Effects of Rearing Conditions on Oxidation-Reduction Potential Change and ATP Degradation of Live and Dead Scallops (*Pecten yessonensis*)

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The possibility of using oxidation-reduction potential (ORP) as freshness index for scallops (*Pecten yessoensis*) was investigated. Scallops were divided into three groups and subjected to different rearing conditions: rearing at higher-than-optimum temperatures (at 15～18℃) with aeration, rearing in seawater but without aeration, or removal from seawater and live storage in air at 10℃. The ORP and pH of the adductor muscle of live scallops during rearing were measured at regular time intervals for 24 h. Then the adductor muscle in each sample was removed from the valves and stored at 0℃. Changes in ORP, K value, ATP degradation, and D-lactic acid content during storage were monitored. The results showed that live scallops had a reductive characteristic in terms of ORP, which varied between 0.16～0.19 V and a pH range from 6.2～7.0. However, ORP generally increased towards a more oxidative state under all the rearing conditions tested. ORP also increased with K value and approached its maximum when K value exceeded 25%. Regardless of rearing conditions, the ORP range that indicates the reasonable freshness of scallops for sashimi was 0.166～0.215 V. The postmortem ATP content was highest in the scallops reared in seawater while the fastest ATP degradation observed in the live scallops stored in air. D-lactic acid accumulation during storage was lowest in the samples reared in seawater with aeration. The absence of oxygen supply during rearing promotes enhanced ATP degradation and D-lactic accumulation.

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Scallops (*Pecten yessoensis*) are one of the major marine products in Japan. Scallop consumption has been increasing considerably since scallop aquaculture was successfully established commercially in the 1970's. In Japan, large amounts of scallops are commonly consumed raw in some popular dishes such as sashimi and sushi. Because scallops are highly susceptible to spoilage by microorganisms owing to their high moisture content and neutral pH, freshness retention becomes a matter of concern. The postmortem biochemical changes of extract components in relation to scallop freshness have been studied extensively. Various ratios of the concentration of ATP to those of and its decomposition products, such as K value, have been widely used as chemical indexes for estimating scallop freshness. However, the measurement of such indexes is complicated and time-consuming. Moreover, the pattern and rate of nucleotide degradation not only differ among scallop species but also depend on the predeath living conditions[1]. *Pecten yessoensis* is a species of Japanese scallop that usually lives in cold water. In general, the optimum rearing temperature range for commercial aquaculture is approximately 8 ～ 10℃ with sufficient water circulation[2]. During spring, scallop eggs and sperm are released after maturing at 5 ～ 8℃ for their fertilization. Unfortunately, the effect of rearing conditions on the postmortem freshness loss of aquaculture scallops still remains unclear especially if scallops need to be cultured in an unusual environment with high water temperature or

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without oxygen supply. Nowadays, the market requires fresher scallops for sashimi, hence, processors generally prefer to get live samples for best quality. However, unlike marine fish, scallops can survive in cold moist atmosphere after harvest, therefore, it is sometimes difficult to determine whether they are dead or alive. We found that in scallops, the oxidation-reduction potential (ORP) and pH of the adductor muscle related to metabolic activity; thus, we investigated the possibility of identifying live and dead scallops by monitoring ORP changes in scallop adductor muscle. Research on post-mortem ORP changes in dead fish muscle has been conducted[3]. However, we have not found reports on ORP data for live samples. Patterns of ORP shift from the live to dead transition are necessary to validate the application of ORP as rapid freshness index in the future. Furthermore, to obtain further understanding of the postmortem biochemical change in muscles of scallops subjected to stressful living conditions and how these influence scallop freshness, in this research, we aim to study effect of rearing conditions on ORP and ATP degradations in scallops particularly at the initial stage of deterioration.

Materials and Methods

1. Preparation of live scallop samples

Large live scallops (*Patinopecten yessoensis*) from Aomori prefecture with an average weight of 33.85 ± 4.30 g, were purchased from a local seafood retailer. The samples were weighed and divided equally into three groups. For initial adjustment to the same living conditions, all samples in each group were reared with aeration supplied by an air pump (Inno™ β4000, 3.5 W, 50/60 Hz) in the optimum rearing temperature range of 8～10℃ for 3 hours before subjecting to further treatments. This initial rearing time is designated as 0 hour in Fig. 1 and 2.

2. Identification of live and dead scallops by monitoring ORP and pH changes in adductor muscle during rearing

To study the effects of stress during living, after rearing under the above conditions, the scallops were subsequently reared under the following unusual living conditions : ① rearing at higher seawater temperatures (than normal optimum temperature) of 15～18℃ with aeration ; ② rearing at 15～18℃ but without aeration ; and ③ removal from seawater and live storage in a refrigerator at 10℃ with a 64% relative humidity. The samples in each treatment were 10. During rearing, seawater in the tank was changed every 8 hours. Rear under these conditions and report the time when all samples were dead for each treatment. ORP and pH were measured at 6-hour intervals on the side surface of the adductor muscle. During the measurement, an electrode was carefully inserted in the shells attached to the adductor muscle. To determine whether a scallop is still alive, the following criteria for judgment were used : ① A live sample should have some response of closing its valves when the electrode's probe touches the muscle. ② The mantle lobe around the adductor muscle should not shrink or detach to the shells. ③ There should be no mucous liquid leakage caused by the postmortem autolytic decomposition of the muscle. If either one of these criteria is present, the authors would consider the scallop "dead". The mantle, an integument responsible for opening and closing the valves, is illustrated in Fig. 1. It is composed of the right and left lobes and is closely connected to the adductor muscle and digestive gland. It is connected to the right and left valves by strands of connective tissues terminating in specialized tendon cells[4]. If the mantle loses its contractile ability to keep the valves closed, it is an indicator that the scallop is dying.

3. Study of effect of antemortem living conditions on biochemical change in adductor muscle

Another batch of scallops were adjusted to the initial living conditions and divided into three groups, as mentioned above. Each group of sample was treated accordingly to the rearing methods described earlier. However, in this experiment, rearing time was controlled to be no longer than 24 hours. After 24 hours of living under the above conditions, the adductor muscle in all the samples was detached from the valves using a knife. Each muscle sample was wrapped in a polyethylene bag and stored at 0℃. From each storage time, samples were collected at different intervals for the determination of K value by the modified method of Ryders and D-lactic acid content according to the enzymatic Boehringer's method.

4. ORP and pH analyse

ORP is defined as the electrode potential for estimating the degree of the oxidation or reduction of a particular foodstuff. The ORP (E_h or ORP) of a reversible redox reaction is given by the equation

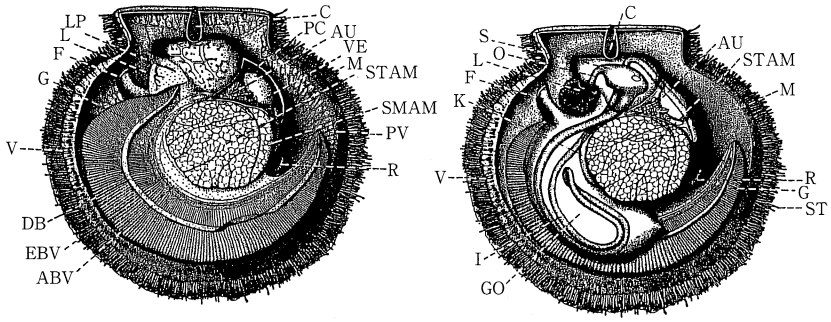

Fig. 1 Schematic general anatomy of scallop (From Bubel, 1984)

Abbreviations : ABV ; afferent branchial vessel, AU ; auricle, C ; chondrophore, DB ; dorsal bend of gill filaments, EBV ; efferent branchial vessel, F ; foot, G ; gill, GO ; gonad, I ; intestine, K ; kidney, L ; lips, LP ; labial pulp, M ; mantle, O ; oesophagus, PC ; pericardium, PV ; pallial vessels, R ; rectum, S ; stomach, ST ; sensory tentacle, SMAM ; smooth adductor muscle, STAM ; striated adductor muscle, V ; velum, VE ; ventricle.

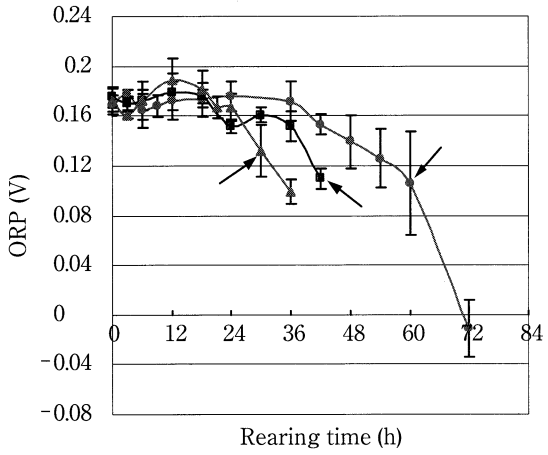

Fig. 2 ORP change in adductor muscle of live *P. Yessoensis* during rearing

(●) reared at 15~18℃ with aeration ; (■) reared at 15~18℃ without aeration ; (▲) stored live at 10℃
Arrows indicate the time at which all samples died (n = 10).

originally derived by NERNST[5], which is expressed as

$$E_h = E_0 + \frac{RT}{nF} \ln \frac{[oxidant]}{[reductant]}$$

in which E_0 is the standard redox potential at pH 0, with other solute components at 1 M ($E_0$is assumed equal to the theoretical E_0 for a redox couple in dilute aqueous solutions) ; R is the molar gas constant; T is the temperature in K; F is the Faraday quantity of electricity; n is the number of electrons transferred in the reaction; and ln is the natural logarithm.

ORP and pH was measured using an electrometer (Toko TPX-90i) consisting of platinum and glass electrodes, respectively. Both electrodes are specially designed for measuring ORP and pH in liquid and solid states. The quinhydron standard solution, which has an ORP of 0.26 + 0.02 V, was used for electrode calibration. Between each reading, the electrode was cleaned and soaked in distilled water to remove excess charge accumulation before conducting the next measurement.

5. K value

K value was determined by high-performance liquid chromatography (HPLC) according to the modified method of RYDER[6]. One gram of scallop muscle tissue was homogenized in 4 mℓ, each of chilled 10% and 5% perchloric acid. The homogenate was centrifuged at 2000X*g* for 10 minutes at 5℃ and the supernatant was immediately neutralized to pH 6.8 with 10N and 1 N KOH solutions. The neutralized mixture was recentrifuged at 2000X*g* for another 10 minutes. The supernatant was diluted to 20mℓ with distilled water and then filtered through Whatman no. 1 filter paper prior to storage at − 46℃ for subsequent analysis.

ATP-related compounds were separated using a reverse-phase HPLC column (GS-320 HQ; Asahipak, Kanagawa, Japan). A buffer of 0.2 M sodium dihydrogenphosphate dihydrate and phosphoric acid (pH 3.6) was used as mobile phase at a flow rate of 1 mℓ/min at 25℃. The eluent was monitored at 258 nm for each ATP-related compound. The concentration of each compound was calculated from its peak area.

6. D-lactic acid determination

Five grams of muscle sample was placed in a homogenizer. After adding 20mℓ of 1 M perchloric acid, the mixture was homogenized for 10 min. The homogenate was transferred into a beaker with 40 mℓ of distilled water. The pH of the mixture was adjusted to 10~11 with 2 M potassium hydroxide

with stirring. The mixture was quantitatively transferred into a 100-mℓ volumetric flask, which was filled up to the mark with water (the fatty layer is above the mark and the aqueous layer must be at the mark). The mixture was shaken and refrigerated for 20 min, to separate the fat and precipitate potassium perchlorate. It was then filtered and a few milliliters of the filtrate were discarded. The clear solution obtained was used for enzymatic assay. D-lactic acid in the scallop muscle was analyzed using D-lactate dehydrogenase (EC 1.1.1.28, Boehringer Mannheim Co./R-Biopharm), according to the method of NOLL[7].

Results

The time courses of the ORP and pH of the live samples during rearing are shown in Fig. 2 and 3. After adjustment to the same initial living conditions, ORP and pH gradually decreased after 24 hours in each rearing treatment and then clearly decreased when scallops died (indicated by arrows). Live scallops generally have reductive characteristic in terms of ORP and these initial values vary slightly within 0.16~0.19 V in the pH range of 6.2~7.0. However, below these ORP and pH regions, none of live sample was observed. In the comparison among the rearing conditions, scallops survived the longest in seawater with aeration supply than without aeration (60 hours, and 42 hours, respectively (Fig. 2). In addition, scallops survived up to 30 hours in cold moist atmosphere at 10℃ without oxygen supply. Moreover, the live scallops stored in air at 10℃, clearly lost their capacity for valves closure. Because there is no respiratory pigment in scallop's blood and there are low concentrations of mitochondria and cytochromes in the scallop's adductor muscle[8),9)], the contractile activity necessary for valves closure largely depends on ATP generation following anaerobic glycolysis. [10)]

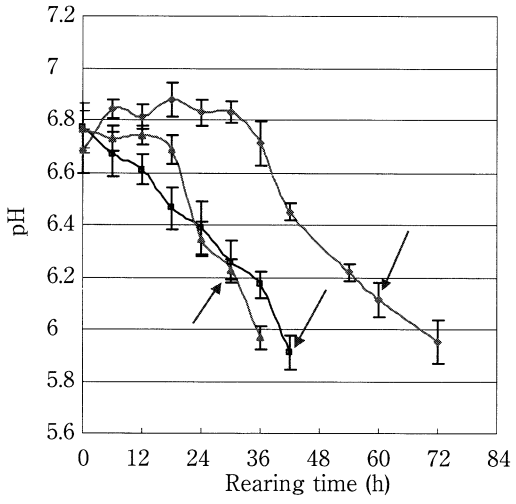

Fig. 3 pH change in adductor muscle of live *P. yessoensis* during rearing

(●) reared at 15~18℃ with aeration
(■) reared at 15~18℃ without aeration
(▲) stored at 10℃
Arrows indicate the time at which all samples died (n=10)

Note that the ORPs of live scallops in Fig. 2 were measured during "rearing" until the scallops eventually died. It was found that the longest rearing time for scallops in all treatments was 24 hours. The ORPs of live scallops during these 24 hours were between 0.16~0.19 V. However, after the samples died, some excretion from the intestine was observed, which caused ORP to drop rapidly. This rapid decrease in ORP was due to bacterial activities in the excretion. Therefore, after determining the optimum rearing time (24 hours), we used such information to conduct the next experiment.

Considering the early change in ATP content during the first 6 hours after killing by the removal of the adductor muscle from the valves (Fig. 4), it was found that ATP is gradually depleted during storage. The initial ATP content (0.3 micromole/g) was highest in the samples reared in seawater with aeration while it was lowest (0.188 micromole/g) in the live samples stored in air. In contrast, the rate of ATP degradation was fastest in the latter treatment.

The time courses of the ORP change in the scallop adductor muscle from the live to dead state transition are illustrated in Fig. 5. As mentioned above, the ORP of the live samples varied within a range of 0.16~0.19 V but the ORP of the dead samples continuously increased and reached an almost constant plateau for different storage durations. The time required for the ORP to reach this constant value appears to be dependent on each rearing treatment. The samples reared in seawater with aeration had significantly higher ORPs than the other samples. The reason for the higher ORPs is believed to be the higher concentration of oxygen accumulated in the adductor muscle of scallops reared with sufficient aeration. Oxygen supplied by aeration is used in the glycolytic respiratory pathway to generate ATP and is also accumulated in the muscle. Therefore when

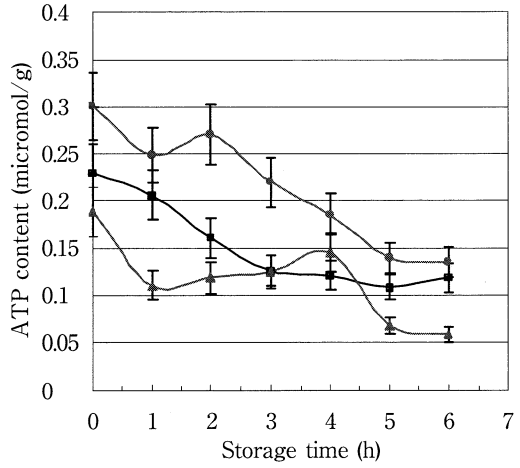

Fig. 4 Early ATP degradation of *P. yessoensis* after removing the adductor muscle out from the valves

(●) reared at 15～18℃ with aeration
(■) reared at 15～18℃ without aeration
(▲) stored live at 10℃ (n = 4)

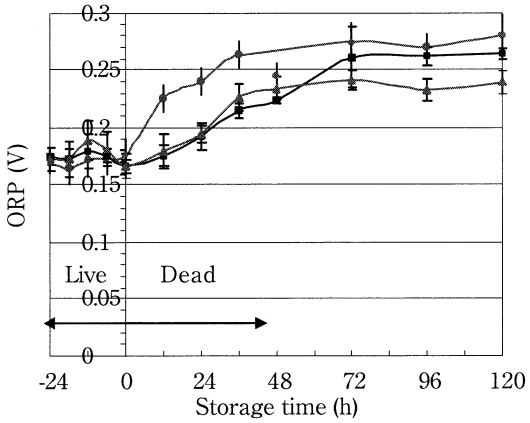

Fig. 5 Time courses of ORP change in adductor muscle of live and dead scallops during storage at 0 ℃

(●) reared at 15～18℃ with aeration
(■) reared at 15～18℃ without aeration
(▲) stored live at 10℃ (n = 4)

measuring the ORP change in the adductor immediately after killing, we found that the ORPs were significantly higher in the sample rears with aeration. On the other hand, the live scallops stored in air, had the lowest ORP values, which reached an almost constant plateau after 36 hours of storage. Moreover, it should also be noted that the ORP changes in Fig. 5 are different from those in Fig 2. The ORPs in Fig. 5 were measured after the scallops were reared for 24 hours and killed immediately by removing the adductor muscle from the valves. Thus, they were considered to be the ORP change "during storage".

Fig. 6 shows the relationship between ORP and K value, in which ORP increased with K value and approached its maximum when K value exceeded 25%. Interestingly, it has been demonstrated that a K value below this level is an indication of limited freshness for consuming as raw scallops (sashimi) [11]. However, the effect of rearing conditions has little effect on K value. The K values of the samples reared without aeration were not significantly different from those reared with aeration. A maximum difference of about 7 % in K value after

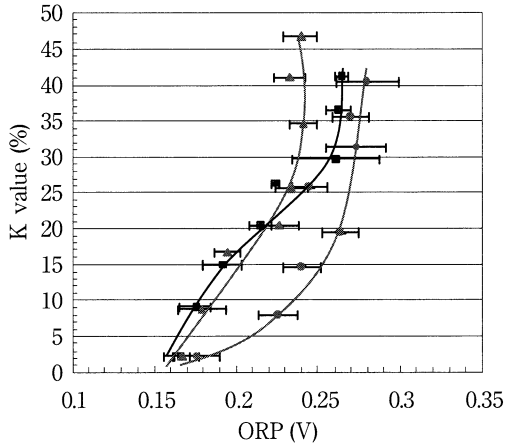

Fig. 6 Change in ORP with K value in adductor muscle during storage at 0 ℃ for each treatment

(●) reared at 15～18℃ with aeration
(■) reared at 15～18℃ without aeration
(▲) stored live at 10℃ (n = 4)

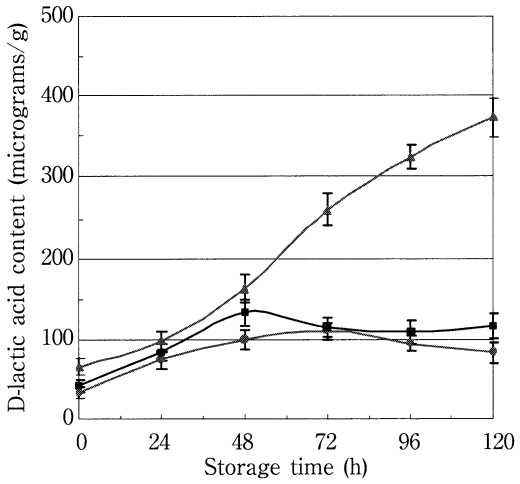

Fig. 7 Accumulation of D-lactic acid in adductor muscle of *P. yessoensis* during storage at 0 ℃

(●) reared at 15～18℃ with aeration
(■) reared at 15～18℃ without aeration
(▲) stored live at 10℃ (n = 4)

5 days of storage was observed between rearing with aeration and live storage at 10℃.

The initial D-lactic acid contents in the adductor muscle of the scallops were low (less than 70 microgram/g) in all rearing treatments. However, the D-lactic content increased steadily during storage in the live samples stored in air and exceeded 350 & micro;/g after 5 days of storage (Fig 7). Although the D-lactic acid content was lowest in the scallops reared in seawater with aeration, the presence or absence of aeration did not significantly influence lactic acid accumulation in the adductor muscle.

Discussion

To investigate the effect of stress during rearing, scallops were allowed to thrive in an unusual habitat either by living at a higher seawater temperature or living without oxygen supply. It was found that factors such as seawater temperature and oxygen supply influence the metabolic change in scallop's adductor muscle, which correlate to ORP with time. The measurements of the ORP change and ATP depletion in the muscle of live scallops gave results concerning the metabolic activity and stress accumulation in scallops. Similarly to our study, OKOUCHI *et al*.'s[12] study showed that fatigue in humans could be evaluated by measuring ORP changes in urine and blood plasma. This suggests that ORP is sensitive to metabolic changes in scallop muscle.

Antemortem living conditions influence the rate of ATP degradation in scallop muscle, as shown in Fig. 4. This implies that the scallops had to use their own source of energy in to survive under such stressful living conditions, which resulted in a higher ATP depletion rate. The effects of habitat temperature on the postmortem change in scallop quality can be observed from a study by KIMURA *et al*[13]. According to their study, when the seawater temperature around the harvesting area was higher in September 1997 (18.8℃) than in April 1998 (4.4℃), the ATP content and pH of scallop muscle decreased, and the development of rigor, and increase in octopine concentration (use as a deterioration index) were faster under the former condition. These results suggest that a change in habitat temperature could induce the rigor of the adductor muscle and hasten the deterioration of scallops after harvest.

The results in Fig. 5 emphasize that in the initial stage of deterioration, the ORP of scallops generally increases to a more oxidative state regardless of antemortem rearing conditions. Takahashi and Mori[14] reported that glycogen serves as the main reserve energy in the adductor muscle of *P. yessoensis*. During anaerobic glycolysis, as shown in Fig. 8, coenzymes such as NADH and NADPH play important roles in maintaining the oxidation-reduction potential balance by either donating or accepting of electrons[15]. However, when the scallops died, the respiratory system is terminated and anaerobic glycolysis occurs. The system finally loses its capacity to maintain the oxidation-reduction potential balance. As deterioration proceeds, pyruvate is changed into its end product of either octopine or D-lactate by the activities of the enzymes SDH and LDH, respectively. In these reactions, NADH is oxidized to NAD^+ and the overall ORP in the system is shifted to a more oxidative state until the equilibrium ORP is approached (Fig. 5). Because ORP is a measure of the tendency of a system to induce donor or accept electron transfer, thus ORP gives some information on deterioration progress.

When considering the potential of ORP as freshness index for scallops by comparison with widely used indexes such as K value, it was found that ORP increased with K value until it reaches its maximum values depending on storage time. Judging from results in Fig. 5, regardless of antemortem rearing conditions, the suitable ORP values range, which indicates the reasonable freshness of scallops as sashimi, is 0.166~0.215 V.

With decomposition progress, D-lactic acid accumulated as possible end product of postmortem biochemical changes in the scallop adductor muscle. KAWASHIMA and YAMANAKA[16] reported the accumulation of D-lactic acid in scallops during refrigerated storage. This is due to the fact that lactic acid formation in shellfish is dependent on glycogen reserves. Struggle prior to death will result in the depletion or loss of endogenous glycogen and yield higher lactic acid as final glycolytic product[17]. Because the effect of antemortem treatment on the D-lactic acid content in scallops has not been studied extensively, the use of D-lactic acid as freshness indicator is limited.

Our overall results show that ORP can be possibly applied as freshness indicator owing to its sensitivity to the change in the metabolic activity of

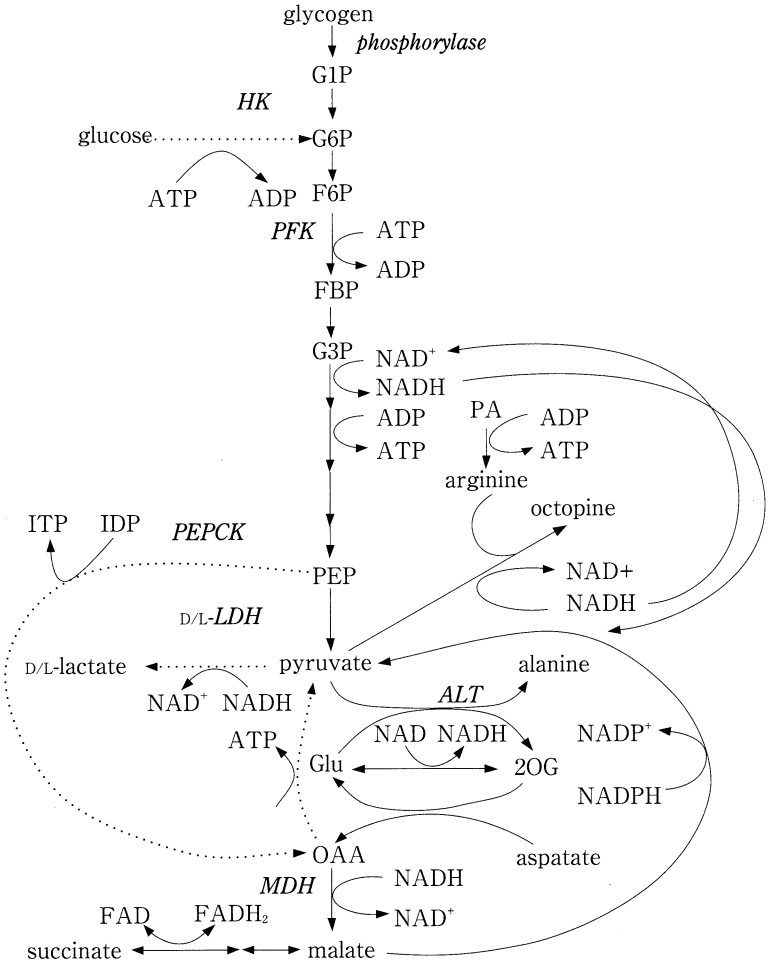

Fig. 8 Anaerobic glycolysis in scallops

G1P: glucose-1-phosphate, G6P: glucose-6-phosphate, F6P: fructose-6-phosphate, FBP: fructose-1, 6-bisphosphate, G3P: glyceraldehydes-3-phosphate, PEP: phosphoenolpyruvate, Glu: glutamate; 2OG: 2-oxoglutarate, OAA: oxaloacetate, PA: phosphoarginine.

scallops. Moreover, scallops can adjust themselves to survive in stressful environments even though there is little or no oxygen supply. The temperature of their habitat seems to influence their ATP degradation and mortality rather than oxygen supply. Therefore, it is recommended that temperature in the aquaculture area should not be too high and that water circulation should be adequate. The absence of oxygen during rearing seems to enhanced ATP degradation and marked lactic acid accumulation.

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異なる環境で生かした活ホタテの生死前後におけるATP量変化とORP変化

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活ホタテ貝鮮度計測法として酸化還元電位（ORP）の利用可能性を調べた。活ホタテを三つのグループに分け，それぞれ異なる環境で維持した。一つは15～18℃の海水中に空気を入れながら保持，また同じ海水温度で空気を与えずに，もう一つは海水から取り出し10℃の空気中で貯蔵した。この状態で24時間までのORPとpHの変化を測定し，その後ホタテの身を貝柱から取り，0℃に貯蔵した。貯蔵中のORP変化，K値，ATP分解，そしてD-乳酸量を分析した。生かした活ホタテは還元的状態にあり，0.16～0.19 Vの範囲内またpHの6.2～7.0にあった。ホタテの死後のORP値はすべての環境において上がった。またORPはK値とともに上がり，K値が25%あたりでORPは最大値を示した。これらのK値との関係から，鮮度のよい刺身用のORP値は0.166～0.215 Vであることがわかった。海水に空気を入れながら飼育したホタテ貝は死後のATP量が最も高いが，10℃の空気中で貯蔵した貝はATP分解が最も速かった。同様に，空気を入れながら飼育したサンプルの貯蔵中のD－乳酸変化は小さかったことから 飼育中のエアレーションはK値変化より，肉内のATP分解とD-乳酸蓄積に影響を与えることが示唆された。

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