METABOLIC FATE OF PYRUVATE DEPENDS ON NUTRITIONAL STATUS IN THE SEA ANEMONE METRIDIUM SENILE

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An important area of research among evolutionary biologists is the demonstration of mechanisms by which genetic variation might lead to differences in fitness among individuals in a population. Previous radiotracer studies showed that genetic variants (allozymes) of glucose-phosphate isomerase differentially modulate levels of pentose shunt metabolism in the sea anemone Metridium senile (Zamer and Hoffmann, 1989, Proc. Natl. Acad. Sci. USA, 86:2737-2741). The modulation by the allozymes occurs only in fed anemones that have been acclimated to low temperature. Thus there are interactive effects of temperature, nutritional status and functional properties of the allozymes on the organization of metabolism in these animals. More information is needed about specific features of metabolism to understand better these interactions, and to determine whether the effects operate in situ. If genetic modulation of metabolism can be demonstrated in natural populations of Metridium, only then may the modulation be interpretable as adaptive. Such modulation under natural conditions has not been demonstrated. On the contrary, previous studies revealed metabolic differences between laboratory-held anemones and those tested soon after collection from a population near MDIBL.

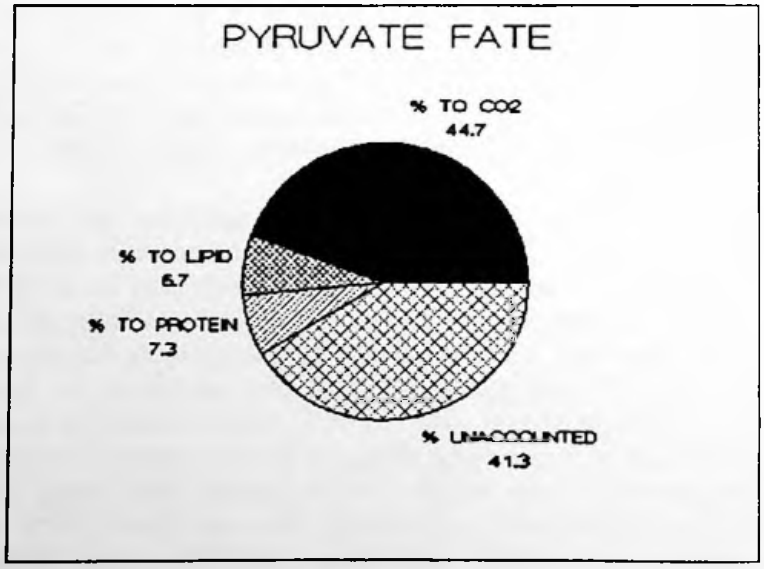
Glycolytic flux measured in field anemones (318 pmol glucose.mg wet $wt^{-1}.h^{-1}$) is greater than the rate measured previously in lab-held anemones that had been fed Artemia sp. nauplii (176 pmol glucose.$mg^{-1}.h^{-1}$), but glucose oxidation to CO_2 is the same in both groups of anemones [(23 pmol glucose.$mg^{-1}.h^{-1}$); Zamer and Hoffmann, 1989]. This difference indicates that more pyruvate is generated by increased glycolytic flux in field anemones compared to lab held-anemones, but that the added amount of pyruvate is not catabolized to CO_2 in the tricarboxylic acid (TCA) cycle.

More pyruvate carbon may be used for lipid synthesis in field anemones than in lab-held anemones. Lipid is the major energy store in cnidarians. Pyruvate carbon is assimilated into citrate in the TCA cycle, and in turn may be used for lipid biosynthesis in the cytosol. To test this hypothesis, radial tissue sections from anemones freshly collected by SCUBA (June and July from Bartlett's Landing) were incubated in a medium containing [^{14}C]-3-pyruvate (1 mM pyruvate, final concentration, in seawater; 15°C; specific radioactivity: 1.811 dpm.pmol $pyruvate^{-1}$). $^{14}CO_2$ was collected in hyamine hydroxide. Glycolytic flux was measured by incubating tissue slices in a seawater medium containing 1 mM glucose (final concentration) to which [^{3}H]-5-glucose had been added (specific radioactivity: 479.3 dpm.nmol $glucose^{-1}$). Glycolytic flux was determined from the amount of $^{3}H_2O$ produced metabolically during incubation (see Zamer and Hoffmann, 1989 for details). Lipid and protein were extracted from the tissues using standard biochemical techniques to determine incorporation rates of pyruvate into these biochemical fractions. Pyruvate utilization from the medium was determined as the difference in radioactivity in the medium before and after tissue incubation. Liquid scintillation methods were used to quantify radioactivity in all of these samples. Similar experiments have been performed on lab-held anemones, fed Artemia nauplii.

Higher glycolytic flux in field anemones compared to lab-held anemones has been confirmed [331 ± 21 (S.E.; n=20 anemones) and 292 ± 17 (n=15) pmol glucose.$mg^{-1}.h^{-1}$, respectively]. Pyruvate utilization was 1.97 ± 0.06 (S.E.) nmol pyruvate.$mg^{-1}.h^{-1}$ (n=8) in field anemones, similar to utilization in lab-held anemones that had been fed within 12 h of the experiment (1.97 ± 0.09 nmol pyruvate.$mg^{-1}.h^{-1}$, n=8). However, pyruvate utilization rate declined in lab-held anemones that had been fed 12-30 h before an experiment (1.26 ± 0.06 nmol pyruvate.$mg^{-1}.h^{-1}$, n=16). Field anemones feed on zooplankton and detritus at this time of year. These results indicate that pyruvate utilization increases with food availability.

Pyruvate oxidation to CO_2 was lowest in field anemones (878 ± 19 (S.E.) pmol pyruvate.$mg^{-1}.h^{-1}$, n=8). Recently-fed lab anemones had a higher oxidation rate (1393 ± 53 pmol pyruvate.$mg^{-1}.h^{-1}$, n=8) than those individuals that had been fed 12-30 h before an experiment (1097 ± 55 pmol pyruvate.$mg^{-1}.h^{-1}$, n=16). Although recently-fed anemones had a higher oxidation rate, it accounts for 71% of the pyruvate utilization rate in these anemones, compared to oxidation rates accounting for 88% of pyruvate utilization in anemones fed 12-30 h previously. The comparable figure for the field anemones is 45%. Therefore, a smaller fraction of the utilized pyruvate was catabolized to CO_2 at the higher levels of pyruvate utilization. No information is available on the relative importance of different pathways that could produce CO_2 from pyruvate, but presumably direct oxidation in the TCA cycle is the primary route.

Approximately 7% of utilized pyruvate was incorporated into each of the lipid and protein fractions (figure). Field anemones are probably growing at this time of year, so that these levels of pyruvate incorporation may reflect a synthetic poise of the tissues. About 45% of the utilized pyruvate was catabolized to CO_2; and forty-one percent is unaccounted. Much of the latter may be incorporated into free amino acids. Tissue studies from lab-held anemones are not available at this time. The large fraction of utilized pyruvate that was catabolized to CO_2 in lab-held anemones may reflect discontinuous food availability or differences in nutritional quality between field and lab diets.



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