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Estimation of CO_2 partial pressure and bicarbonate concentration in the hemolymph of the noble scallop *Mimachlamys nobilis*

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Abstract：We examined hemolymph pH, total CO_2 content (Tco_2, mM/*l*), CO_2 partial pressure (Pco_2, mmHg) and bicarbonate concentration ([HCO_3^-], mM/*l*) in order to evaluate the acid-base balance of the noble scallop *Mimachlamys nobilis* in normoxic conditions. Hemolymph was collected anaerobically through a cannula after catheterization of the adductor muscle. The mean values of hemolymph pH and Tco_2 were 7.442 and 1.50 mM/*l*, respectively. Using the CO_2 solubility coefficient and apparent dissociation constant of carbonic acid determined in this study, Pco_2 and [HCO_3^-] were calculated as 1.55 mmHg and 1.44 mM/*l*, respectively. These values were in same range as those of the hemolymph of other marine bivalves of Mytiloida, Pterioida and Ostreoida.

Key words：*Mimachlamys nobilis*, cannulation, hemolymph, acid-base balance, Pco_2, dissociation constant of carbonic acid

Introduction

The noble scallop *Mimachlamys nobilis* is a filibranchial bivalve classified in the Pectinidae, Ostreoida.[1)] This species inhabits rock reefs or sandy-bottomed shores up to a depth of 20 m in the littoral zone from the Boso Peninsula to Okinawa in Japan along the Kuroshio and Tsushima currents.[1-2)] These warm ocean currents maintain a moderate climate across this region, with annual water temperatures of 17–29℃.[2)] Noble scallops are produced in southwest Japan, including Wakayama, Ohita, Nagasaki, Kagoshima and Okinawa prefectures, and are importance species for aquaculture.[1-3)] The many species of the Pectinidae distributed around the world are widely found in high latitudes between 30° and 55° in the Northern and Southern hemispheres and are economically important aquatic resources in cold-water sea areas.[4)] The noble scallop is, therefore, an unusual resource as a Pectinidae species produced in warm-water sea areas. Noble scallop has been a subject of previous research in terms of reproductive cycle,[5)] induction of oviposition,[6)] seedling production,[6)] early food,[7)] karyotype,[8)] genetic variation,[9)] gametogenesis and triploid induction.[10,11)] The ciliary movement of the ctenidium in hypoxic and anathermal conditions has been studied.[12)] The anatomical structures of the ctenidium and circulatory system were clarified recently.[13)] However, there are few reports on the respiratory mechanism from the viewpoint of CO_2 dynamic phase and acid-base balance. Research into the acid-base status could contribute to efficient CO_2 utilization, which is related to respiration, and calcification for the formation of the shell valves. The estimated CO_2 partial pressure of the hemolymph was 0.9 mmHg in sea mussel *Mytilus edulis*,[14)] 2.3 mmHg in Asian freshwater clam *Corbicula fluminea*,[15)] 1.7–2.3 mmHg in akoya pearl oyster *Pinctada fucata*,[16,17)] and 1.5 mmHg in black-lip pearl oyster *Pinctada margaritifera*.[18)] Because the CO_2 partial pressures in these bivalves were very low, it was supposed that the CO_2 partial pressure in the noble scallop would also be similarly very low. The direct measurement of CO_2 partial pressure is difficult when there is small quantity of hemolymph sample and CO_2. The estimation of CO_2 partial pressure by application of the Henderson–Hasselbalch equation is used in studies of the acid-base balance owing to the relative ease and accuracy of the estimates.[19)] In the equation, the characteristic values of the CO_2

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solubility coefficient (αCO_2) and apparent dissociation constant of carbonic acid (pKapp) in the hemolymph are required for the experimental animal. Therefore, we examined noble scallop hemolymph pH, total CO_2 content, CO_2 partial pressure and bicarbonate concentration using the hemolymph αCO_2 and pKapp, which were determined in this study. By means of catheterization of the adductor muscle, hemolymph was collected anaerobically from noble scallop underwater.

Materials and Methods

Experimental animals and conditions

The experiments used 36 noble scallops (shell length: 86.1 ± 5.0 mm [Mean ± SE]), shell height: 88.0 ± 5.1 mm, and total wet weight: 102.7 ± 11.8 g). The animals were obtained from the coast of Nagasaki prefecture, Japan. After cleaning the shell valves, they were reared for 2 months at 24°C in aerated seawater with added cultivated phytoplankton.[17,18,20,21] Twenty-four hours before collecting hemolymph, the noble scallop were transferred to seawater that was particle-free (>0.45 μm). All experiments were conducted in the seawater with a salinity of 33 psu, water temperature 24.0°C, O_2 saturation 99%, pH 8.15, and total CO_2 content 1.2 mM/*l*.

Surgical procedures and hemolymph collection

Hemolymph was collected from the adductor muscle using a cannula (polyethylene tubing, 0.96 mm outer diameter, 0.58 mm inner diameter, PE-50, Clay Adams). A small hole (2 mm diameter) was made on adjacent shell valves at the center of the posterior margin. The cannula with a stylet was inserted through the hole into the adductor muscle, which is part of the large anterior muscle, and was advanced 1.0 – 1.2 cm toward the center of the adductor muscle. The stylet was removed, and the end of the cannula was closed. The cannula was gently fixed to the left shell valve using denture adhesive (Kobayashi Pharmaceutical Co., Ltd.) in order to prevent effects from movement of the shell valves. This surgical operation was completed within 5 minutes. The cannulated scallop was transferred to a darkened acrylic respiratory chamber and was allowed to recover for 3 h at 24.0°C in normoxic conditions. A hemolymph sample was then drawn through the cannula using a gas-tight micro syringe (Model 1750, Hamilton Co. Ltd.). The volume of hemolymph collected was 0.4 m*l*.

Hemolymph analysis

Hemolymph pH and total CO_2 content (Tco_2, mM/*l*) were measured immediately after each collection. The pH was measured using a blood gas meter (BGM200; Cameron Instruments), pH glass and reference electrodes (E301, E351; Cameron Instruments) at 24.0°C. Tco_2 was measured using a total CO_2 analyzer (Capnicon 5; Cameron Instruments). The hemolymph CO_2 partial pressure (Pco_2, mmHg) and bicarbonate concentration ($[HCO_3^-]$, mM/*l*) were calculated by rearranging the Henderson–Hasselbalch equation.[19,22] In the equation, the CO_2 solubility coefficient (αCO_2, μM/*l*/mmHg) and apparent dissociation constant of carbonic acid (pKapp) of noble scallop were required. The determinations of αCO_2 and pKapp were performed *in vitro*.

The αCO_2 was determined using noble scallop hemolymph that was adjusted to pH 2.5 by the addition of lactic acid (Wako Pure Chemical Industries, Ltd.). The acidified sample was transferred to a tonometer flask and equilibrated using humidified standard CO_2 gas (CO_2, 15.0%; O_2, 20.9%; N_2 Balance) using an equilibrator (DEQ-1; Cameron Instruments) at 24.0°C, and subsequently the total CO_2 content of each equilibrated sample was measured with the total CO_2 analyzer. The CO_2 partial pressure of the equilibrated sample was calculated from known CO_2 concentration standard gas (15.0%), prevailing barometric pressure and water vapor pressure at 24.0°C. The CO_2 solubility coefficient was calculated using the equation:

$$\alpha CO_2 = \text{Total } CO_2 \text{ content} \bullet CO_2 \text{ Partial pressure}^{-1}$$

For determination of the apparent dissociation constant of carbonic acid, hemolymph was transferred to a tonometer flask and equilibrated with humidified standard CO_2 gases (CO_2, 0.5, 1.0, 2.0, 5.0 and 10.0%; O_2, 20.9%; N_2 Balance) using an equilibrator at 24.0°C. After equilibration, the pH and total CO_2 content of the sample were measured with the blood gas meter and the total CO_2 analyzer. Using the sample pH, total CO_2 content and αCO_2 calculated using the above equation, the pKapp was determined by rearrangement of Henderson–Hasselbalch equation[19,22] as follows:

$$pKapp = pH - \log [(\text{Total } CO_2 \text{ concentration} - \alpha CO_2 \bullet CO_2 \text{ Partial pressure}) \bullet (\alpha CO_2 \bullet CO_2 \text{ Partial pressure})^{-1}]$$

where CO_2 partial pressure is calculated from known CO_2 concentration standard gases.

The αCO_2 and pKapp obtained in this study were used for calculation of hemolymph Pco_2 from measured pH and Tco_2:

$$Pco_2 = Tco_2 \bullet [\alpha CO_2 \bullet (1+10^{(pH-pKapp)})]^{-1}$$

$[HCO_3^-]$ was calculated from Tco_2, αCO_2 and Pco_2 using the following equation[23]:

$$[HCO_3^-] = Tco_2 - \alpha CO_2 \bullet Pco_2$$

Statistical analysis

All data are expressed as means ± standard error. Kruskal-Wallis one-way analysis of variance was used to test for changes in hemolymph properties using the standard CO_2 gases. Mann-Whitney *U* test was used for the comparison of mean values of hemolymph parameters. Statistically significant differences were set at $P<0.01$.

Results

Hemolymph samples were collected from the adductor muscles of noble scallops through cannulae. The collection volume was 0.4–0.5 *ml* from each individual. The hemolymph pH and Tco_2 in normoxic conditions were 7.442 ± 0.0094 and 1.50 ± 0.043 mM/*l*, respectively (Table 1), and the hemolymph αCO_2 was 39.0 ± 0.820 μM/*l*/mmHg. The hemolymph pKapp at known CO_2 partial pressures (standard gases) and the corresponding measured pH and Tco_2 values are shown in Table 2. The calculated pKapp from all hemolymph samples was 6.064140 ± 0.013876. Pco_2 and $[HCO_3^-]$ were calculated by substitution of the mean value of hemolymph αCO_2 and pKapp in the rearranged Henderson–Hasselbalch equation as follows:

$$Pco_2 = Tco_2 \bullet [0.039 \bullet (1+10^{(pH-6.064140)})]^{-1}$$

$$[HCO_3^-] = Tco_2 - 0.039 \bullet Pco_2$$

where the units of the parameters in the equations are mmHg for Pco_2, mM/*l* for Tco_2 and for $[HCO_3^-]$.

Hemolymph Pco_2 and $[HCO_3^-]$ at 24.0°C in normoxic condition were 1.55 ± 0.070 mmHg and 1.44 ± 0.041 mM/*l*, respectively

Table 1. Hemolymph pH, total CO_2 content (Tco_2), CO_2 partial pressure (Pco_2) and bicarbonate concentration ($[HCO_3^-]$) of noble scallop (*Mimachlamys nobilis*) at 24°C in normoxic conditions

		Mean	SE	N
pH		7.442	0.0094	6
Tco_2	mM/*l*	1.50	0.043	6
Pco_2	mmHg	1.55	0.070	6
$[HCO_3^-]$	mM/*l*	1.44	0.041	6

αco_2, 39.01 μM/*l*/mmHg; pKapp, 6.0641396 ± 0.0138764

Table 2. Mean values of measured pH, total CO_2 content (Tco_2) and calculated apparent dissociation constant of carbonic acid (pKapp) of hemolymph in the adductor muscle of the noble scallop (*Mimachlamys nobilis*) with known Pco_2 standard gases

Standard gas		Hemolymph			
CO_2 (%)	Pco_2 (mmHg)	pH	Tco_2 (mM/*l*)	pKapp	N
0.5	3.64	7.213	1.791	6.1532	6
1.0	7.28	6.873	1.937	6.1089	6
2.0	14.60	6.629	2.766	6.0495	6
5.0	36.40	6.265	4.104	6.0111	6
10.0	72.80	5.990	5.973	5.9980	6

Water temperature 24.0°C; Barometric pressure 759 mmHg; Water vapor pressure 22.37 mmHg

(Table 1). In in vitro experiments (Table 2), the pH decreased significantly with the increase in Pco_2 (P<0.01). At the same time, the interaction between pKapp and pH was analyzed (Fig. 1), and the correction equation of pKapp was obtained as follows:

$$pKapp = 45.217 - 17.721 \cdot pH + 2.6463 \cdot pH^2 - 0.1303 \cdot pH^3$$

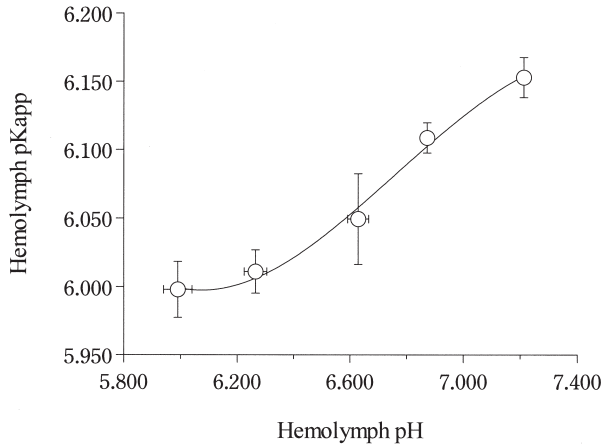

Fig 1. Relationship between pH and apparent dissociation constant of carbonic acid (pKapp) in the hemolymph of noble scallop *Mimachlamys nobilis* at 24° C (n=30). Values are means ± SE. The solid line is fitted to the data and the equation: $pKapp = 45.217 - 17.721 \cdot pH + 2.6463 \cdot pH^2 - 0.1303 \cdot pH^3$ (R^2=0.992)

For the comparison, using the hemolymph pH and Tco_2 measured immediately after collection, Pco_2 and [HCO_3^-] were estimated using the correction equation and are shown in Table 3. The hemolymph Pco_2 calculated using the correction equation was higher than that from the mean value of pKapp (P<0.01). There was no difference in hemolymph [HCO_3^-] calculated by the two methods (Table 3).

Discussion

We collected noble scallop hemolymph from the adductor muscle and examined hemolymph pH, total CO_2 content, CO_2 partial pressure and bicarbonate concentration in order to evaluate the acid-base balance of noble scallops. The hemolymph was collected anaerobically through a cannula from noble scallops kept underwater after pretreatment by adductor muscle catheterization. The mean values of pH and Tco_2 measured immediately after hemolymph collection were 7.442 and 1.50 mM/*l*, respectively. Previously reported mean values of hemolymph pH include 7.65 in sea mussel *M. edulis* at 12°C,[14] 7.36 in Pacific oyster *Crassostrea gigas* at 15°C,[24] 7.55 in *M. galloprovincialis* at 18°C,[25] 7.284–7.375 in akoya pearl oyster *P. fucata* at 28°C,[17] and 7.563 in black-lip pearl oyster *P. margaritifera* at 26°C.[18] Although there are few descriptions of hemolymph total CO_2 content in marine bivalves, Handa and Yamamoto (2011, 2012, 2015) reported the mean values in akoya pearl oyster *P. fucata* and black-lip pearl oyster *P. margaritifera* as 1.90–2.10 mM/*l*[16,17] and 2.04 mM/*l*,[18] respectively. The hemolymph pH in noble scallop was almost the same as that in other marine bivalves, and the contents of carbonic acid and CO_2 in noble scallop hemolymph appeared to be less than in pearl oysters.

Cameron (1986) reported the CO_2 solubility of seawater as a function of temperature, and the solubility coefficient at 24°C was 39.24–40.13 μM/*l*/mmHg at 30–35 salinity (psu).[26] The hemolymph αCO_2 in noble scallop was in the range of the coefficient of seawater. The mean value of hemolymph pKapp in this study was 6.0641396, whereas the hemolymph pKapp

Table 3. Compariosn of the hemolymph Pco_2 and [HCO_3^-] calculated from the mean value of pKapp and using the correction equation

	Pco_2 (mmHg)	[HCO_3^-] (mM/*l*)	N
the mean value of pKapp	1.55	1.44	6
the correction equation of the pKapp	2.05 *	1.42	6

* : statistically significant difference (Mann–Whitney U test, P <0.01)

values of other marine bivalves were 5.8191 in the akoya pearl oyster *P. fucata* at 28°C,[17] 5.99878 in black-lip pearl oyster *P. margaritifera* at 26°C,[18] and 6.114 in sea mussel *M. edulis* at 12 °C.[14,27] The apparent dissociation constant of carbonic acid is equal to the pH at which it shows the most effective as a buffer.[28] The most effective buffer pH of noble scallop hemolymph seemed to be lower than that in sea mussel *M. edulis* but higher than that in pearl oyster *P. fucata*.

Using the hemolymph αCO_2 and pKapp in this study, Pco_2 and $[HCO_3^-]$ of the hemolymph of noble scallops were calculated. The mean values of hemolymph Pco_2 and $[HCO_3^-]$ were 1.55 mmHg and 1.44 mM/*l*, respectively (Table 1). In other marine bivalves, the mean values of hemolymph Pco_2 and $[HCO_3^-]$ were 0.9 mmHg and 1.8 mM/*l* in sea mussel *M. edulis* at 12°C,[14] 0.15 kPa (2.0 mmHg) and 1.37 mM/*l* in Pacific oyster *C. gigas* at 15°C,[24] 1.15 mmHg and 1.62 mM/*l* in *M. galloprovincialis* at 18°C,[25] 2.08–2.33 mmHg and 1.83-2.04 mM/*l* in the akoya pearl oyster *P. fucata* at 28°C,[17] and 1.50 mmHg and 1.98 mM/*l* in black-lip pearl oyster *P. margaritifera*,[18] respectively. The acid-base status of the noble scallop will be almost same as it of those bivalves regardless of water temperature.

In decapod crustaceans, the hemolymph Pco_2 was 0.21 kPa (1.57 mmHg) in green crab *Carcinus maenas*, 0.32 kPa (2.4 mmHg) in blue crab *C. sapidus*, 0.28–0.46 kPa (2.1–3.45 mmHg) in Chinese mitten crab *Eriocheir sinensis*,[29,30] 0.44 kPa (3.3 mmHg) in European lobster *Homarus gammarus*,[31] and 0.268 kPa (2.01 mmHg) in American lobster *H. americanus*.[32] The hemolymph $[HCO_3^-]$ of these decapods were 5.5 mM/*l* in green crab *Carcinus maenas*, 4.2 mM/*l* in blue crab *C. sapidus*, 7.09–8.0 mM/*l* in Chinese mitten crab *Eriocheir sinensis*,[29,30] and 9.2–9.3 mM/*l* in European lobster *H. gammarus*.[31] In teleosts, blood Pco_2 was 2.0 mmHg in red sea bream *Pagrus major*,[33] 2.0–4.0 mmHg in starry flounder *Platichthys stellatus*,[34] 0.42–0.48 kPa (3.2–3.6 mmHg) in Atlantic cod *Gadus morhua*,[35] 3.47–4.98 mmHg in rainbow trout *Salmo gairdneri* (*Oncorhynchus mykiss*),[34] and 3.8–4.2 mmHg in the common carp *Cyprinus carpio*.[36,37] The $[HCO_3^-]$ was 8.3–9.2 mM/*l* in Atlantic cod *G. morhua*,[35] 8.8–9.3 mM/*l* in rainbow trout *S. gairdneri* (*O. mykiss*), [34] and 10.2–10.3 mM/*l* in the common carp *C. carpio*.[37] Marine bivalves could contain CO_2 and bicarbonate levels that are less than crustacean and teleosts; therefore, noble scallop seemed to utilize CO_2 for calcification of shell valves and discharge CO_2 to control the acid-base balance of the hemolymph.

On the other hand, the pH and Tco_2 changed significantly with the increase in Pco_2 (Table 2). The relationship of hemolymph pH and pKapp was shown (Fig. 1), and Pco_2 and $[HCO_3^-]$ were estimated on the basis of the correction equation. Hemolymph Pco_2 estimated by the correction equation was higher than that from the mean value of pKapp, nevertheless there was no significant difference in $[HCO_3^-]$ (Table 3). The αCO_2 and pKapp vary with ionic strength and temperature,[19] and estimation of Pco_2 could be affected by temperature and salinity. The hemolymph pH of noble scallops in normoxic conditions in this study (7.442, Table1) was higher than the *in vitro* pH range (5.990–7.213, Table 2). Therefore, it is necessary to examine these parameters in various temperature, salinity and pH conditions in order to increase the accuracy of the calculation of Pco_2 and to formulate the correction equation for pH, salinity and water temperature.

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