rate in per cent of the control rate (in 1/30 M buffer).
A: 1/4×D (8.3×10^{-3} M), B: 1/3×D (1.1×10^{-2} M), C: 1/2×D (1.7×10^{-2} M), D: 3.3×10^{-2} (1/30) M, E: 2×D (6.7×10^{-2} M).

4. Effects of extracellular substrates

In order to obtain some information concerning the individual enzyme system in these myxamoebae, the effects of added substrates were investigated (Table 3). O_2 uptake before and after the addition of substrates was compared. A marked increase in the respiratory rate was observed with the addition of extracellular substrates such as glucose, pyruvic acid, succinic acid, malic acid, lactic acid, acetic acid, serine and glutamic acid. A sparking effect of pyruvic acid and succinic acid was also found.

Table 3. Effects of extracellular substrates on respiration.

Added substrate	Concentration	Respiration
Glucose	3.3×10^{-3} M	160–220%
Pyruvic acid	3.3×10^{-3}	175–185
Citric acid	3.3×10^{-2}	25–30
Citric acid	3.3×10^{-3}	80–95
Citric acid	1.7×10^{-3}	98–102
Sodium citrate	3.3×10^{-3}	97–103
Succinic acid	3.3×10^{-3}	170–190
Sodium succinate	3.3×10^{-3}	128–131
Malic acid	3.3×10^{-3}	140–175
Lactic acid	3.3×10^{-3}	120–140
Sodium acetate	3.3×10^{-3}	135–145
Serine	3.3×10^{-3}	145–155
Glysine	3.3×10^{-3}	98–99
Sodium glutamate	3.3×10^{-3}	142–148
Sodium propionate	3.3×10^{-3}	101–102

O_2 uptake of myxamoebae, as percentage of the control rate before addition of substrate.

5. Effects of inhibitors

The respiration of the myxamoebae was inhibited by some respiratory poisons.

1) Monoiodoacetate

The respiratory rate of these myxamoebae was considerably inhibited by monoiodoacetate at concentrations above 4×10^{-3} M. At low concentrations of this inhibitor, below 1.5×10^{-3} M, a slight increase in O_2 uptake was observed (Fig. 3).

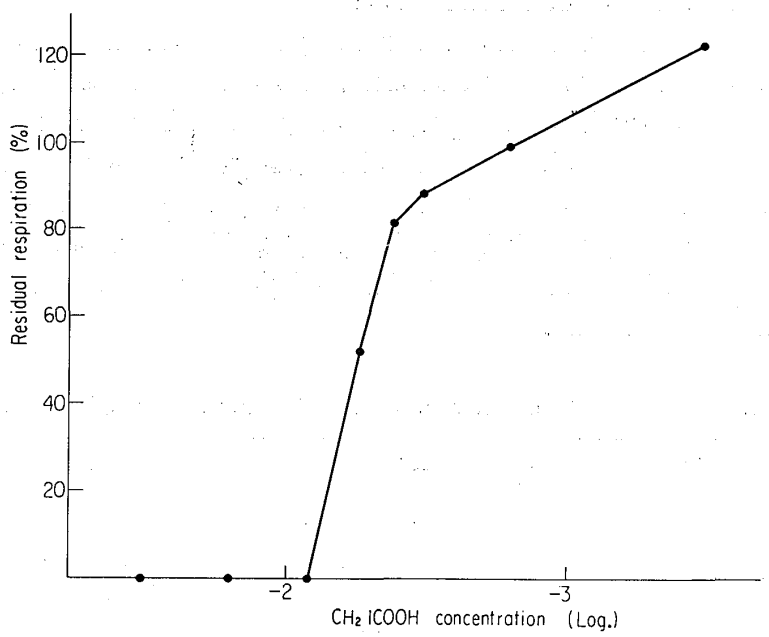

Fig. 3. Effect of monoiodoacetate on respiration of myxamoebae. Abscissa: concentration of monoiodoacetate (Log.). Ordinate: residual respiration of myxamoebae, as percentage of the control rate before addition of monoiodoacetate.

2) Sodium azide

Sodium azide also inhibited the respiration (Fig. 4).

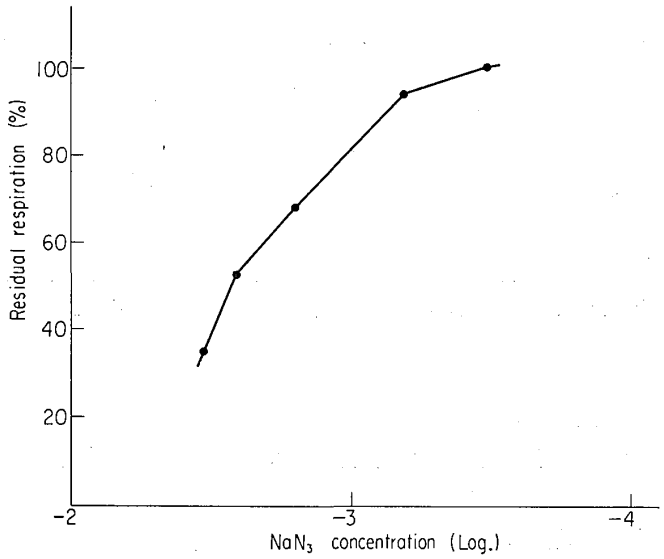

Fig. 4. Effect of sodium azide on respiration of myxamoebae. Abscissa: concentration of sodium azide (Log.). Ordinate: residual respiration of myxamoebae, as percentage of the control rate before addition of sodium azide.

3) 2, 4-dinitrophenol

The respiratory rate was inhibited at concentrations of 2, 4-dinitrophenol above 5×10^{-4} M. However, a considerable increase in O_2 uptake was observed at low concentrations of this inhibitor (Fig. 5).

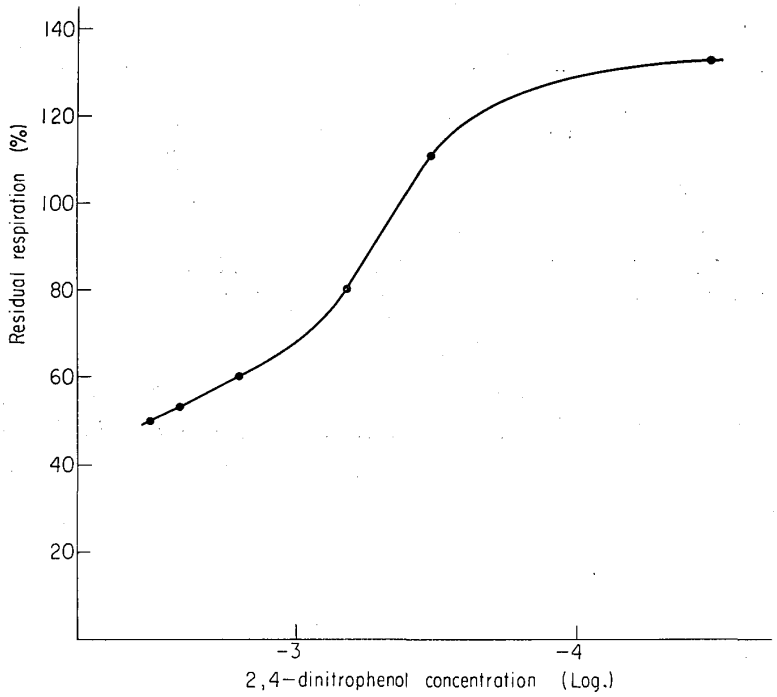

Fig. 5. Effect of 2, 4-dinitrophenol on respiration of myxamoebae. Abscissa: concentration of 2, 4-dinitrophenol. Ordinate: residual respiration of myxamoebae, as percentage of the control rate before addition of 2, 4-dinitrophenol.

4) Cyanide

Inhibition was observed with this substance at pH 8.0. The respiration was very sensitive to cyanide as shown in Table 4, but there remained some residual respiration at high concentrations, even at 2×10^{-2} M.

Table 4. Effect of cyanide on respiration of myxamoebae.

	Concentration of KNC		
	2×10^{-2}	10^{-2}	10^{-3}
Residual O_2 consumption	30.5%	40.3	58.1

Residual respiration of myxamoebae, as percentage of the control before addition of KCN.
The suspension was buffered at pH 8.0 with 1/10 M phosphate buffer.

5) Other inhibitors

A slight decrease in the respiratory rate was observed in the presence of high concentrations of sodium fluoride. Malonate, regulated at pH 6.0 with 1/10 M phosphate buffer at pH 8.0, inhibited slightly.

6. Anaerobic metabolism

1) The value of $Q^{N_2}_{CO_2}$

Amounts of anaerobic CO_2 production were small, and the difference between the value of $Q^{N_2}_{CO_2}$ in 100% N_2 and that in 95% N_2+ 5% CO_2 was slight. The $Q^{N_2+CO_2}_{CO_2}$ averaged 0.014. This value was only about one-hundredth of that of aerobic CO_2 production.

2) Production of acids

No acid formation was observed with 7.1×10^{-3} M $NaHCO_3$ in 1/30 M phosphate buffer (pH 7.0) under 95% N_2+ 5% CO_2.

7. Detection of cytochrome system

Hydrosulfite was added to either homogenized or sonicated myxamoebae. The absorption spectra of the samples (Figs. 6 and 7) show

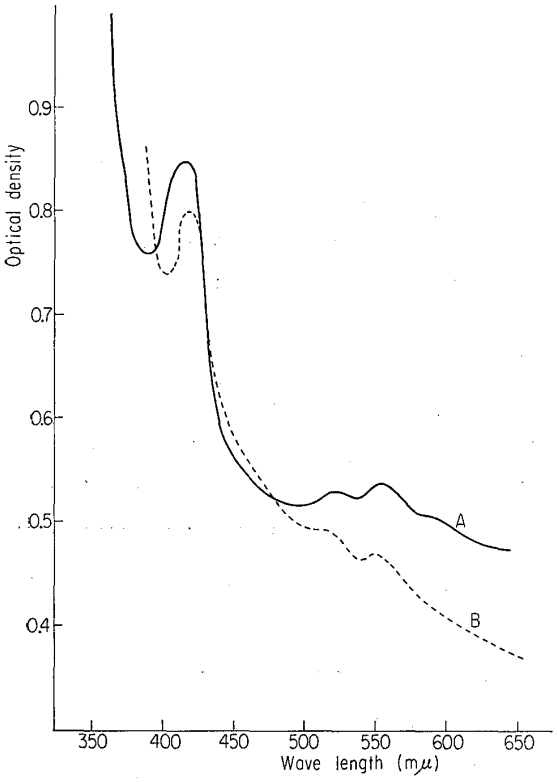

Fig. 6. Absorption spectra of homogenized myxamoebae. Abscissa: wave length (mμ). Ordinate: optical density. A: homogenized myxamoebae. B: homogenized myxamoebae with added hydrosulfite.

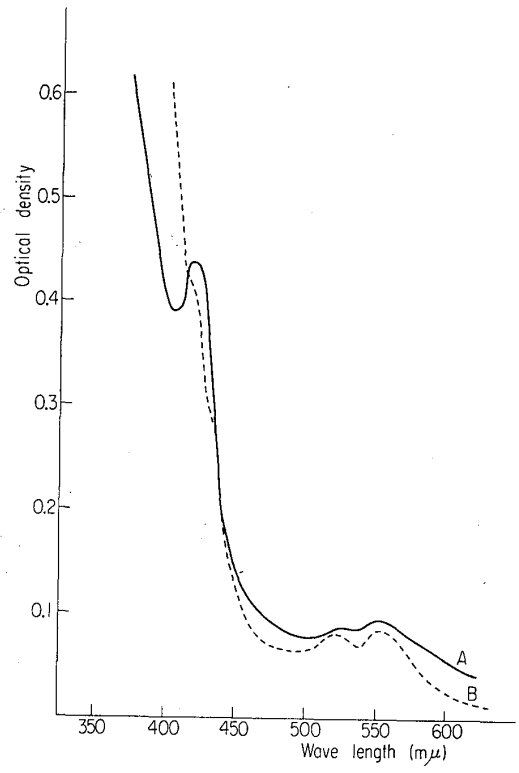

Fig. 7. Absorption spectra of sonicated myxamoebae. Abscissa: wave length (mμ). Ordinate: optical density. A: sonicated myxamoebae. B: sonicated myxamoebae with added hydrosulfite.

two peaks at about 500–550 mμ and one peak at about 400–430 mμ. The former are ascribed to the α-band and β-band of cytochromes, and the latter is ascribed to their soret-band. As reported above, cyanide and azide inhibited the respiratory rate of the myxamoebae to a considerable extent. The further finding that cytochromes are present makes it likely that a cytochrome system participates in the oxidative process of this organism.

Discussion

In these experiments, the respiratory rate of this myxamoebae was found to increase with the addition of extracellular substrates, such as glucose, pyruvic acid, succinic acid, malic acid, lactic acid, acetic acid, serine and glutamic acid. Moreover, this rate was strongly inhibited by various metabolic poisons, such as cyanide, sodium azide, monoioidoacetate and 2, 4-dinitrophenol, and was slightly inhibited by sodium fluoride and malonic acid. Spectrophotoscopically, the presence of cytochromes was found. From these results, the main metabolic pathway of the myxamoebae seems to be as follows: Glucose is decomposed to pyruvic acid through a series of glycolytic enzymes, then pyruvic acid goes into the TCA cycle and is oxidatively decomposed to carbon dioxide with the aid of a cytochrome system. In addition to these results, the optimum pH, Qo_2 value and R.Q. value on the respiration of the myxamoebae were found to be similar to those of the plasmodium[2]. All these facts suggest that the metabolic system in this myxamoebae resembles that of the plasmodium.

However, a difference between the two stages was found in that no acids were produced by the myxamoebae under anaerobic conditions, although a considerable amount of acids is produced by the plasmodium[2]. This result suggests that a fermentation with accompanying acid formation takes place in the plasmodium, but not in the myxamoebae.

The myxamoebae show amoeboid movements on a solid substratum. The problem concerning the connection between such movements and the metabolism of the organism remains to be investigated in the future.

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Summary

The metabolism of the myxamoebae, the gamete of the Myxomycete *Physarum polycephalum*, was investigated by using a Warburg manometer. The following results were obtained.

1. The Qo_2 value of the myxamoebae on a dry weight basis was about 1.00–1.10 in a medium of 1/30 M phosphate buffer at pH 6.0. The Qo_2 value of encysted myxamoebae was very low.

2. The average R.Q. value at pH 5.0 with 1/30 M citrate phosphate buffer was found to be 0.99.

3. The optimum pH for the respiration of these myxamoebae was pH 6.0. This rate was almost constant at pH's between 4.0 and 8.0.

4. The respiratory rate of these myxamoebae was enhanced by the addition of extracellular substrates such as glucose, pyruvic acid, succinic acid, malic acid, lactic acid, acetic acid, serine and glutamic acid. The O_2 uptake was strongly inhibited by some metabolic poisons, such as cyanide, natrium azide, monoiodoacetate and 2, 4-dinitrophenol, and was slightly inhibited by sodium fluoride and malonic acid. The presence of cytochromes was confirmed spectrophotoscopically. From these results, the main metabolic pathway of the myxamoebae seems to be as follows: Glucose is decomposed to pyruvic acid through a series of glycolytic enzymes, then pyruvic acid goes into the TCA cycle and is oxidatively decomposed to carbon dioxide with the aid of a cytochrome system.

5. Anaerobic CO_2 production was slight and anaerobic acid production was not observed at all.

6. The metabolism of the myxamoebae is found to be similar to that of the plasmodium, except that they fail to produce acid anaerobically.

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