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SYSTEMS OF L-LEUCINE TRANSPORT INTO *SACCHAROMYCES CEREVISIAE* PROTOPLASTS

N. KOTLIAR and E.H. RAMOS *

Instituto de Química Biológica, Facultad de Medicina, Universidad the Buenos Aires, Paraquay 2155, 1121 Capital Federal (Argentina)

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L-[^{14}C]Leucine transport into *Saccharomyces cerevisiae* protoplasts involves two systems (1 and 2) with different kinetic parameters. The K_T values for these systems are of the same order as those for intact yeast cells. These results suggest that the proteins related to the affinity constants are located in the cytoplasmic membrane.

Amino acid uptake by *Saccharomyces* has been studied with regard to the influx and efflux processes, their specificity and their metabolic regulation [1–5].

In *Saccharomyces cerevisiae,* the transport of L-leucine involves two transport systems as has previously been described [6,7].

According to these results, one system (S_1) has high affinity and low velocity and the other (S_2) has low affinity and high velocity. The two systems are characterized by their kinetic parameters, energy requirements, and their different response to specific inhibitors [8] and thiol reagents [9].

In order to extend our previous observations and to investigate the location of the SH-dependent transport proteins on the outer surface of the cell permeability barrier, we have studied the kinetics of L-leucine transport into whole yeast cells and protoplasts.

The yeasts employed were *S. cerevisiae* strain JB65 wild type, and JB64, a pet 9/α pet 9, a strain deficient in the ATP/ADP mitochondrial translocator, kindly provided by Dr. J.R. Mattoon, University of Colorado, Colorado Springs.

Experiments with energized yeast cells and assay of radioactive samples were performed as described before [8,9].

Protoplasts were prepared from yeast cells pretreated with 50 mM dithiothreitol in 100 mM Tris/5 mM EDTA buffer (pH 8.9) at 30°C for 30 min; after this treatment, the cell wall was enzymatically removed by 40 min incubation with lyophylized Helicase (Reactifs IBF, France). Protoplast formation was followed by cell counting before and after dilution with water; about 97% of protoplasts were obtained.

The kinetics of L-[^{14}C]leucine entrance were studied in freshly prepared protoplasts, which were kept in 1 M sorbitol as osmotic stabilizer solution.

The experiments were carried out at 30°C in a New Brunswick Gyratory Water Shaker (Model T-76). The incubation mixture (200 μl), in an Eppendorf tube, contained yeast protoplasts (2–4 $\cdot 10^8$ cells/ml), 0.8 M sorbitol, 20 mM potassium phthalate buffer (pH 4.5) and L-[^{14}C]leucine as specified in each case. At times T_0 and $T_0 + 3$ min after L-[^{14}C]leucine addition to the incubation medium, the reaction was stopped by dilution with

* To whom correspondence should be addressed at: Casilla de Correo 176, Sucursal 53(B), 1453 Buenos Aires, Argentina.

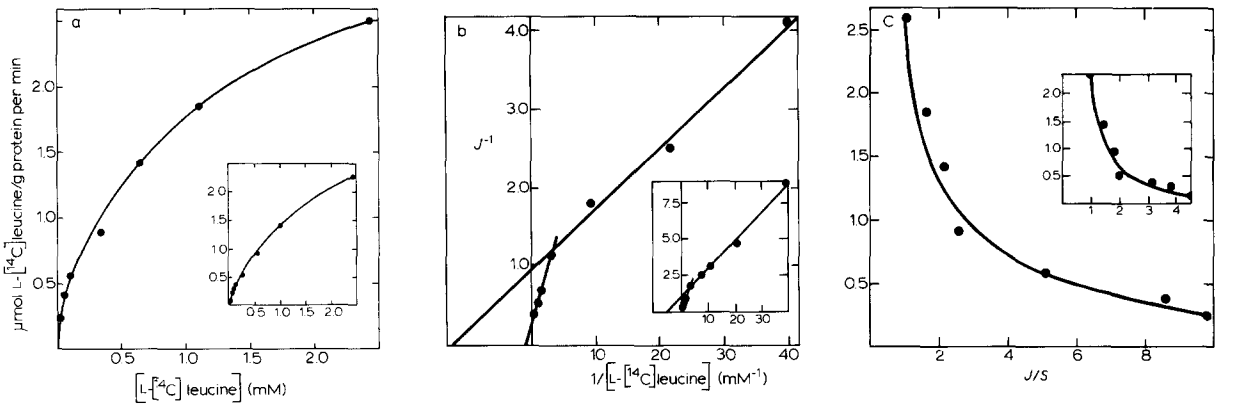

Fig. 1. Effect of L-[^{14}C]leucine concentration on amino acid transport by yeast protoplasts. (a) Protoplasts from the JB65 strain were preincubated with 5 mM glucose for 15 min at 30°C. After centrifugation, cells were resuspended in 20 mM potassium phthalate buffer (pH 4.5) containing L-[^{14}C]leucine at the concentrations stated. The translocation rates were determined as described in the text; $v = (T_0 + 3\ \text{min} - T_0)/3$. (b) Double-reciprocal plot of the transport values. (c) Hofstee representation of the transport values. Inset: Same as in (a), (b) and (c), but for the JB64 strain.

1 ml 1 M ice-cold sorbitol and immediately centrifuged at maximal speed (10000 × g). The supernatant was discarded and the pellet, after being washed three times with the osmotic stabilizer, was resuspended in 0.5 ml methanol/H_2O (9 : 1). Radiactivity was measured by Bray's [12] method and proteins by the method of Lowry et al. [13].

In all experiments T_0 samples were taken immediately after L-[^{14}C]leucine addition and the corresponding L-[^{14}C]leucine values are taken to represent the amino acid binding to active binding-sites of the permeability barrier. The L-[^{14}C]leucine values obtained at $T_0 + 3$ min were termed 'entrance' values and $T_0 + 3\ \text{min} - T_0$ values were considered the translocation values [9].

Fig. 1a shows the effect of L-[^{14}C]leucine external concentration on the amino acid translocation into yeast JB65 protoplasts. Representation of L-[^{14}C]leucine translocation velocities as a function of amino acid concentration yielded hyperbolic curve, indicating that the translocation system was saturable.

Similar results were obtained with yeast JB64 protoplast as is shown in the inset of the same Fig. 1a. The double-reciprocal plots presented in Fig. 1b as well as the Hofstee plots presented in Fig. 1c point to the existence of two kinetically different L-[^{14}C]leucine translocation systems. In both yeast strains K_T values are lower for one system than for the other; high-affinity systems (S_1) have lower velocities than low-affinity systems (S_2).

Similar results were obtained in the study of the kinetics of L-[^{14}C]leucine translocation into whole

TABLE I

KINETIC PARAMETERS OF L-[^{14}C]LEUCINE TRANSLOCATION IN ENERGIZED WHOLE CELLS AND PROTOPLASTS

Cells were energized by pretreatment with 4 mM D-glucose for 15 min. Results by Lineaweaver-Burk method for kinetic data analysis. Experimental conditions were as described in the text and Fig. 1. (a) = μmol/min per g protein; (b) = μmol/min per g cells.

Yeast		K_{T1} (mM)	J_{max1} (μmol/min per g)	K_{T2} (mM)	J_{max2} (μmol/min per g)
JB65	Protoplasts	0.084	1.06 (a)	0.93	3.77 (a)
	Whole cells	0.034	0.53 (b)	0.41	1.59 (b)
JB64	Protoplasts	0.170	1.46 (a)	1.16	3.59 (a)
	Whole cells	0.069	0.52 (b)	0.53	1.09 (b)

cells of JB65 and JB64 strains as can be seen from Table I where the K_T and J_{max} values are presented.

These results suggest that protoplasts devoid of yeast cell wall and periplasmic space kept their two L-[^{14}C]leucine translocation systems. Moreover, when K_T values of systems S_1 and S_2 in protoplasts (Fig. 1b and 1c) are compared to those in whole yeasts cells (Table I), it will be observed that the former are somewhat larger but of the same order. On this basis, it seems reasonable to presume that the proteins concerning affinity constants are actually located in the cytoplasmatic membrane.

In this connection it is worth recalling that, in experiments with yeast *S. carlsbergensis* subjected to osmotic shock, Wainer and Ramos [10] showed that cytoplasmic membrane proteins were concerned with K_T values, while other compounds from the periplasmatic space were probably involved in J_{max} values. On the other hand, absence of inorganic phosphate high-affinity transport system in *Candida tropicalis* was reported by Jeanjean and co-workers [11]. These authors, in addition, demonstrated the presence of P_i-binding proteins in the supernatants of the protoplast formation medium.

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