The characteristics of lactate dehydrogenase (LDH) and the relationship between LDH and its inhibitor in mosquito larvae (Diptera: Culicidae)

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Abstract: Lactate dehydrogenase (LDH) activity is generally detected by reversible reaction using a combination of a substrate and a coenzyme, that is lactate and NAD (NAD-dependent LDH), or pyruvate and NADH (NADH-dependent LDH). In *Anopheles stephensi* larvae, NAD-LDH and NADH-LDH activities were high and were easily detected after polyacrylamide gel electrophoresis (PAGE), while in *Culex pipiens molestus* larvae, NADH-LDH could be detected, but NAD-LDH showed very low activity. According to enzyme histochemical studies, *Cx. p. molestus* had NAD-LDH at the same localities as *An. stephensi* did, but its activity was very low. NAD-LDH activity of *Cx. p. molestus* larvae is, therefore, greatly inhibited by the LDH-inhibitor, which is present in the cytosol of the gastric caeca and midgut, and also in the haemolymph. Among the eight mosquito species examined, LDH-inhibitory activity was highest in the species with low NAD-LDH activity, such as *Cx. p. molestus*. The LDH-inhibitor was seen to specifically inhibit NAD-LDH activity. Although lactate accumulated in *An. stephensi* larvae, both NAD-LDH and NADH-LDH were active in this species. While in *Cx. p. molestus* larvae, although neither lactate nor pyruvate was accumulated, NAD-LDH activity was very low. The LDH-glycolysis could be less active. These results suggested that *Cx. p. molestus* larvae use some other glycolysis.

INTRODUCTION

Lactate dehydrogenase (LDH; EC 1.1.1.27) is widely present in animals, plants and microorganisms, and is one of the terminal enzymes of the glycolysis. In mammals, LDH is distributed mostly in the cytosol of the cells in the heart and skeletal muscles. LDH is known as a clinical marker for the detection of cancer and some other diseases. LDH has, therefore, been analyzed routinely in the clinical field for humans (Eropkin et al., 1995). In *Drosophila melanogaster* (Meigen), higher LDH activities were found in the skeletal muscles and imaginal discs of larva, and

developing somatic muscles of embryos (Rechsteiner, 1970a, Abu Shumays and Fristrom, 1997). LDH is also probably one of the most important enzymes for mosquitoes and is used for diagnosis of the mosquitoes infected by pathogens. Recently, LDH has drawn attention as a possible marker of *Plasmodium* infection in malaria vector mosquitoes (Riandey et al., 1996). However, few enzymatic studies have been performed on mosquito LDH, and these were made in connection with isozyme polymorphism in the field of the population genetics (Adak et al., 1994).

LDH catalyzes the reversible reaction between a substrate of pyruvate or lactate

using a coenzyme of NADH or NAD, namely, NADH-dependent LDH activity (NADH-LDH) and NAD-dependent activity (NAD-LDH), respectively. NAD-LDH activity in almost all mosquito larvae can be easily detected after polyacrylamide gel electrophoresis (PAGE) by a reaction using lactate and NAD. However, Tsukamoto (1982) reported that larvae of some mosquito species, such as the *Culex pipiens* (L.) group, showed no detectable NAD-LDH activity band using PAGE. In a study of *Cx. p. molestus* (Forskål), when the digestive tract was removed from the larval whole body, the enzyme preparations of the remainder demonstrated NAD-LDH activity as an electrophoretic band on the gel. Tsukamoto (1982) indicated the presence of a large amount of a "natural inhibitor" to LDH activity in the digestive tract of *Cx. p. molestus* larvae. The present study demonstrates the relationship between these two activities of LDH and the LDH-inhibitor of mosquito larvae and discusses the substance of the inhibitor.

MATERIALS AND METHODS

Mosquitoes used. *Anopheles stephensi* (Liston) (Beach strain) obtained from Sumitomo Chemical Co. Ltd. (Takarazuka, Japan) has been maintained at our laboratory according to the methods of Tsukamoto (1982, 1984). *Anopheles balabacensis* (Baisas) Perlis form (IMR) (Kanda et al., 1983) was from St. Marianna University School of Medicine, Kawasaki, Japan. *Culex p. molestus* and *Cx. p. pallens* (Coquillett) were obtained from the Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, and have been maintained at our laboratory. *Culex tritaeniorhynchus* (Giles) was obtained from the National Institute of Infectious Diseases, Tokyo, Japan. *Aedes togoi* (Theobald) was collected at Shirashima Island of Kitakyushu City, Kyushu Island, Japan, in 1991. *Aedes aegypti* (L.) was obtained from the Institute of Tropical Medicine, Naga-

saki University, and has been maintained at our laboratory. *Toxorhynchites klossi* (Edwards) was collected at Cameron Highland, Malaysia, in 1988, and has been maintained at our laboratory according to the methods of Tsukamoto and Horio (1985a).

Larvae at the end of the 4th-instar were used in this study. During the experiments, all species of larvae were fed on a powdered mixture of Ebios (dried brewer's yeast, Tanabe Pharmaceutical, Osaka, Japan), liver powder (baby food, Yukijirushi, Hokkaido, Japan), and Tetramin (tropical fish food, Tetra-Werke, Melle, Germany).

Dissection. The digestive tract including feces (D) was removed from the whole body (W) of a larva, and the remainder (W minus D: W-D) was dissected on a slide glass under a stereo microscope. The feces were squeezed out from the digestive tract, and the D sample was separated into feces (F) and the true digestive tract. The digestive tract was rinsed in phosphate buffered saline (PBS) (128 mM NaCl, 5 mM KCl, 2 mM CaCl₂) for a few minutes, and cut into 3 parts: gastric caeca (GC), midgut (MG) and hindgut (HG). To collect haemolymph (H), the thorax of a larva was injured and the larva was put into a micro tube (1.8 ml Eppendorf tubes) containing 20 μ l of PBS. The larva was centrifuged in PBS at 1,000 *g* for 5 min. All samples were rinsed in PBS for a few minutes and homogenized in 20 μ l of PBS, except for W-D of *An. stephensi*, which was homogenized in 30 μ l of PBS, using a Teflon homogenizer on ice and then centrifuged at 10,000 *g* for 10 min at 4°C. Each supernatant was decanted into a new tube and stored at -80°C until analysis.

Measurement of LDH activity. Both NAD-LDH and NADH-LDH activities were separately detected by PAGE and/or spectrophotometry. A 5% horizontal PAGE was performed at 4°C according to the method of Tsukamoto (1982). Five microliters of each sample was loaded into the

sample slot. For the measurements of NAD-LDH and NADH-LDH activities, sodium DL-lactate (Nacalai Tesque Inc., Kyoto, Japan) and NAD (Kohjin Co., Ltd., Tokyo, Japan), and pyruvic acid (Sigma Chemical Co., St. Louis, MO, USA) and NADH (Kohjin Co., Ltd., Tokyo, Japan) were used, respectively. After the electrophoresis and enzyme band detection, the gel films were dried, and each activity was measured using a densitometer (Amersham Pharmacia Biotech Inc., Uppsala, Sweden). For NADH-LDH activity, 0.1 ml of each sample with PBS was agitated into 2.85 ml of a phosphate-pyruvate mixture (50.2 mM K_2HPO_4 , 8.3 mM KH_2PO_4 , 0.34 mM $CH_3COCOONa$) and 50 μ l of NADH solution (10 mg NADH $\cdot$ 2Na, 1.5 ml phosphate-pyruvate mixture) for ca. 5 min. NADH-LDH activity was then measured using a spectrophotometer at 340 nm absorbance. Each value obtained by densitometry and spectrophotometry was calculated from a standard curve on L-lactic dehydrogenase Type II (Sigma Chemical Co.).

Gel filtration. W-D sample obtained from 50–70 larvae was homogenized with 1 ml of PBS using a teflon homogenizer on ice, and centrifuged at 10,000 g for 10 min at 4°C. The supernatant was fractionated by gel filtration using a Sephadex G-100 column (40 $\times$ 1.6 i.d. cm) equilibrated with 0.05 M Tris-HCl, pH 7.4, and LDH activity was detected using a spectrophotometer at 280 nm absorbance. Each fraction of the gel filtration was eluted at 15 ml per hr. at 4°C, and 10 ml was collected from each. Both NAD-LDH and NADH-LDH activities were measured by 5% PAGE as described above.

Histochemical localization of LDH. For fixation, a mosquito larva was immersed in 100 mM of PBS (pH 7.4) containing 4% paraformaldehyde (PFA) for 30 min at room temperature. The fixed larva was rinsed extensively in 100 mM PBS before embedding. One drop of O.C.T. compound (Tissue Tek, 4583, Miles) was dropped

onto a paper frame (8 $\times$ 8 mm) placed on a holder. The holder was immersed halfway into liquid N_2 until the O.C.T. compound began to freeze at the bottom. The holder was removed from the liquid N_2 and placed on a flat surface. The fixed larva was placed on the unfrozen surface inside of the paper frame, and a second drop of O.C.T. compound was added. The holder was immersed again halfway into liquid N_2 until the frame froze completely. The reaction for NAD-LDH activity was carried out by the methods of Evans and Butcher (1979), Frederiks et al. (1983), and Frederiks and Marx (1989).

Measurement of lactate and pyruvate. Ten W-D samples were homogenized with 0.5 ml of PBS using a teflon homogenizer on ice. The homogenate was agitated in 1.5 ml of 5% metaphosphoric acid (Nacalai Tesque Inc.) on ice and then centrifuged at 10,000 g for 10 min at 4°C. For lactate, 0.1 ml of supernatant was agitated in 2 ml of a glycine-hydrazine mixture (1 M glycine, 2% hydrazine hydrate, NaOH [pH 9.0]). Thirty microliters of LDH solution (3 mg/ml of L-lactic dehydrogenase with 0.85% PBS) and 0.2 ml of 20 mg/ml NAD were incubated for 14 min, and then measured using a spectrophotometer at 340 nm absorbance (A_{sample}). Both absorbances of blank (A_{blank}) and lactate standard (A_{standard}) were measured using 0.1 ml of 3% metaphosphoric acid and 1.0 mM L-lactate in 5% metaphosphoric acid, respectively, after agitating with 2 ml of a glycine-hydrazine mixture. Values were calculated as follows:

$$\text{Lactate (mM)} = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{standard}} - A_{\text{blank}}) \times 1.0 \times 4$$

For pyruvate, 1.0 ml of the sample supernatant was agitated with 0.5 ml of 0.75 M tris (hydroxymethyl) aminomethane hydrochloride and 30 μ l of NADH solution (10 mg/ml of NADH with 1% $NaHCO_3$) and then measured using a spectrophotometer at 340 nm absorbance. Thirty microliters of LDH solution was added

and measured at 340 nm absorbance after 2 min. The value of each sample (ΔA_{sample}) was indicated as the difference between the initial absorbance and the final absorbance. For the blank (ΔA_{blank}) and the pyruvate standard ($\Delta A_{\text{standard}}$), 1.0 ml of 3% metaphosphoric acid and 1.0 ml of 0.05 mM sodium pyruvate in 5% metaphosphoric acid were used, respectively, instead of samples, and measured using a spectro-

photometer at 340 nm absorbance. Values were calculated as follows:

$$\text{Pyruvate (mM)} = (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) / (\Delta A_{\text{standard}} - \Delta A_{\text{blank}}) \times 0.05 \times 4$$

RESULTS

Mosquito NAD-LDH and its inhibitor. Electrophoretic bands of NAD-LDH activity were obtained from *An. stephensi* and *Ae. aegypti* W-D samples on the gel at different band mobility (Fig. 1). The highest activity was shown in *An. stephensi*, followed by *Ae. aegypti*. NAD-LDH activity was also observed in *Cx. p. molestus* W-D, but it was almost unmeasurable on the gel (the PAGE profile was omitted in this text). When the D samples were mixed with each W-D, the inhibitory activity of the D sample to NAD-LDH showed that *An. stephensi* was the lowest, followed by *Ae. aegypti*, and *Cx. p. molestus* was the highest.

The inhibitory activity to NAD-LDH on the separated digestive tract was estimated after dissection. For *Cx. p. molestus*, the highest LDH-inhibitory activity was obtained from the feces, showing nearly 100% inhibition, followed by haemolymph (Fig. 2). In the digestive tract

Fig. 1. LDH and LDH-inhibitor activities of *An. stephensi* and *Ae. aegypti* larvae detected by 5% PAGE using lactate and NAD. Lanes 1 and 5, W-D samples of *An. stephensi* and *Ae. aegypti*, respectively. Lanes 2 and 6, each W-D mixed with D of *An. stephensi* (1:1, v/v); lanes 3 and 7, mixed with D of *Ae. aegypti*; lanes 4 and 8, mixed with D of *Cx. p. molestus*. Dissections are described in "Materials and Methods".

Fig. 2. The presence of LDH-inhibitor in mosquito larvae. Ten *Cx. p. molestus* larvae were individually dissected and the inhibitory activity was measured using a densitometer after 5% PAGE using lactate and NAD. Each value indicates the average with 95% CI.

GC, gastric caeca; MG, midgut; HG, hindgut; F, feces; H, haemolymph.

Fig. 3. Enzyme histochemical localization of LDH in the abdomen of *An. stephensi* (left) and *Cx. p. molestus* (right) larva.

MG, midgut; FB, fat body; F, feces.

Fig. 4. PAGE analysis of LDH activity in larvae of *An. stephensi* (Ans) and *Cx. p. molestus* (Cpm). Lanes 1, 3, detection using lactate and NAD; lanes 2, 4, using pyruvate and NADH. Each W-D sample used was obtained from one and three larvae of *An. stephensi* and *Cx. p. molestus*, respectively. Arrows indicate electrophoretic bands of LDH activity, which are not clear but are visible for Cpm.

after feces were removed and rinsed with PBS, 47.1 and 44.7% inhibitions were obtained from GC and MG, respectively, indicating significantly higher inhibitory activities than that from HG (12.6%) (GC:

$\chi^2=28.42$, MG: $\chi^2=25.20$, $df=1$, $P<0.001$ in both cases).

As shown in Fig. 3, the enzyme histochemical profiles in *An. stephensi* showed high activity of NAD-LDH localized in the fat body and the midgut epithelium, and low activity in feces. While in *Cx. p. molestus*, NAD-LDH activity was observed at the same localities as in *An. stephensi*, the activity was much lower than that in *An. stephensi*. *Culex p. molestus* had a comparatively smaller amount of NAD-LDH than *An. stephensi*, but the activity was at detectable levels, as shown in the PAGE profile in Fig. 1.

Comparison between NAD-LDH and NADH-LDH. The LDH activity is also detected by another reaction using pyruvate and NADH (NADH-LDH). In *An. stephensi*, W-D sample obtained from one larva showed both NAD-LDH and NADH-LDH activities at the same band mobility on the gel and showed the same level of activity by densitometry (Fig. 4). On the other hand in *Cx. p. molestus*, when W-D samples from three larvae were applied, NAD-LDH activity appeared as only a single narrow band having very low activity, but NADH-LDH activity was detected as many narrow bands.

Fig. 5. Gel filtration for LDH activity in W-D of *An. stephensi* and *Cx. p. molestus* larvae. Each W-D sample was prepared from 50–70 larvae and applied to gel filtration on Sephadex G-100. Both NAD-LDH and NADH-LDH activities of each fraction were assayed by 5% PAGE. See "Materials and Methods" for details.

Fig. 6. Pyruvate and lactate in *An. stephensi* (Ans) and *Cx. p. molestus* (Cpm) larvae. Both activities of pyruvate (open bar) and lactate (slashed bar) were measured from each 10 W-D sample using a spectrophotometer at 340 nm absorbance. Each value indicates the average with 95% CI.

The elution profiles of both NAD-LDH and NADH-LDH activities were obtained by gel filtration and compared between *An. stephensi* and *Cx. p. molestus* (Fig. 5). In *An. stephensi*, both LDH active peaks overlapped in elution volume and enzyme activity (height). In *Cx. p. molestus*, a

larger and faster peak fraction was obtained from NADH-LDH, but a smaller and slower peak for NAD-LDH. These elution profiles show that all LDH activities were observed during 25–35 ml of elution volume, except for the NADH-LDH of *Cx. p. molestus*, which was less than 20 ml. Their molecular weights were calculated as 40–48 KDa, except for the NADH-LDH of *Cx. p. molestus*, the molecular size of which was 146 KDa.

When either side of the lactate and pyruvate-catalytic reactions is inactive, the corresponding pyruvate or lactate metabolite is expected to be accumulated. Figure 6 shows the amounts of pyruvate and lactate. *Anopheles stephensi* had a significantly higher amount of lactate (34.667 mM) than pyruvate (0.255 mM) ($t=20.084$, $df=18$, $P<0.001$). This result shows that lactate accumulated in *An. stephensi*, but pyruvate did not. On the other hand in *Cx. p. molestus*, neither lactate (1.372 mM) nor pyruvate (1.127 mM) accumulated ($t=0.31$, $df=18$, $P>0.5$).

LDH and LDH-inhibitory activities in

Fig. 7. The relationship between LDH-inhibitory activity and LDH activity in eight strains of larvae derived from 4 different species of mosquitoes. Cpm, *Cx. p. molestus*; Ct, *Cx. tritaeniorhynchus*; Cpp, *Cx. p. pallens*; At, *Ae. togoi*; Ae, *Ae. aegypti*; Anb, *An. balabacensis*; Ans, *An. stephensi*; Tx, *T. klossi*. The abscissa describes the relative activity of NADH-LDH to NAD-LDH. For inhibitory activity to LDH, each D sample was incubated with a W-D sample of *An. stephensi* (1:1, v/v). NAD-LDH activity was measured using a densitometer after 5% PAGE using lactate and NAD, and NADH-LDH by spectrophotometry at 340 nm absorbance using pyruvate and NADH.

mosquito species. The correlation between the LDH-inhibitory activity and the relative activity of NADH-LDH to NAD-LDH is shown in Fig. 7. A highly positive correlation was obtained among the eight different species ($r=0.894$, $t=4.887$, $df=6$, $P<0.01$). The inhibitory activity to NAD-LDH was highest in the species with high NADH-LDH activity. This inhibitor specifically inhibited NAD-LDH activity.

DISCUSSION

Tsukamoto (1982) reported in a previous study that larvae of some *Culex pipiens* groups had a large amount of a "natural inhibitor" to NAD-LDH activity in their digestive tract. We reconfirmed in the present study that *Cx. p. molestus* larvae have the LDH-inhibitor in the cytosol of the gastric caeca and midgut. This

inhibitor acts when the larval whole body is homogenized, and is also present in the inside of the gut and haemolymph of mosquito.

LDH is one of the terminal enzymes of the glycolysis and is widely present in animals, plants and microorganisms. It is evident that LDH is present in the mosquito species studied. However, for larvae of *Cx. p. molestus*, almost unmeasurable NAD-LDH activity was obtained from the whole body (W) enzyme preparations, and when the digestive tract (D) was removed from the whole body, the remainder (W-D) preparations demonstrated NAD-LDH activity. We, therefore, assumed that LDH activity would be inhibited when the whole body homogenates of mosquito larvae were used for PAGE analyses. If the digestive tract and haemolymph were removed completely, LDH activity would be clearly obtained from the remainder (W-D). As we expected, the enzyme histochemical profiles showed that NAD-LDH localized also in the *Cx. p. molestus* larval abdomen at the same localities as in *An. stephensi*. However, the NAD-LDH activity of *Cx. p. molestus* was much lower than that of *An. stephensi*. This result suggests that *Cx. p. molestus* NAD-LDH could be inhibited by some kind of endogenous inhibitor of the larvae. This inhibitor seems to be produced mostly in the gastric caeca and midgut, and then secreted into the inside of the gut and haemolymph. To know the biochemical nature of this inhibitor, we need to discuss not only its enzymatic inhibitory activity, but also the controlling LDH-gene expression.

LDH activity is inhibited by some toxins (cocaine; LeDuc et al., 1994), medicines (mercuric chloride; Gill et al., 1990) and chemicals (oxalate and glutamate; Javed et al., 1995). Some substances present in human body and in the haemolymph of insects could also act as a natural inhibitor to LDH. These include proteins such as trypsin and chymotrypsin (Wang and Srivastava, 1994), and metal ions such as I^- (Arnold and Kaplan, 1974)

and Cu^{2+} , Co^{2+} and Mn^{2+} (Javed et al., 1995). Our results suggested that the natural inhibitor to LDH in mosquitoes is not metal ions but, rather, some protein-like substances, because, although the same diet was supplied to the larvae of all the species used in this study, some degree of LDH-inhibitory activity was obtained from almost every mosquito species. It is difficult to imagine that great differences are found in the metal ion content of different species reared under the same conditions. However, reliable evidence that this LDH-inhibitor is not a metal ion has not been obtained. More detailed studies are needed to confirm this point, and we plan to study this as the next step of our investigation.

It has been known that LDH activity is generally detected using either bi-directional reactions of NAD-LDH or NADH-LDH. However, in *Cx. p. molestus*, NADH-LDH indicated a very different characteristic from NAD-LDH in both PAGE and elution profiles. The LDH had several isozymes or sub-units, e.g. a lizard, *Uromastix hardwickii*, has six and four isozymes in the skeletal muscles and liver, respectively (Javed et al., 1995), and a bacteria, *Nocardia asteroides*, shows LDH trimmer (Ike et al., 1992). However, in mosquitoes, the band patterns of NAD-LDH showed some variation such as in numbers and mobility (Tsukamoto and Horio, 1985b). We thus speculate that the NADH-LDH of *Cx. p. molestus* could also be constructed by multiple loci, and/or shows several biochemical characteristics. In addition, there is a possibility that some physiological actions inside the mosquito body may affect the appearance of the enzyme active band. Further information is needed to discuss these and other possibilities. The results of this study suggest that, at least for *Cx. p. molestus*, the target of LDH-inhibitory action should be considered separately as NAD-LDH and NADH-LDH.

In *An. stephensi*, lactate was accumulated. However, as both NAD-LDH

and NADH-LDH were very active, we can easily predict that the accumulated lactate would gradually be converted to pyruvate. In *Cx. p. molestus*, on the other hand, neither lactate nor pyruvate accumulated. However, as NAD-LDH activity was very low in this species, the lactate-pyruvate reversible reaction is suggested to be less active, contrary to *An. stephensi*. *Culex p. molestus* larvae may use a different glycolysis from *An. stephensi*.

In the glycolysis, coenzyme NADH competes between LDH and alpha-glycerolphosphate dehydrogenase (α -GPDH). Either LDH or α -GPDH is activated by some internal conditions, such as pH, aerobiosis/anaerobiosis, developmental stage, specific organs and tissues. Under a high NADH-LDH condition, NADH is used for the reduction of pyruvate, and lactate is finally produced. On the other hand, under a low NADH-LDH (high- α -GPDH) condition, NADH reduces dihydroxyacetone-phosphate and finally produces glycerol. Rechsteiner (1970b) reported for *D. melanogaster* that LDH activity is higher than α -GPDH in larvae, while in adults, α -GPDH is higher than LDH. The α -GPDH activity appears mainly at the fat body and flight muscles (Rechsteiner, 1970; Hansford and Sacktor, 1970), and associates with the lipid synthesis. An analysis of the relationship between LDH and α -GPDH in mosquitoes is in progress.

Sawabe and Moribayashi (2000) reported that *Cx. p. molestus* larvae had a higher level than *An. stephensi* larvae of neutral lipids and composition of linoleic acid (C 18:2), which is a precursor of arachidonic acid. We discussed that these characteristics of the lipid utilization for *Cx. p. molestus* might play a role in inducing ovarian development in autogenous mosquitoes. We, therefore, speculate that the relationship between two activities of LDH and the LDH-inhibitor could be associated with some mosquito physiological characteristics requiring lipid reserves, such as autogeny, overwintering and desiccation resistance.

The LDH-inhibitory activity was strongly related to the level of relative activity of NADH-LDH/NAD-LDH among the eight mosquito species examined. LDH-inhibitory activity was highest in the species with low activity of NAD-LDH. This indicates that LDH-inhibitor specifically inhibited NAD-LDH activity. Among the examined species, two anopheline mosquitoes, *An. balabacensis* and *An. stephensi*, having a low level of LDH-inhibitor, are anautogenous. *Culex p. molestus* and *Ae. togoi* display an obligatory autogeny. *Culex p. pallens* and *Cx. tritaeniorhynchus* do not show the autogeny, but the *Culex pipiens* group shows variation in the degree of autogeny. In addition, an adult of *Cx. tritaeniorhynchus* shows overwintering, and *Cx. p. pallens* enters diapause at the adult stage in Japan. *Aedes aegypti* populations are known to be mostly anautogenous. However, some wild African populations include some autogenous females (Trpis, 1977). For almost all *Toxorhynchites* species, sugar is not an essential nutritional requirement for autogenous ovarian development (O'Meara, 1985). *Aedes aegypti* can survive even under certain dry conditions at the egg and adult stages. Increased lipid content is associated with starvation, and desiccation resistance was reported in *D. melanogaster* by Service et al. (1985, 1988) and in *Ae. aegypti* by Sawabe and Mogi (1999). In the next study, we hope to clarify the glycolysis for *Cx. p. molestus*.

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