As well as sucrase, other glucose-releasing hydrolases can be assayed by the continuous method. Experiments were carried out with purified α-glucosidase and β-fructosidase from yeast and β-glucosidase from sweet almonds. In the case of β-glucosidase, on the basis of the results shown in figure 1, B, mutarotase has been omitted from the reaction mixture. For α- and β-glucosidase a proportionality has been observed between the measured activity and the amount of enzyme used, up to a value of 0.2 µmoles/min. For β-fructosidase a proportionality that reaches 0.6 µmoles/min has been obtained.

The continuous method can also be usefully applied to crude homogenates. The table shows the activities of some disaccharidases in a crude homogenate of intestine, determined with the continuous method and compared with 2 discontinuous methods. With sucrose as substrate there is a good agreement between the activities determined by the present procedure and these determined by discontinuous methods. With trehalose and maltose as substrates, the activities determined by discontinuous methods are slightly higher.

Discussion. The present work describes a continuous optical method for the assay of sucrase activity, which can be applied to all other glucose-releasing hydrolases. The presence of glucose dehydrogenase in the auxiliary system has 2 main consequences. Firstly, the interference of other enzymes possibly present in the crude extracts and/or in the auxiliary system is greatly reduced. For instance, the assay of sucrase with hexokinase/glucose-6-phosphate dehydrogenase can be greatly affected by the presence of glucose-phosphate isomerase or 6-phosphogluconate dehydrogenase. Secondly glucose dehydrogenase is specific for the β-anomer of glucose. Therefore, whenever the α-anomer of glucose is released, in the absence of mutarotase an exceedingly long lag will be observed. In our case, with the addition of mutarotase to the reaction mixture, the time lag is noticeably shortened, becoming about 30 sec.

It is also known that disaccharidases, besides their hydrolytic activity, are endowed with a transglucosidase activity[6], that may interfere in the assay and in the determination of kinetic parameters. With the present method, the glucose formed is oxidized by the auxiliary system and therefore cannot become an acceptor for the transferase activity.

In conclusion, when compared with the discontinuous methods used until now, the present method has noticeable advantages of rapidity and accuracy, especially in kinetic studies, in which it is essential to determine the initial rate of the reaction.

1 Present address: Istituto di Chimica biologica, University of Florence, viale Morgagni 50, Florence, Italy.
2 Present address: Istituto di Fisiologia generale e di Chimica biologica, University of Milan, via Saldini 50, Milan, Italy. To whom correspondence should be sent.
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The distribution of pyrroline carboxylate reductase and proline oxidase in the larva of the blowfly, *Aldrichina grahami*

S. Tsuyama, T. Higashino and K. Miura

Laboratory of Biochemistry, Department of Agricultural Chemistry, University of Osaka Prefecture, Sakai-City, Osaka 591 (Japan), 27 December 1979

Summary. P5C reductase and proline oxidase in the larva of the blowfly, *Aldrichina grahami,* were found to be localized mainly in the fat body mitochondrial matrix and the muscle, respectively.

Arginine is required in the diet of all insects, and during the larval growth of the blowfly, *Aldrichina grahami,* it is metabolized mainly to proline in vivo[1]. Δ^1-Pyrroline-5-carboxylate (P5C) has been suggested to be an intermediate formed during this conversion. It is well known that P5C is metabolized to proline by P5C reductase and that proline oxidase is its reverse enzyme. In insects, it has been reported that P5C reductase is localized in the cytosol of the fat body of the adult silkmoth, *Hyalophora gloveri*[2], and that proline is oxidized in the mitochondria of the flight muscle of the blowfly, *Phormia regina*[3]. However, the distribution of these enzymes during larval growth has not been elucidated. In this paper, we report on the distribution of P5C reductase and proline oxidase in the larva of the blowfly, *A. grahami.*

Materials and methods. Blowflies were reared aseptically on semi-synthetic diets at 25 °C as described previously[4]. The 4-day-old larvae were dissected in a cold saline solution [120 mM NaCl, 5 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.2 mM $NaHCO_3$, 0.13 mM NaH_2PO_4 and 5 mM N-2-hydroxyethylpiperadine-N′-2-ethanesulfonic acid (HEPES), pH 7.4] and the fat body, gut, malpighian tube, haemolymph, muscle and other tissues containing cuticle and trachea were isolated. These isolated organs were homogenized in 9 volumes of 0.4 M sorbitol solution containing 1 mM ethylendiamine tetracetic acid, 0.2%

Table 1. Tissue distribution of enzymes involved in the metabolism from arginine to proline in the 4-day-old larvae of blowfly

	Arginase (μmole/h/insect)	Ornithine aminotransferase (μmole/h/insect)	P5C reductase (μmole/min/insect)	Proline oxidase (nmole/h/insect)
Whole body	2.35±0.18	0.54±0.05	9.37±0.25	38.2±4.3
	100%	100%	100%	100%
Malpighian tube	0.01±0.00	0.05±0.01	0.20±0.01	4.0±0.4
	0.5%	9.0%	2.1%	10.5%
Gut	0.03±0.01	0.14±0.01	1.05±0.12	3.0±0.7
	1.3%	26.0%	11.2%	7.9%
Fat body	2.28±0.08	0.32±0.03	5.93±0.21	3.2±0.1
	96.8%	60.3%	63.3%	8.4%
Haemolymph	0.15±0.02	0.04±0.01	0.35±0.07	4.0±0.6
	6.5%	7.8%	3.7%	10.5%
Muscle	0.01±0.00	0.03±0.00	0.05±0.01	35.7±1.5
	0.3%	5.0%	0.5%	93.5%
Residue	0.02±0.00	0.07±0.01	0.75±0.07	0.7±0.1
	0.6%	12.6%	8.0%	1.8%
Recovery	106.0%	120.4%	88.8%	132.6%

Each value represents the mean of 5 determinations ± SD.

Table 2. The submitochondrial distribution of P5C reductase and some enzymes in the fat body mitochondria

	P5C reductase (μmole/min/ml)	Ornithine aminotransferase (μmole/h/ml)	Arginase (μmole/h/ml)	Cytochrome oxidase (nmole/min/ml)	Fumarase (nmole/min/ml)
Whole mitochondria	40.84±1.17	3.49±0.37	15.78±0.24	84.13±3.41	71.79±1.69
	100%	100%	100%	100%	100%
Outer membrane	5.51±0.14	0.21±0.03	0.05±0.00	0.02±0.01	1.44±0.02
	13.5%	6.0%	0%	0%	2.0%
Inter membrane space	1.10±0.03	1.12±0.34	0.31±0.01	0	5.77±0.43
	2.7%	32.0%	2.0%	0%	8.0%
Inner membrane	4.41±0.72	2.48±0.13	0.19±0.00	28.64±3.02	5.57±0.25
	10.8%	71.1%	1.2%	34.0%	7.8%
Matrix	53.79±2.67	22.45±1.07	4.10±0.39	1.65±0.06	70.23±2.28
	131.7%	643.3%	26.0%	2.0%	97.8%

Each fraction was suspended in 1.5 ml of homogenizing medium. Each value represents the mean of 3 determinations ± SD.

bovine serum albumin and 5 mM HEPES (pH 7.4) by a glass homogenizer. The methods of subcellular and submitochondrial fractionation were the same as those used previously[5,6]. P5C reductase and proline oxidase were assayed by the methods of Peisach et al.[7] and Johnson et al.[8]. Arginase and ornithine aminotransferase were assayed by the methods of Reddy et al.[2] and Van Slyke et al.[9]. Fumarase and cytochrome oxidase were assayed by the methods of Hill et al.[10] and Smith[11].

Results and discussion. P5C reductase and proline oxidase were distributed mainly in the fat body and the muscle, respectively. In addition, P5C reductase was recovered in the submitochondrial fractionation in the matrix of the fat body mitochondria. These findings suggest that P5C reductase is localized in the matrix space of fat body mitochondria. The difference of P5C reductase localization in the insect fat body between that described in the present paper and that reported by Reddy and Campbell[2] may arise from a difference in the developmental stage examined or in the species of insect used. Already, we have shown that arginase and ornithine aminotransferase are localized in the intramitochondrial site in the larval fat body of the blowfly, *A. grahami*[6,12]. These facts and the data presented in table 2 lead us to conclude that the enzyme system responsible for the formation of proline from arginine is present in the fat body mitochondria during the larval stage of the blowfly. On the other hand, many reports show that in the rat P5C reductase and proline oxidase are distributed in the liver[7,8,13], and that these enzymes isolated from adult or larval blowfly are controlled by some metabolites[14,15]. The evidence provided in table 1 shows that proline oxidase occurs mostly in the muscle in this larva. This indicates, therefore, that the above 2 enzymes are further controlled by their different distribution in the tissues, which prevents a futile consumption of energy in the larva of the blowfly. Sacktor[3] suggests that proline in the adult insect may be utilized at the initiation of flight. Therefore, the difference of the tissue distribution of proline oxidase between rat and insect may be due to the difference in the way proline is utilized.

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