Utilization of Carbohydrate by *Eimeria tenella* Sporozoites

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ABSTRACT. Utilization of carbohydrate by *Eimeria tenella* sporozoite was investigated under aerobic condition. The decrease of amylopectin reserve in the sporozoite cultivated at 37°C was suppressed by glucose, fructose, mannose and maltose but not by galactose, glycogen, sucrose, lactose, pyruvate and glycerol. When the sporozoites were incubated at 41°C in the presence of ¹⁴C-glucose, the change of radio activity showed that the sporozoites took in glucose from out of their body and metabolize it to excrete briskly. CO₂ production from glucose was observed at the beginning of incubation.

Sporozoites of *Eimeria brunetti* [7], *E. tenella* [3, 13], *E. acervulina* [12], *E. auburnensis* and *E. bovis* [2] were shown to contain polysaccharide granules stained by periodic acid-Schiff (PAS) stain. By the chemical analysis, polysaccharides of sporozoites of *E. tenella* [9] and of oocysts of *E. brunetti* [10], *E. bovis* and *E. stiedai* [11] were identified as amylopectin. We also confirmed cytochemically that PAS positive granules of *E. tenella* was amylopectin [6].

Ryley [8] incubated *E. tenella* sporozoites anaerobically at 41°C, and observed that the decrease of amylopectin reserve in the parasites correlated with the production of lactic acid and lesser amount of CO₂ and glycerol. When the sporozoites were incubated with certain substrates anaerobically, he found that the production of lactic acid was stimulated by glucose and lactose and to a lesser extent by fructose and mannose, and that the consumption of amylopectin was suppressed by addition of glucose, fructose and mannose but not by lactose.

On the other hand, cytochrome *a* and *b* in sporozoite, and cytochrome oxidase

on the tubular cristae of the mitochondria of sporozoite have been detected [1, 8]. The stimulation of aerobic respiration of sporozoites by glucose and fructose, and its depression by KCN were also investigated [8]. The consumption of amylopectin reserve under aerobic condition was observed [5].

These investigations suggest that the sporozoites can utilize aerobically carbohydrates presenting around their bodies as well as amylopectin in them.

Thus we investigated the utilization of carbohydrate by sporozoite under aerobic condition.

MATERIALS AND METHODS

Organisms: *Eimeria tenella* K-2 strain which originated in NIAH Japan and was serially passaged at the Department of Animal Science, Faculty of Agriculture, Tohoku University, was used.

Preparation of sporozoite suspension: Sterilized sporozoites were prepared by the method mentioned previously [4], and suspended in 0.1 M pH 7.0 phosphate buffer (PB).

Observation of substrate utilization by

sporozoites: Sporozoites were suspended in PB with or without 1% (w/v) substrate, and prepared to be 2×10^6 sporozoites/ml. In each test tube of 5 ml capacity, 0.3 ml of the sporozoite suspension was placed with the atmosphere. The tubes were stoppered tightly and settled in a water bath at 37°C. After 16 and 24 hr the sporozoites were smeared, fixed, and stained by PAS stain. By the microscopical observation of these sporozoites, the PAS indices were calculated as mentioned previously [5].

Measurement of glucose intake of sporozoites: Sporozoites were suspended in PB which contained 0.05 M ^{14}C -glucose (1 $\mu\text{Ci/ml}$), resulting to be 5×10^6 sporozoites/ml. In each test tube of 5 ml capacity, 0.5 ml of the sporozoite suspension was placed with the atmosphere. After stoppering tightly, the tubes were settled in a water bath at 41°C. At intervals of 30 min, 3 tubes of the sporozoite suspension were cooled in an ice bath, and PB at 4°C was added to each tube. The suspension was centrifuged at $700 \times g$ for 10 min at 4°C and the supernatant was removed. After washing twice by the centrifugation as mentioned above, the remaining pellet was removed to a counting vial by a Pasteur pipette after resuspended in ethanol and ethyl ether. This pellet was dried at 60°C, and heated at 50°C for 30 min in 1 ml of Scintilamine (Scintilamine-OH-1 M methanol solution: Dojindo laboratories) to lyse the sporozoites. After cooling, 10 ml of liquid scintillation cocktail was added to the vial, and its radio activity was counted.

Detection of CO_2 from glucose: Sporozoites were suspended in PB which contained 0.05 M ^{14}C -glucose (0.1 $\mu\text{Ci/ml}$), resulting to be 3.3×10^5 sporozoites/ml. With the atmosphere each 1 ml of sporozoite suspension was placed out of a cen-

ter well in a Erlenmeyer flask of 20 ml capacity which had a well at the center of the bottom, and in a center well 0.2 ml of Scintilamine was placed. The flask was stoppered tightly and shaken horizontally in a water bath at 41°C. Following incubation, 0.05 ml of 5N-HCl was injected into the sporozoite suspension and settled for 2 hr at the room temperature. The Scintilamine in the center well was dissolved in 10 ml of the scintillation cocktail, and the radio activity was counted.

Radio activity determination: Radio active glucose was D- ^{14}C (U)-glucose (329 mCi/mM: New England Nuclear Corp.). Liquid scintillation cocktail contained 5 g DPO (Dojindo) and 100 mg POPOP (Dojindo) in 1,000 ml toluen. Liquid scintillation counter was Model 3385 Packard instrument Co.

Confirmation of sterility: In each experiment, a part of the sporozoite suspension was injected to the thioglycolate medium to confirm the sterility.

RESULTS

Amylopectin content of the sporozoites incubated at 37°C was shown in Fig. 1. In all 6 trials, at the beginning of incubation sporozoites contained a large amount of amylopectin, and their PAS indices gave about 5. At 16 hr of incubation, the sporozoites incubated without any substrates contained little amount of amylopectin, and showed nearly 0 of the PAS index.

The sporozoites incubated with galactose, glycogen, sucrose, lactose, pyruvate and glycerol lost almost their amylopectin at 16 hr of incubation as well as in the sporozoites without any substrates.

Amylopectin of the sporozoites incubated with glucose, fructose, mannose and maltose was, however, consumed slowly, and several amount of amylopec-

Fig. 1. Effect of substrate to amylopectin reserve of sporozoite. Sporozoites were incubated at 37°C with substrate (▲, ■), or not (●).

tin remained at 24 hr of incubation. Their PAS indices gave above 1. This may suggest that these carbohydrates are utilized by the sporozoites as substitutes for amylopectin.

By use of ^{14}C -labeled glucose, it was investigated whether sporozoites take glucose in their body or not.

In the first place, the extent of cleanliness of washed sporozoites was examined. After addition of ^{14}C -glucose the sporozoite suspension was immediately washed by centrifugation as mentioned above, and the radio activity of the resulting sporozoite pellet was measured. The disintegration per minute (dpm) of the pellet was similar to the dpm of the back ground. This showed that ^{14}C -glucose could be removed from the sporozoite pellet by this washing method.

By this method, the radio activity in the sporozoites incubated with ^{14}C -glucose was measured (Fig. 2). The radio activity in the sporozoites increased as the incubation proceeded. This shows clearly that ^{14}C -glucose was taken in by the sporozoites.

The increase of radio activity slowed

after 60 min of incubation, so the metabolic turn-over at that time was investigated. Following sporozoites were incubated with 0.05 M non-labeled glucose for 60 and 120 min. They were incubated with 0.05 M ^{14}C -glucose (1 $\mu\text{Ci}/\text{ml}$), and their radio activity was measured (b and c in Fig. 2). In each case, the radio activities of the parasites increased as rapid as in the case when the fresh sporozoites were incubated with ^{14}C -glucose (a in Fig. 2). This fact shows that the speed of the glucose intake of the sporozoites after 60 min of incubation was the same as that at the beginning of incubation, though the apparent speed of glucose intake slowed. Therefore it is clear that the sporozoites briskly take in glucose from out of their body and metabolize it to excrete.

Figure 3 shows the radio activity of the Scintilamine which trapped CO_2 produced by sporozoites in PB with ^{14}C -glucose. High level of the radio activity of CO_2 was detected at 30 min of incubation, which shows that sporozoites produce CO_2 from glucose in a short time.

However, since the radio activity of

Fig. 2. ^{14}C -glucose intake of sporozoite. Sporozoites were incubated at 41°C with ^{14}C -glucose (0.05 M : $1\text{ }\mu\text{Ci/ml}$) from the beginning of incubation (a), or after incubation with non-labeled glucose (b, c).

Fig. 3. $^{14}\text{CO}_2$ production from ^{14}C -glucose by sporozoite. Sporozoites were incubated at 41°C with ^{14}C -glucose (0.05 M : $0.1\text{ }\mu\text{Ci/ml}$).

CO₂ did not increase significantly at 30–240 min of incubation, no glucose may be catabolised to CO₂ at this term. This finding shows that sporozoites catabolise glucose to CO₂ only at the beginning of incubation.

DISCUSSION

It had been investigated that *E. tenella* sporozoites consumed amylopectin in their body when they were incubated aerobically in PB without any substrate [5]. In this observation, the consumption of amylopectin in the parasites was depressed by addition of glucose, fructose, mannose, and maltose to the sporozoite suspension (Fig. 1). Although such a depression is able to be done by the action of the metabolic inhibition, these substrates may be utilized in respiration as sparing the stored polysaccharide, because these substrates can come into the reaction series of glycolysis. This finding corresponds with the results under anaerobic condition by Ryley [8] except for the result concerning maltose.

When sporozoites were incubated with ¹⁴C-glucose, the radio activity in the parasites increased rapidly for 60 min of incubation, and slowly after 60 min of incubation. The radio activity of the sporozoites which were pre-incubated with non-labeled glucose increased, however, as fast as that of sporozoites which were not pre-incubated (Fig. 2). This suggests that in the course of this experiment the sporozoites rapidly take glucose in their body continually, and briskly metabolize and excrete it.

CO₂ production from glucose by the sporozoites was observed (Fig. 3). Although CO₂ was remarkably produced at the beginning of incubation, it was scarcely produced after 30 min of incubation. As the glucose was shown to be taken in always, at the beginning of incu-

bation it may be catabolised briskly to CO₂ and after that it may be excreted as intermediate products in spite of continuous intake. However, further study such as analysis of the metabolic products of the parasites should be done.

From the value of radio activity, the glucose retention of sporozoites at 30 min of incubation was calculated to be 0.12 μg/10⁶ sporozoites. Amount of the glucose catabolised to CO₂ within 30 min was calculated to be 5.97 μg/10⁶ sporozoites. Although it is difficult to compare these values because of different condition of cultivation of sporozoites, it is suggested that the metabolic turn-over of glucose in the parasite is quick.

The present study showed clearly that the sporozoite take glucose from the medium in their body and catabolise it to CO₂ under aerobic condition. It was also suggested that fructose, mannose and maltose might be able to be utilized by sporozoites aerobically as well as glucose.

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要 約

Eimeria tenella スポロゾイトの炭水化物利用性: 中井 裕・扇元敬司¹⁾ (茨城大学農学部家畜衛生学教室, ¹⁾東北大学農学部家畜衛生学教室)——好気条件下における *Eimeria tenella* スポロゾイトの炭水化物利用性を検討した。スポロゾイトを 37°C で培養したとき、虫体内の貯蔵アミロペクチンの消費は、ガラクトース・グリコーゲン・シュウクロース・ラクトース・ピルビン酸塩およびグリセリンを添加しても影響されなかった。しかし、グルコース・フラクトース・マンノースおよびマルトースの添加によってアミロペクチン消費は抑制され、これらの糖がスポロゾイトに利用される可能性が示唆された。つぎにスポロゾイトを ¹⁴C-グルコース存在下 41°C で培養したところ、虫体内放射能は経時的に上昇し、非放射性グルコース添加培養後に ¹⁴C-グルコースを添加しても虫体内の放射能の急速な上昇が認められ、スポロゾイトはグルコースを速やかに取込み、代謝・排泄するものと考えられた。また添加したグルコースの一部は CO₂ に分解された。