Result of the Moore-Stein analysis of amino acid mixture deriving from *Candida lipolytica* grown on deuteriated substrate

Lysine	31.32 nmoles/ml
Histidine	11.06 nmoles/ml
Arginine	20.65 nmoles/ml
Tryptophan*	
Aspartic acid	55.55 nmoles/ml
Threonine	47.17 nmoles/ml
Serine	44.25 nmoles/ml
Glutamic acid	53.73 nmoles/ml
Proline	12.85 nmoles/ml
Glycine	54.49 nmoles/ml
Glucosamine	60.00 nmoles/ml
Alanine	61.74 nmoles/ml
Valine	32.50 nmoles/ml
Cystine**	4.04 nmoles/ml
Galactosamine	15.00 nmoles/ml
Methionine**	6.64 nmoles/ml
Isoleucine	14.44 nmoles/ml
Leucine	41.41 nmoles/ml
Tyrosine	15.34 nmoles/ml
Phenylalanine	21.40 nmoles/ml

* Compound destroyed during hydrolysis. ** Compound partly destroyed during hydrolysis.

culture in media containing a high concentration of heavy water, *C. lipolytica* was grown and transplanted successively on gelatinous media in Petri dishes. These media were composed of a normal long-chain deuterio-alkane, the above mentioned mineral salts, vitamins[18], agar-agar, and mixtures of water-heavy water of the following proportions (by v/v): 50:50, 40:60, 35:65, 30:70, 27.5:72.5 and 20:80. In these cultures the fungus-cell size was, on average, larger than that originating from a non-deuteriated culture in the same growth period. This phenomenon became more apparent when the deuterium content of the water in the culture media reached the lethal levels for the yeast – beyond 80% D_2O. However, frequent subculturing on substrates with increasing D_2O concentration may result in a higher adaptability of the fungus toward deuterium, thus the expected yield of deuteriated amino acids could be increased. Further development of the biosynthesis along these lines is now in progress, and the results will be communicated later.

The present results indicate that *C. lipolytica* can assimilate the perdeuteriated alkanes as its sole carbon source for biosynthesis. This fact is important, not only because it leads to a suitable approach for obtaining deuteriated lipids and amino acids by a biosynthetic method, but also because it offers new possibilities for tracing microbial metabolic pathways by the use of isotope-labelled substrates with subsequent analysis by GC/MS.

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2 Address for reprint requests: Ng. D.-Ng., University of Göteborg, Structural Chemistry, Medicinaregatan 9, S-400 33 Göteborg 33, Sweden.

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Correlations between glucose-inhibition and control parameters of α-glucosidase kinetics in *Apis mellifica* haemolymph (Hymenoptera: Insecta)

M. Bounias

Laboratory of Biochemistry, INRA, Avignon Research Center, F-84140 Montfavet (France), 7 May 1979

Summary. Kinetics studies of haemolymph α-glucosidase inhibition by D. glucose led to general correlations between inhibition and control parameters, all over the honeybee development. Maximum velocities are not affected by the inhibition, while affinity constants are always significantly increased, and Hill coefficients tend to decrease, especially in foraging adults and in prenymphs. In this later case, the 'n-type' effect tends to break the manifestation of the 'K-type' mechanism.

The haemolymph α-glucosidase contributes to the maintenance of Insects glycaemic levels, and we have shown that D. glucose is a natural inhibitor of this enzyme in honeybees haemolymph[1]. Evidence was given for a 'K-type' (competitive) inhibition in emerging adults[2] and for a mixed mechanism rather affecting the Hill coefficient in young nymphs[3]. Other cases of glucose inhibition have been reported by Sinha[4] for midgut invertase and by Giebel and Domnas[5] for whole larvae trehalase, both in Diptera, but the kinetics mechanisms have not been studied. Previously to mathematical studies of the 'n-type' molecular transitions, it seemed necessary to determine more thoroughly the

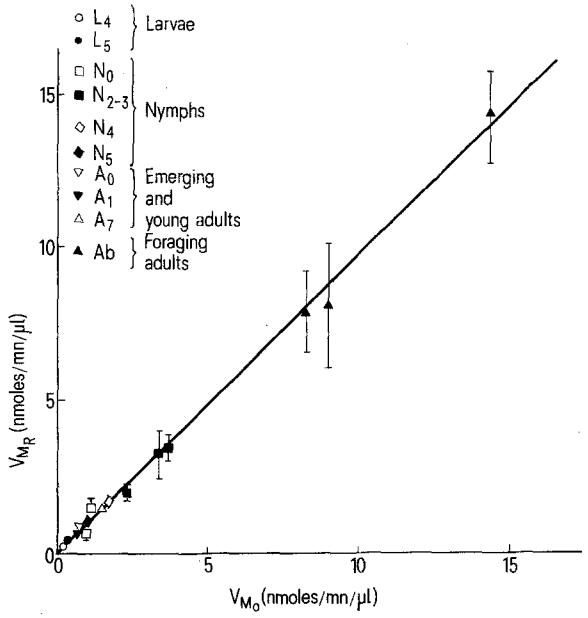

Fig. 1. Correlation between the α-glucosidase maximum velocities determined under glucose inhibition (V_{MR}) vs control ones (V_{M0}). Each point results from 9 replications; the variation coefficient is: $S_\kappa/\bar{\kappa} = 0.152$

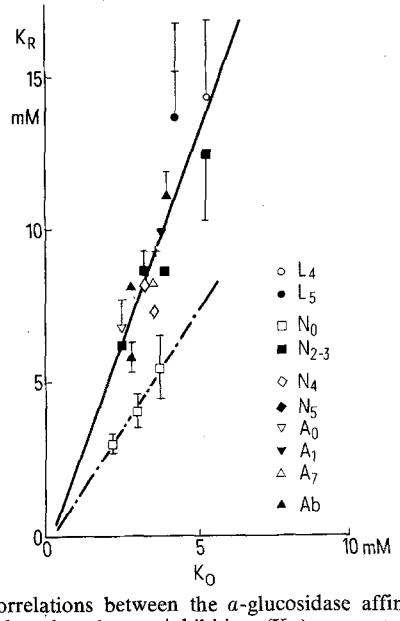

Fig. 2. Correlations between the α-glucosidase affinity constants determined under glucose inhibition (K_R) vs control ones (K_0). Each point results from 9 replications; the variation coefficient is: $S_\kappa/\bar{\kappa} = 0.247$.

kinetics parameters, comparatively under control and inhibition conditions, all over the different developmental stages of the honeybee.

Materials and methods. Honeybees of the *mellifica* race were kept from hives placed in natural conditions. The haemolymph α-glucosidase kinetics were determined in phosphate buffer, 50 mM, pH 6.75, by continuous microphotometric recording of the irreversible hydrolysis of p.nitro-phenyl α-D.glucopyranoside, as previously described[2,3]. The main 3 parameters: maximum velocity (V_M), affinity constant (K) and Hill coefficient (n) of the reaction have been recalculated according to a new rigorous algebraic method[6] such as: V_1, V_p, V_q being initial velocities

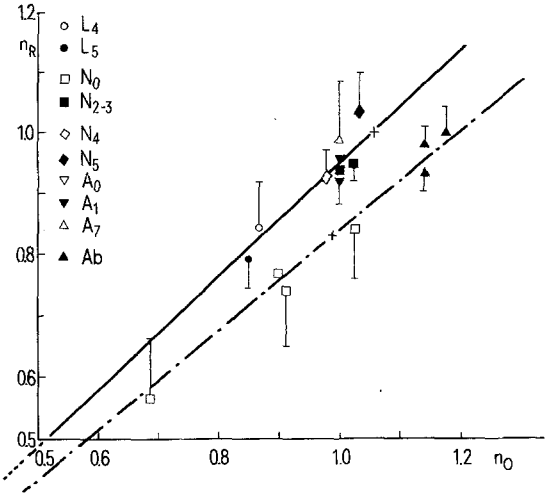

Fig. 3. Correlations between the α-glucosidase Hill coefficients determined under glucose inhibition (n_R) vs control ones (n_0). Each point results from 8 replications; the variation coefficient is: $S_\kappa/\bar{\kappa} = 0.083$.

associated to the respective substrate concentrations: $S_1 = 1$ unit, $S_p = p$, $S_q = q$, 2 conditions allow V_M to be calculated from simple 1st order equations derived from the Hill form:

1st case: $p\,q = 1$

$$V_M = [2/V_1 - (1/V_p + 1/V_q)]/[1/V_1 2 - 1/V_p \cdot V_q] \quad [1]$$

2nd case: $q = p^2$

$$V_M = [2/V_p - (1/V_1 + 1/V_q)]/[1/V_p^2 - 1/V_1 \cdot V_q] \quad [2]$$

Then, the determination of K proceeds from:

$$K = (V_M/V_1) - 1 \quad [3]$$

and for each pair (S_i, v_i), n can be calculated from:

$$n = [LN(K \cdot V_i) - LN(V_M - V_i)]/LN\ S_i \quad [4]$$

In the correlations and regression calculations, the natural-parameters are considered as the controlled variable (average from 12 determinations), and inhibition-parameters as aleatory variable (average from 8–9 replications). The applied glucose concentrations have been first empirically chosen as decreasing the initial reaction rate by 50% (in presence of substrate concentrations adjusted to the natural apparent values of K). They were: 75 mM in larvae; 80 mM in young nymphs; 60 mM in last stages nymphs; 75 mM in emerging adults; 95 mM in foraging adults. The rigorous mathematical determination of these values (namely: I_{50}) will be possible, only following the present identification of the inhibition types.

Results and discussion. The maximum velocities resultant from glucose-inhibition (V_{MR}) are highly correlated with control ones (V_{MO}) all over the development (figure 1). The correlation coefficient is: $\rho = 0.998$ and the regression equation:

$$V_{MR} = 0.976 \cdot V_{M0} - 0.03.$$

The general correlation between the affinity constants K_R and K_0 would be:

$$\rho = 0.830 \text{ and } K_R = 3.00 \quad K_0 - 2.19 \quad [6]$$

but pre-nymphs appear to belong to different curves than do others stages (figure 2):

– in pre-nymphs:

$$\rho = 0.995;\ K_R = 1.50 \quad K_0 - 0.39 \quad [7]$$

- all other stages: [8]
$p=0.886$; $K_R=2.75$ $K_0-0.58$.

It is noticable that these equations differ by their slopes but not their origin. The natural Hill coefficients (n_0) are lower than 1 in larvae and pre-nymphs, and higher than 1 in foraging adults (figure 3). In this case, the general correlation with inhibition-resultant values (namely n_R) would be:

$$p=0.849;\ n_R=0.81 \quad n_0-0.09 \qquad [9]$$

but both pre-nymphs (N_0) and foraging adults (Ab) on one hand, and all other stages on the other, can be gathered on two curves, again with distinct slopes and same origin:

- in N_0 and Ab:
$p=0.990$; $n_R=0.82$ $n_0+0.02$ [10]

- in other stages:
$p=0.85$; $n_R=0.93$ $n_0+0.02$. [11]

The equations [5]-[8] indicate that the inhibition mechanism is rather of the 'K' type over the whole development range, but less marked at the N_0 stage; then, equations [9]-[11] show a general tendency towars the 'n-type' inhibition, especially in pre-nymphs and adults, in which the Hill coefficient are more significantly decreased than at other stages. It thus appears that for pre-nymphs, the 'n-type' effect protects the enzyme substrate affinity under inhibition conditions: the regression coefficient for K_R/K_0 is the lower as the coefficient for n_R/n_0 is itself decreased.

These correlations suggest that the molecular mechanism of haemolymph α-glucosidase activity might proceed from a common general molecular event, inflected in some cases by physiological factors probably related to developmental requirements (metamorphosis metabolism, and adults nutrition for instance).

In natural conditions, the α-glucosidase activity partly results from the equilibrium between the substrate (α-glucoside, mainly trehalose) and the reaction-product (D. glucose as inhibitor). The wide range of the observed values of the resultant Hill coefficients suggests that it might exist different specific coefficients: n_s and n_i respectively for substrate and inhibitor binding, in parallel to their respective affinities for the enzyme: K_s and K_i.

Our purpose is now to determine these parameters, in order to clarify the molecular mechanism of the observed modifications of α-glucosidase kinetics, taking into account both the competitive inhibition-factor: $f_i=1+(I/I_{50})^{ni}$, in agreement with Chou[8] and the 'n-type' effect described by Bounias[3]. This seems the more interesting as another (slightly specific) α-glucosidase purified from whole honeybees by Huber and Mathison[9] did not appear to be controlled by glucose.

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tRNA in developing human placenta

M. Woźniak and Wanda Dobryszycka

Department of Biochemistry, Institute of Bioanalysis and Environmental Research, Medical Academy, Szewska 38/39, PL-50-139 Wrocław (Poland), 11 May 1979

Summary. Amino acid acceptor activity of tRNA in the human placenta as measured throughout gestation was found to be the lowest in post-term placenta. Aminoacylation of tRNA proceeded with maximum activity in the stage of formation of the placenta.

The placenta is probably the most complex mammalian tissue. It is characterized inter alia by the rapid growth, that is the vigorous protein biosynthesis, not observed in other organs, and bearing some resemblance to malignant growth. The magnitude of protein biosynthesis in cell-free placental extracts is known to be correlated with gestational age, DNA content, placental weight, birth weight[1], and RNA[2], respectively. In view of changes in human tRNA during normal and abnormal cellular development[3], and its function in regulation and control[4], it seemed worthwhile to detect changes in amino acid acceptor activity and aminoacylation of tRNA of human placenta from different gestational ages. This is reported in the present paper.

Materials and methods. Human placentas were obtained from the Institute of Obstetrics and Gynecology immediately after delivery. The placenta was washed with cold water from adherent blood, and was separated from fetal membranes. Uniformly ^{14}C-labeled amino acids were from UVVVR Czechoslovakia. tRNA and aminoacyl-tRNA synthetases were isolated from the placental tissue[5,6], the procedures being carried out at 4 °C. ^{14}C-Amino acid acceptor activity of the tRNA was measured by the method of Yang and Novelli[7] at individual Mg^{2+} concentrations for particular amino acids[5]. The periodate procedure was applied to placental tRNA to compare the relative proportions of tRNA charged with amino acids. Periodate treatment was carried out according to Kędzierski and Pawełkiewicz[8], the oxidation mixture containing 200 mM sodium acetate pH 5.0, 20 mM $NaIO_4$ and 5 mg tRNA. Acceptor activities of periodate treated tRNAs were compared to activities of control tRNA preparations subjected to all the procedure, except that $NaIO_4$ was omitted in the oxidation mixture.

Results and discussion. In the Table results of amino acid acceptor activity determinations are shown as well as those of aminoacylation of tRNA from human placentas of different gestational ages. As can be seen, total amino acid acceptor activities of tRNA from post-mature placentas were about 50% lower than those of developmental and term placentas. Levels of tRNA aminoacylation accounted