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TISSUE DISTRIBUTION OF GLUCOSE-6-PHOSPHATASE AND FRUCTOSE-1,6-BISPHOSPHATASE IN *HELIX POMATIA* L. AND *POMACEA BRIDGESII* (REEVE)

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ABSTRACT: Key enzymes of gluconeogenesis, D-glucose-6-phosphatase (G-6-Pase) [EC 3.1.3.9] and D-fructose-1,6-bisphosphate 1-phosphohydrolase (Fru-1,6-Pase) [EC 3.1.3.11], were investigated in *Helix pomatia* L. and *Pomacea bridgesii* (Reeve). Fru-1,6-Pase and G-6-Pase activities were determined measuring with malachite green the concentration of inorganic phosphate produced by substrate hydrolysis in homogenates of hepatopancreas, kidney and foot muscle. Determined Fru-1,6-Pase activities (U/g wt.) in *H. pomatia* were as follows: hepatopancreas 1.01, kidney 0.19, foot muscle 0.24, in *P. bridgesii* the respective values were: 0.94, 0.34 and 0.22. Activities of G-6-Pase (U/g wt.) in *H. pomatia* were: 1.65 in hepatopancreas, 0.64 in kidney and 0.21 in foot muscle, in *P. bridgesii* the respective values were: 0.79, 0.31, 0.21. Thus the highest gluconeogenic capacity in both species was found in hepatopancreas. Fru-1,6-Pase from *H. pomatia* and *P. bridgesii* hepatopancreas was inhibited by AMP. Determined $I_{0.5}$ were 8.4 μM for *H. pomatia* and 7.3 μM for *P. bridgesii*, and the values were comparable with those of mammalian liver Fru-1,6-Pase. K_m determined for hepatopancreas G-6-Pase from *H. pomatia* and *P. bridgesii* were 1.3 mM and 4.1 mM, respectively and were also comparable with K_m of mammalian liver enzyme.

KEY WORDS: fructose-1,6-bisphosphatase (Fru-1,6-Pase), glucose-6-phosphatase (G-6-Pase), *Helix pomatia, Pomacea bridgesii,* gluconeogenesis

INTRODUCTION

A number of papers is available on gluconeogenesis in vertebrates (MORRISON et al. 1972, PHILLIPS & HIRD 1977, FINOL & CHACIN 1980, DOHM & NEWSHOLME 1983, HERS & HUE 1983, GOLDMAN & WITKOVSKY 1987, GOLDMAN 1988, PILKIS et al. 1988, BAANANTE et al. 1991, PILKIS & CLAUS 1991). Most research has focused on mammals but birds and lower vertebrates have also been studied. On the other hand, much less information is available on gluconeogenesis in invertebrates and only few papers have been published on this pathway in Gastropoda.

Glucose-6-phosphatase (G-6-Pase) [EC 3.1.3.9] and D-fructose-1,6-bisphosphate 1-phosphohydrolase (Fru-1,6-Pase) [EC 3.1.3.11] are key enzymes of vertebrate gluconeogenesis. G-6-Pase catalyzes hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate. Fru-1,6-Pase in the presence of divalent ions hydrolyzes D-fructose-1,6-bisphosphate to fructose-6-phosphate and P_i. G-6-Pase activity has been detected in liver, kidney and intestine (VORHABEN & CAMPBELL 1979, LEONI et al. 1990, DZIEWULSKA et al. 1997), the activity of this enzyme in other tissues being very low.

Tissue distribution pattern of Fru-1,6-Pase is more complicated. Three isoenzymes of Fru-1,6-Pase have been found in mammals (TEJWANI 1983), birds (HAN et al. 1976, VAN TONDER et al. 1991) and lower vertebrates (DZIEWULSKA et al. 1997, DZIEWULSKA & DŻUGAJ 1999). The liver isoenzyme, recognized as regulatory enzyme of gluconeogenesis, has been found in liver (DŻUGAJ & KOCHMAN 1980, DŻUGAJ et al. 1985), kidney (DZIEWULSKA et al. 1997), intestine

(MIZUNUMA & TASHIMA 1978, MIZUNUMA & HASEGAWA 1980) and lung (SKAŁECKI et al. 1999). Fru-1,6-Pase isoenzyme, found in muscle tissue, participates in synthesis of glycogen from lactate (HERMANSEN & VAAGE 1977, MCLANE & HOLOSZY 1979) and supposedly in regulation of glycolysis via futile cycle. Physiological role of the brain isoenzyme (BISWAS & MAJUMDER 1985, LIU & FROM 1988) is unknown. Despite different primary structure, a great similarity of kinetic properties of all Fru-1,6-Pase isoenzymes has been observed. The isoenzymes are characterized by a high affinity to substrate, are activated by monovalent cations and inhibited by fructose-2,6-bisphosphate and AMP. The basic difference between liver and muscle isoenzyme concerns their sensitivity to AMP inhibition. $I_{0.5}$ for the muscle Fru-1,6-Pase is approximately ten to hundred times lower than $I_{0.5}$ for the liver isoenzyme (SKAŁECKI et al. 1995).

The primary objective of the present study was the investigation of G-6-Pase and Fru-1,6-Pase activity in hepatopancreas, kidney and foot muscle of *Helix pomatia* L. and *Pomacea bridgesii* (Reeve). Since a different sensitivity to AMP of hepatopancreas, kidney and foot muscle Fru-1,6-Pase could be expected, $I_{0.5}$ was also determined.

MATERIAL AND METHODS

All biochemicals were purchased from Sigma (St. Louis, MO, U.S.A.)

Adult individuals of two snails species – a pulmonate *Helix pomatia* L., and a prosobranch *Pomacea bridgesii* (Reeve) – were used. Twenty eight individuals of *H. pomatia* were caught in the wild in spring, kept at room temperature in a terrarium with humid soil and fed with lettuce. Fifteen specimens of *P. bridgesii,* coming from the laboratory culture of the Dept. of Physiology, Wrocław University, were kept in an aquarium, water temperature 24°C, and fed with vagetable fodder Vitabin and superVit, *Tubifex* larvae and pieces of raw ox heart.

The snails were weighed and their shells measured. The shells were then crushed, cerebral ganglia removed and the hepatopancreas, kidney and foot muscle were excised. The organs were then cleaned of adhering fragments of other tissues and weighed. The tissues (100 mg) were homogenized in 20 mM Tris, 0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol and 0.3 mg/ml BSA (1 ml), pH 6.8, at 4°C, in the Ultra Turax homogenizer (Janke & Kunkel IKA Labortechnik, Germany) during 30 sec., at 13,000 rpm. The homogenate was divided in two parts. One was centrifuged for 25 min at 16,000 g, and the resulting supernatant was used for determination of Fru-1,6-Pase activity. The remaining part of the homogenate was used for measurements of G-6-Pase activity. To extract G-6-Pase, deoxycholate was added at the concentration of 0.4% w/V (FOURNIER & GUDERLEY 1992).

The activity of Fru-1,6-Pase was determined colorimetrically through measurements of the quantity of released inorganic phosphate P_i, according to the method of TASHIMA & YOSHIMURA (1975). The assay mixture contained 50 mM BisTrisPropane, 150 mM KCl, 2 mM $Mg^{+2}(CH_3COO^-)_2$, 1 mM EDTA, 50 µM Fru-1,6-P_2, pH 7.5 or 9.3 at 25°C. The centrifuged supernatant was added to the assay mixture not containing substrate and incubated for 10 min at 25°C. The reaction was started by adding the substrate, continued for 4–5 min and terminated by adding malachite green (3% water solution of ammonium molybdate $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ mixed with a 0.06% solution of malachite green in 6 M HCl, as 1:1). Malachite green solution 0.5 ml was added to 1 ml assay mixture, and the absorbance was measured at 650 nm after 15 min. The reaction rate was a linear function of the amount of added homogenate.

The activity of G-6-Pase was also determined colorimetrically through measurements of the quantity of released inorganic phosphate P_i, using the same method (TASHIMA & YOSHIMURA 1975). The assay mixture (final concentration) contained 40 mM cacodylate, 10 mM glucose-6-phosphate and 6 mg/ml BSA, pH 6.5. Tissue homogenate 20 µl was added to the assay mixture not containing substrate and incubated for 10 min at 25°C. The reaction was started by adding the substrate.

Protein concentration was measured with the method of LOWRY et al. (1951). One unit of enzyme activity was the amount that catalyzes the formation of 1 µmole of product per minute. K_m and $I_{0.5}$ values were calculated with the program GraFit (LEATHERBARROW 1992).

RESULTS AND DISCUSSION

Mean weight of the whole body, hepatopancreas, kidney and foot muscle of both investigated species is presented in Table 1. It may be calculated that hepatopancreas of *H. pomatia* makes ca 7% whole body weight. The corresponding value for *P. bridgesii* is within the same range.

The results of G-6-Pase investigation are presented in Table 2. In both species the activity was the highest in hepatopancreas, lower in kidney and the lowest in foot muscle. It is comparable with vertebrates in which the highest activity of G-6-Pase was found in liver, lower in kidney and the lowest in muscle (DZIEWULSKA et al. 1997). Although it was postulated that mammalian lung and brain are gluconeogenic organs, no convincing evidence supporting this hypothesis has ever been presented. The presence of G-6-Pase in lung or muscle tissues is rather doubtful and the observed hydrolysis of glucose-6-phosphate by muscle or lung homogenate may have been caused by nonspecific phosphatases which do not hydrolyse glucose-6-phosphate in physiological conditions.

The presence of G-6-Pase in gastropod tissue has been previously reported. KASPRZYK et al. (1977) found a relatively high activity of G-6-Pase the hepatopancreas of *H. pomatia* and described basic kinetic properties of this enzyme. The reported K_m value (0.8 mM) was comparable with K_m of vertebrate G-6-Pases. Our results (Table 2) are in a good agreement with those obtained by KASPRZYK et al. (1977), thus supporting the idea on the similarity between the snail and vertebrate G-6-Pase kinetic properties.

The results of investigation of Fru-1,6-Pase activity are presented in Table 3. The activity of Fru-1,6-Pase was also the highest in the hepatopancreas of *H. pomatia* and *P. bridgesii*. It was five times higher than in the muscle tissue. It is of interest that the activity in muscle and kidney is nearly the same. Determining its $I_{0.5}$ required determination of 5'nucleotidase which might decrease AMP concentration. Taking into account the obtained activities of the latter enzyme, its effect on $I_{0.5}$ determination is rather negligible. $I_{0.5}$ was calculated from Hill plot and the results are presented in Table 3. The values of $I_{0.5}$ for hepatopancreas Fru-1,6-Pase of *H. pomatia* and *P. bridgesii* are comparable with those of vertebrate liver Fru-1,6-Pases (TEJWANI 1983, DZIEWULSKA et al. 1993, DZIEWULSKA & DŻUGAJ 1999). Contrary to our expectation, $I_{0.5}$ determined for the muscle Fru-1,6-Pase of *H. pomatia* was nearly the same as $I_{0.5}$ hepatopancreas Fru-1,6-Pase. This may indicate that in Gastropoda only one Fru-1,6-Pase isoenzyme is present.

Besides G-6-Pase and Fru-1,6-Pase, pyruvate carboxylase [EC 6.4.1.1] and phosphoenolopyruvate carboxylase [EC 4.1.1.31] are also regarded as regulatory enzymes of gluconeogenesis (PILKIS et al. 1988). Since glucose may be synthesized not only from pyruvate, lactate or gluconeogenic amino-acids, but also from glycerol, regardless of the substrate, only G-6-Pase and Fru-1,6-Pase are indispensable enzymes for glucose synthesis and may be regarded as marker enzymes of gluconeogenesis.

Table 1. Mean weight of whole body and examined organs of two investigated species

	Helix pomatia (n = 28)	*Pomacea bridgesii* (n = 15)
Whole body [g]	18.87 ± 3.70	10.80 ± 6.09
Hepatopancreas [mg]	1349.00 ± 328.26	827.80 ± 504.45
Kidney [mg]	383.50 ± 95.90	103.80 ± 44.01
Foot muscle [mg]	2898.00 ± 772.05	1381.50 ± 633.71

Table 2. Tissue distribution and K_m of *Helix pomatia* and *Pomacea bridgesii* G-6-Pase

	Helix pomatia (n = 28)			*Pomacea bridgesii* (n =15)		
Tissue	hepatopancreas	kidney	muscle	hepatopancreas	kidney	muscle
Activity [U/g wt.]	1.65 ± 0.51	0.64 ± 0.32	0.28 ± 0.15	0.79 ± 0.12	0.31 ± 0.08	0.21 ± 0.04
K_m [mM]	1.29 (n = 4)	n.d.*	1.13 (n = 4)	4.10 (n = 3)	n.d.*	n.d.*

*not determined

Table 3. Tissue distribution and $I_{0.5}$ of Fru-1,6-Pase in *Helix pomatia* and *Pomacea bridgesii*

	Helix pomatia (n = 28)			*Pomacea bridgesii* (n = 15)		
Tissue	hepatopancreas	kidney	muscle	hepatopancreas	kidney	muscle
Activity in pH=7.5 [U/g wt.]	1.01 ± 0.14	0.19 ± 0.06	0.24 ± 0.07	0.94 ± 0.20	0.34 ± 0.07	0.22 ± 0.04
Protein [mg/g]	41.30 ± 4.14	23.70 ± 3.92	30.10 ± 8.87	81.60 ± 13.00	40.50 ± 4.34	49.00 ± 5.63
Specific activity pH=7.5 [mU/mg]	23.80 ± 3.48	9.70 ± 2.07	8.20 ± 1.64	11.60 ± 2.51	8.40 ± 1.54	4.70 ± 1.10
$I_{0.5}$ for AMP [μM]	8.40 (n=8)	n.d.*	10.40 (n=9)	7.30 (n=3)	n.d.*	n.d.*

CONCLUDING REMARKS

The presence of relatively high activities of G-6-Pase and Fru-1,6-Pase in *H. pomatia* and *P. bridgesii* strongly supports the idea that in this organ gluconeogenesis takes place.

The weight ratio of hepatopancreas and whole body in *H. pomatia* and *P. bridgesii* is even higher than the corresponding values in amphibians, fishes, birds or mammals, indicating a high gluconeogenic capacity of Gastropoda.

The determined kinetic parameters: K_m for G-6-Pase and $I_{0.5}$ for Fru-1,6-Pase inhibition by AMP are quite similar to the corresponding values of vertebrate enzymes.

Probably only one Fru-1,6-Pase isoenzyme is present in Gastropoda.

Further studies are necessary to determine G-6-Pase and Fru-1,6-Pase kinetic properties in *H. pomatia* and *P. bridgesii* more precisely, and particularly to determine Fru-1,6-Pase inhibition by fructose-2,6-bisphosphate. Ascertaining the number of Fru-1,6-Pase isoenzymes which are present in Gastropoda would be also desirable.

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