Ureogenic function and periodic urea excretion in a gobiid fish, *Mugilogobius abei*

*KATSUYA IWATA¹, MAKIKO KAJIMURA² AND TATSUYA SAKAMOTO³

¹Biological Labo. Faculty of Education, Wakayama University, Wakayama, 640-8510, Japan (katsuiw@center.wakayama-u.ac.jp), ²Department of Bio-and Geo-Sciences, Graduate School of Science Osaka City University, 558-8585, Japan, ³Graduate school of Biosphere Science, Hiroshima University, Higasi-Hiroshima, 739, Japan

SUMMARY: *M. abei* has a high ability to produce a large amount of urea, when exposed to 2mM NH_4HCO_3 or 1mM $^{15}\text{N}-(\text{NH}_4)_2\text{SO}_4$ solution. After exposure to ^{15}N -ammonia, ^{15}N -concentration of the amide-N in glutamine in tissues was virtually the same as that of ammonia-N, which were about 2-fold that of urea-N in the tissues as well as in the excreta. Glutamine contents and glutamine synthetase activities in the liver and muscle increased greatly following exposure to ammonia. CPSaseIII mRNA expression as well as CPSaseIII activity was found to be present in muscle, skin and gill, but not in liver, spleen and intestine. Furthermore, it was found that *M. abei* excreted urea with a periodic rhythm which might be mediated by a periodic enhancement of urea permeability at excretion sites.

KEY WORD: CPSaseIII, gobiid fish, diel periodicity, ornithine-urea cycle, urea, ureogenesis

INTRODUCTION

Only a very few teleost species are known to produce urea *via* ornithine-urea cycle (O-UC). Among the ureogenic teleosts discovered to date, ^{1,2,3)} the Lake Magadi tilapia *Alcolapia grahami* is known to be the only teleostean fish excreting all its nitrogenous waste as urea, i.e. ureotelic.⁴⁾ The other ureogenic teleosts are a functional ureogenesis, i.e. they can produce urea, only when exposed to adverse conditions such as hyper ammonia environment and crowding. Recently, we have revealed that a goby, *Mugilogobius abei*, Abehaze in Japanese has a high ability to produce urea through O-UC, when the fish is exposed to 2 mM NH_4HCO_3 .⁵⁾ This is the first finding of a ureogenic goby among gobiid fishes in the world. *M. abei* is a small goby (normally less than 1g) and its external features are those of water breathing gobiid fishes. *M. abei* lives in mud flat around the mouth of rivers, where Tobihaze, *Periophthalmus modestus* also lives. *M. abei* can be found out even in a heavily polluted area receiving sewage, where it is the sole fish fauna. In such place, the black

mud covers over the surface, so that redox potential values in the mud are usually strong negative. In the interstitial water of such sediment, ammonia concentration is usually very high occasionally reaching 2 mM or more. Indeed, this fish can live indefinitely in 2 mM NH_4HCO_3 without any adverse symptoms, but the majority of fish died in 4 mM NH_4HCO_3 within 24 hours. In this paper, we compare several aspects for urea production of *M. abei* with those of other teleostean species including ureotelic or ureogenic ones.

AMMONIOTELIC VS UREOGENIC

Fig.1 shows a comparison of urea excretion between a ureogenic teleost, *M. abei* and an ammoniotelic teleost, *P. modestus*, when they are exposed to 2mM NH_4HCO_3 dissolved in 20% sea water (SW). Urea excretion in *M. abei* following exposure to ammonia increased with time and reached 7-8-fold higher levels of the control fish (20%SW) on day 4 through 8. While those levels of

P. modestus remained unchanged, although an ability for ammonia tolerance of *P. modestus* was similar to that of *M. abei*.

Fig. 1 Comparison of urea excretion between ureogenic (*M. abei*) and ammoniotelic (*P. modestus*) teleosts following exposure to 2 mM NH_4HCO_3 . Both species have similar high ammonia tolerance.

To make it clear whether *M. abei* can efficiently incorporate external ammonia into the urea molecule, the fish was exposed to ^{15}N - $(\text{NH}_4)_2\text{SO}_4$ solution (^{15}N - $(\text{NH}_4)_2\text{SO}_4$ dissolved in 10% sea water and adjusted pH to 8 with NaHCO_3). Following exposure to ^{15}N - $(\text{NH}_4)_2\text{SO}_4$ solution, increasing manner of urea excretion with time was similar to that of Fig.1 and ^{15}N -concentration in excreted urea increased with time and reached a plateau level at day 3 to 4. After *M. abei* was exposed to ^{15}N -ammonia solution for 4 days, liver, muscle and whole remaining tissues were dissected, and ^{15}N -concentration in ammonia-N, amide-N in glutamine, urea-N and α -amino-N in amino acid fraction in these tissue extracts were measured. ^{15}N -concentration in ammonia-N and amide-N in glutamine were similar in all the tissues examined (60-64 atom% excess), but that of α -amino-N was low (2-3 atom% excess). ^{15}N -concentration in urea-N in tissues as well as the excreta showed just the middle values between ammonia- and amide-N and α -amino-N. These results indicate that ammonia entered in *M. abei* is rapidly incorporated into amide-N in glutamine, which is then converted to half part of urea-N. Glutamine formation is particularly important for

urea synthesis, since carbamoyl phosphate synthetase III (CPSaseIII), the key enzyme of O-UC, requires glutamine as nitrogen donor.

SITE OF UREA PRODUCTION

Table 1 shows the activities of enzymes related to O-UC in various tissues in *M. abei*. CPSaseIII activity could be detected in skin, gill and muscle, but liver had virtually no activity. Similarly, when CPSaseIII mRNA expression in various tissues of *M. abei* was surveyed by RT-PCR using specific primer of *M. abei*, CPSaseIII mRNA expression was found to be present in muscle, skin and gill, but not in liver, intestine and spleen. In accord with relatively higher CPSaseIII activities, skin and gill also have higher ornithine transcarbamoylase (OTCase) activities (Table 1). Thus, *M. abei* has a fully O-UC enzymes, but exhibited two unusual aspects. Firstly, CPSaseIII activities as well as CPSaseIII gene expression were found in extra-hepatic tissues. Secondly, when CPSaseIII is assayed with ammonia as nitrogen donor, enzymes from skin and muscle exhibited similar levels to those of glutamine.

Table 1 Activities of enzymes related to OUC in liver, muscle, skin and gill following exposure to ammonia for 4 to 5 days.

	Liver	Muscle	Skin	Gill
CPSaseIII	nmol/min/g	nmol/min/g	nmol/min/g	nmol/min/g
with glutamine	0.1 ± 0.0	2.7 ± 0.7	2.9 ± 0.4	1.9 ± 0.2
with ammonia	N.D.	1.5 ± 0.3	2.2 ± 0.5	N.D.
OTCase	µmol/min/g	µmol/min/g	µmol/min/g	µmol/min/g
ASSase + ASLase	0.13 ± 0.02	0.63 ± 0.13	3.13 ± 1.12	3.29 ± 1.21
ARGase	0.04 ± 0.01	0.12 ± 0.03	0.09 ± 0.00	0.26 ± 0.04
ARGase	7.16 ± 1.07	0.03 ± 0.01	0.40 ± 0.30	2.71 ± 0.54

Means ± S.E.M. (n=4-8), N.D. (not determined)

ASSase, argininosuccinate synthetase; ASLase, argininosuccinate lyase; ARGase, arginase.

Activities of enzymes in O-UC in the tissues of ammonia exposed fish were not different from those of control.

Among the ureogenic teleosts studied to date, except the Lake Magadi tilapia, the liver of ureogenic species is primary responsible for urea production as in mammal.^{2,3,7)} However, the muscle of the Lake Magadi tilapia is known to have very high CPSase III activity and to be a main site of urea production.⁶⁾ Furthermore, CPSaseIII

from the muscle of the Lake Magadi tilapia has a high capacity to use ammonia as a substrate, in addition to glutamine as the more usual substrate. Thus, CPSaseIII in muscle and skin from *M. abei* seems to have similar properties to those of the Lake Magadi tilapia, although activity in *M. abei* is extremely low as compared with that of the Lake Magadi tilapia. In *M. abei*, the muscle seems to play very important role for overall urea production in fish body, when tissue mass is taken into account.

STORAGE OF AMMONIA AND GLUTAMINE FORMATION IN MUSCLE

Table 2 shows changes in ammonia, urea and free amino acids contents in tissue extracts following exposure to ammonia. Ammonia and urea in muscle and liver increased greatly following exposure to ammonia. Among free amino acids examined, only glutamine showed a great increase in muscle and liver of the exposed fish. The level of alanine was low compared with glutamine, but significantly increased in muscle. On the other hand, other amino acid levels remained unchanged following exposure to ammonia. Ammonia and glutamine in muscle of the exposed fish account 61% and 65% of those in the whole body, respectively (if muscle make up 50% of body mass).

Table 2 Changes in ammonia, urea and free amino acids in liver and muscle following exposure to ammonia

	Liver		Muscle	
	Control μmol/g	Ammonia μmol/g	Control μmol/g	Ammonia μmol/g
Tau	7.74 ± 0.94	7.01 ± 0.64	12.6 ± 1.11	9.81 ± 0.82
Asp	0.33 ± 0.05	0.25 ± 0.02	0.37 ± 0.08	0.23 ± 0.06
Thr	0.55 ± 0.06	0.34 ± 0.03	1.14 ± 0.13	0.94 ± 0.18
Ser	0.51 ± 0.06	0.34 ± 0.04	0.77 ± 0.10	0.74 ± 0.07
Glu	2.89 ± 0.63	1.97 ± 0.24	0.58 ± 0.06	0.68 ± 0.14
Gln	1.73 ± 0.59	5.60 ± 1.22 **	1.12 ± 0.20	6.86 ± 1.53 **
Pro	0.59 ± 0.16	0.33 ± 0.12	0.61 ± 0.13	0.37 ± 0.11
Gly	0.89 ± 0.26	0.58 ± 0.18	9.39 ± 1.38	8.74 ± 0.73
Ala	0.97 ± 0.28	0.81 ± 0.11	0.87 ± 0.21	1.89 ± 0.29 *
	μmol-N/g	μmol-N/g	μmol-N/g	μmol-N/g
Am	3.59 ± 0.40	7.83 ± 1.00 *	4.98 ± 1.04	13.95 ± 1.16 **
Urea	3.51 ± 0.84	2.04 ± 0.42	2.85 ± 1.31	6.81 ± 1.35 **
Mean ± S.E.(n=10), * p<0.01, ** p<0.001				

Glutamine synthetase activity (GSase) in liver and muscle increased greatly following exposure to ammonia. Tissue specific activity

(unit/g tissue mass) for GSase was 15-fold higher in liver than that in muscle. However, total muscular activity of the ammonia-exposed fish was approximately 2-fold larger than total hepatic activity. These facts strongly suggest that the conversion of ammonia into the amide-N in glutamine, i.e. the first step of ammonia detoxification, occurs mainly in muscle. Thus, in *M. abei*, muscle plays a pivotal role not only in converting a large amount of ammonia into the amide-N in glutamine, but also in feeding this nitrogen into O-UC as the primary substrate for CPSaseIII.

Fig. 2 A hypothetical role of muscle, skin and gill of *M. abei* in producing urea under a hyper ammonia environment.

Fig.2 shows a hypothetical role of muscle, gill and skin for urea production under a hyper ammonia environment. When ammonia elimination from gill or skin is disturbed under a hyper ammonia environment, ammonia entered to fish body accumulates mainly in muscle, and a part of ammonia is then converted into glutamine and finally, amide-N in glutamine or probably ammonia is converted to urea molecule mainly in muscle. Furthermore, glutamine formed in muscle is carried away to gill or skin through circulation and then converted there to urea for excreting it. Thus, *M. abei* has unusual properties about urea production. Firstly, extra-hepatic tissues, particularly muscle are main site of urea production. Secondly, multiple tissues seem to cooperate for producing urea when ammonia elimination from the fish into environ-

ment is disturbed.

Finally, another unique aspect of urea excretion in *M. abei* is in that this goby excretes urea with a daily periodicity under a 12h light-dark cycle as shown in Fig. 3. A peak of urea excretion always occurred the middle of light phase. The periodicity in urea excretion was maintained even in continuous darkness, suggesting that this periodicity is controlled by a circadian rhythm. In contrast, the daily periodicity was not found in ammonia excretion. Among the ureogenic teleosts studied to date, the gulf toadfish, *Opsanus beta* is known to excrete urea once in a day, but with an irregular pulsatile manner. Therefore, the daily periodicity of urea excretion as found in *M. abei* seems to be unique even among ureogenic species. There is not enough space to write the detail, but in brief, some evidences we have found suggest that the periodicity for urea excretion is not caused by a periodic production of urea, but a periodic activation for urea permeability at the sites of excretion, probably gill and/or skin. However, the precise mechanism and role of periodic urea excretion in this goby are still unclear. Further studies are required.

Fig. 3 Urea and ammonia excretion of *M. abei* under a 12h light-dark cycle. A daily periodicity was found only in urea excretion.

ACKNOWLEDGMENT

This research was partly supported by Grants-in-Aid from the Japan Society for the Promotion of Science and the Fisheries Agency of Japan.

REFERENCES

1. Walsh JP, Danulat E, Mommsen TP. Variation in urea excretion in the gulf toadfish *Opsanus beta*. *Mar. Biol.* 1990; **106**: 323-328.
2. Saha N, Ratha BK. Active ureogenesis in a freshwater air-breathing teleosts, *Heteropneustes fossilis*. *J. Exp. Zool.* 1987; **241**: 137-141.
3. Saha N, Das L, Dutta S. Types of carbamoyl phosphate synthetase and subcellular localization of urea cycle and related enzymes in air-breathing walking catfish, *Clarias batrachus*. *J. Exp. Zool.* 1999; **283**: 121-130.
4. Randall DJ, Wood CM, Perry SF, Bergman H, Maloiy GMO, Mommsen TP, Wright PA. Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature* 1989; **337**: 165-166.
5. Iwata K, Kajimura M, Sakamoto T. Functional ureogenesis in the gobiid fish, *Mugilogobius abei*. *J. Exp. Biol.* 2000; **203**: 3703-3715.
6. Lindley TE, Scheiderer CL, Walsh PJ, Wood CM, Bergman HL, Bergman AL, Laurent P, Wilson P, Anderson PM. Muscle as the primary site of urea cycle enzyme activity in an alkaline lake-adapted tilapia *Oreochromis alcalicus grahami*. *J. Biol. Chem.* 1999; **274**: 29858-29861.
7. Walsh PJ, Milligan CL. Effects of feeding and confinement on nitrogen metabolism and excretion in the gulf toadfish *Opsanus beta*. *J. Exp. Biol.* 1995; **198**: 1559-1566.