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SHORT COMMUNICATION

COMPARTMENTATION OF LIVER PHOSPHOENOLPYRUVATE CARBOXYKINASE IN THE AQUATIC TURTLE *PSEUDEMYS SCRIPTA ELEGANS*: A REASSESSMENT

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Phosphoenolpyruvate carboxykinase (PEPCK) catalyses the conversion of oxaloacetate to pyruvate in the first step of gluconeogenesis. Since oxaloacetate is impermeable to the inner mitochondrial membrane, the localisation of PEPCK within the cell plays a major role in defining substrate preferences for gluconeogenesis. As a result, the immunochemically distinct isoenzymes of PEPCK found within the mitochondrial and the cytosolic cell fractions vary in their proportion to one another between organs and species and with prandial state.

For animals that routinely undergo periods of prolonged anoxia supported by anaerobic glycolysis (with the accumulation of tissue lactate), the localisation and activity of PEPCK may have an influence on the rate at which lactate is used to replete glycogen on recovery. The red-eared turtle (*Pseudemys scripta elegans*) is such an animal, with anoxic dives characteristic of this species lasting up to 4 weeks at 3˚C (Ultsch, 1985). During 24h of forced anoxic submergence at 22˚C, liver lactate accumulates to 45mmol l^{-1} and liver glycogen is depleted by 83% (Penney, 1974). In this species, a previous study reported that 72% of hepatic PEPCK activity was localised in the cytosol (Penney and Kornecki, 1973). This would tend to suggest that gluconeogenesis is not specific in its source of carbon for pyruvate formation. Since previous studies from our laboratory have noted a predominant proportion of liver mitochondrial PEPCK activity in the western painted turtle, a related aquatic turtle species (*Chrysemys picta bellii*, Buck *et al.* 1993), we undertook to re-examine the intracellular localisation of PEPCK in the red-eared turtle.

Adult red-eared turtles were obtained from a local supplier (Delta Aquatics, BC) and kept in an outdoor tank equipped with a flow-through water supply at 20˚C and with a basking platform. Animals were fed chopped beef heart and liver *ad libitum* and were sampled over a 4 week period in mid summer.

After the turtle had been killed by decapitation and the plastron had been removed, a 1 g sample of liver tissue was taken from the right hepatic lobe and placed in 5 volumes of

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ice-cold homogenisation buffer (in mmol l^{-1}): 250 sucrose, 2 EDTA and 20 Hepes (pH7.2). The tissue was finely minced using chilled razor blades and homogenised with one pass of 10s duration using an Ultra Turrax homogeniser set to a medium speed. The resulting slurry was centrifuged at 300 ***g*** for 10min on a Sorvall RC-5 centrifuge to remove large particulate matter. The supernatant was retrieved and centrifuged at 8000 ***g*** for 10min to pellet mitochondria. The supernatant resulting from this final centrifugation step was decanted and retained on ice for assay (cytosolic fraction). The mitochondrial pellet was resuspended in homogenisation buffer and sonicated twice for 10s, centrifuged to remove cellular debris and assayed as above (mitochondrial fraction). Activities of PEPCK, citrate synthase (CS) and lactate dehydrogenase (LDH) were determined immediately after the preparation of each fraction by the method of Suarez *et al.* (1985) using a Perkin Elmer Lambda 3 ultraviolet/visible spectrophotometer (Norwalk, CT) at 20˚C.

Table 1 demonstrates the distribution of PEPCK, CS and LDH activities between the intramitochondrial fractions and the cytosol. Since CS is exclusively mitochondrial and LDH is exclusively cytosolic, these enzymes serve as markers of contamination of each compartment during the isolation procedure. The results indicate that 88% of PEPCK activity is found within the mitochondria. Based on a PEPCK:CS ratio of 1.03 and the 10–15% contamination of each compartment by LDH and CS, this indicates that PEPCK is exclusively mitochondrial in red-eared turtles. This is in contradiction to the previously published results of Penny and Kornecki (1973), who reported that 72% of the hepatic PEPCK activity was in the cytosolic compartment. However, it should be noted that their study employed an inappropriate technique for the isolation of intact mitochondria. Specifically, mitochondria were isolated after two high-speed centrifugation steps at 24500 ***g*** for 1h, which is likely to cause severe loss of mitochondrial integrity. Current techniques employ a low-speed centrifugation step, to remove large cellular debris, followed by centrifugation at 8000–10000 ***g*** for 10min to pellet the mitochondria (for a review of techniques, see Rickwood *et al.* 1987).

In the present study, the intramitochondrial location of PEPCK suggests a substrate preference for lactate over amino acids as the gluconeogenic precursor. This is based on the observation that the conversion of alanine to pyruvate does not generate NADH that

Table 1. *Total and compartmentalised activities of hepatic PEPCK, CS and LDH in* Pseudemys scripta elegans

	Mitochondrial activity		
Enzyme	(%)	(Units*)	Total activity
PEPCK	88.3	3.2	3.6±0.6
CS	85.3	2.6	3.1±0.4
LDH	10.5	9.3	89.0±1.8

Values are mean ± S.E., *N*=6 in each case.
*Units of enzyme activity are given as μmol min^{-1} g^{-1} wetmass at 20°C.
PEPCK, phosphenolpyruvate carboxykinase; CS, citrate synthase; LDH, lactate dehydrogenase.

can be utilised in the reversal of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) further along the gluconeogenic pathway. Formation of pyruvate from lactate, however, generates cytosolic NADH that balances the reversed G3PDH reaction. Consistent with this is the observation that gluconeogenesis from lactate proceeds at higher rates in species with intramitochondrial PEPCK (reviewed by Suarez and Mommsen, 1988).

The results found in this study agree with a previous study conducted on the western painted turtle (Buck *et al.* 1993). In that species, when corrected for cytosolic CS leakage, hepatic PEPCK is virtually 100% mitochondrial, with gluconeogenesis from lactate proceeding at $2\,\mu\text{mol glucose}\,\text{g}^{-1}\,\text{cells}\,\text{h}^{-1}$. This is of particular relevance when considering protein turnover during anoxia in aquatic turtles. In the painted turtle, anoxia results in the depression of hepatic protein synthesis rates by 92% (Land *et al.* 1993). The apparent preference of gluconeogenesis for lactate in this tissue suggests that amino acids derived from intracellular protein degradation during this time are not lost towards regenerating glycogen. Therefore, if lactate is the likely preferred gluconeogenic substrate in turtle liver, we tentatively suggest that depleted hepatic glycogen stores after an anoxic dive may be repleted preferentially from accumulated plasma and intracellular lactate during normoxic recovery, rather than from dietary or intracellular derived amino acids.

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References

BUCK, L. T., LAND, S. C. AND HOCHACHKA, P. W. (1993). Anoxia tolerant hepatocytes: A model for the study of reversible metabolic suppression. *Am. J. Physiol.* (in press).

LAND, S. C., BUCK, L. T. AND HOCHACHKA, P. W. (1993). Response of protein synthesis to anoxia and recovery in anoxia tolerant hepatocytes. *Am. J. Physiol.* (in press).

PENNEY, D. G. (1974). Effects of prolonged diving anoxia on the turtle, *Pseudemys scripta elegans*. *Comp. Biochem. Physiol.* **47**A, 933–941.

PENNEY, D. G. AND KORNECKI, E. H. (1973). Activities, intracellular localisation and kinetic properties of phosphoenolpyruvate carboxykinase, pyruvate kinase and malate dehydrogenase in turtle (*Pseudemys scripta elegans*) liver, heart and skeletal muscle. *Comp. Biochem. Physiol.* **46**B, 405–415.

RICKWOOD, D., WILSON, M. T. AND DARLEY-USMAR, V. M.(1987). Isolation and characteristics of intact mitochondria. In *Mitochondria: A Practical Approach* (ed. V. M. Darley-Usmar, D. Rickwood and W. T. Wilson), pp. 1–16. Washington, DC: IRL Press.

SUAREZ, R. K., MALLET, P. D., DAXBOECK, C. AND HOCHACHKA, P. W. (1985). Enzymes of energy metabolism and gluconeogenesis in the pacific blue marlin, *Makaira nigricans. Can. J. Zool.* **64**, 694–697.

SUAREZ, R. K. AND MOMMSEN, T. P. (1987). Gluconeogenesis in teleost fishes. *Can. J. Zool.* **65**, 1869–1882.

ULTSCH, G. R.(1985). The viability of nearctic freshwater turtles submerged in anoxia and normoxia at 3 and 10°C. *Comp. Biochem. Physiol.* **81**A, 607–611.