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Transport System of Specific Neutral Amino Acids in Suspension-Cultured Cells

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현탁배양 세포내에서 특수 중성 아미노산의 수송

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The influx of glycine, valine, alanine, and histidine was inhibited by all tested neutral amino acids competitively and the reciprocal inhibitory studies showed the neutral amino acids possess the same transport system as neutral amino acids compete to the same catalytic site of one carrier to each other. The molecules of histidine were transported actively as a neutral form through the neutral amino acid transport system, but were not transported as a charged form.

The Km values of the neutral amino acid transport system have been divided into three different catagory on basis of the affinity to the carrier, below 0.1 mM, between 0.1 mM - 0.5 mM and above 0.5 mM. The V_{max} was between 3.12 μmole · h^{-1} · g fresh weight^{-1} - 15.1 μmole · h^{-1} · g fresh weight^{-1}.

Neutral amino acids cotransported with one H^+ per one molecule and one K^+-efflux per one molecule for charge compensation. Histidine cotransported with proton per one molecule, however the movement of cotransported proton can't detectable because of the release of proton from the charged molecules of histidine in the medium.

Key words : cotransport, histidine

Investigation of the amino acids transport by higher plant cells have been performed with cotyledons (Robinson and Beevers, 1981a, b), Oat coleoptiles (Etherton and Rubinstein, 1978), soybean root cells (King and Hirji, 1975), barley root cells (Nissen, 1978), sugarcane suspension culture cells (Wyse and Komor, 1984), tobacco suspension culture cells (Harrington and Benke, 1981), and *Ricinus* suspension culture cells (Cho and Komor, 1984). Other studies of the kinetics and mechanisms of amino acids transport have utilized suspension cultures of higher plant cells (Maretzki and Thom, 1970: King and Hirji, 1975: Harrington and Henke, 1981). The suspension culture cells have been emploid, because it was very convenient for the uptake studies in comparison with the whole plants. The kinetics of amino acid transport may be multiphasic (Maretzki and Thom, 1970: Nissen, 1978). The amino acid transport by higher plant cells appeared to involve a different number of transport systems in different plants. One general transport system (McDaniel et al., 1982), or two transport systems (Harrington and Henke, 1981: Kinraide and Etherton, 1980) or three different transport systems for amino acids transport systems (Wyse and Komor, 1984) have been reported. Several of these studies indicated that the transport of amino acids was dependent on metabolic energy and that the uptake was coupled to the movement of ions across the plasma membrane (Robinson and Beevers, 1981: Etherton and Rubinstein, 1978: Jung et al., 1982: Eddy and Novacky, 1971: Lanyi, 1977). The influx of neutral amino acids has been performed by the proton uptake with the same direction within the cells and the potassium ion efflux on the basis of ion compensation (Cho, 1989). A depolarization of the membrane potential has also been associated with amino

acids transport (Fischer and Luttge, 1980: Kinraide and Etherton, 1981).

This paper describes the uptake of amino acids in order to characterize the mechanism, kinetics, and the number of amino acid transport systems utilized by these cells when grown as populations of undifferentiated cells in suspension culture.

MATERIAL AND METHODS

Plant Materials and Cell Culture

The cell suspension used for these studies was originated from cotyledons of *Brassica rapa* and has been maintained in suspension culture cells and in solid agar. Suspensions of undifferentiated parenchym cells may be grown in a liquid medium containing White's basal salts, vitamins, 2,4-dichlorophenoxyaceticacid (2,4-D), sucrose, arginine and yeast extract (Nickel and Maretzki, 1969). The cultures were maintained in 100 ml of medium in 250 ml flasks shaken on a rotary shaker at 160 rpm at 27 °C in the dark, and were transferred at 10 days intervals as a subculture.

Measurement of Transport Activity

Cells to be used for transport measurement were grown for 7-10 days and used directly or transferred to a basal medium without sucrose overnight. The cells were harvested by filtering through paper filters using a vacuum filter manifold (Hoefer Scientific Instrument, FH 225V). After filtration the cells were washed with buffer system and 1 g of cells was resuspended in 7 mls of 25 mM sodium-phosphate buffer, pH 6.0. The uptake was strated the addition of amino acids in medium with a specific radioactivity of 0.2 μCi of [^{14}C]-4-glycine (10 mCi/mmol), 0.3 μCi of [^{14}C]-L-valine (10 mCi/mmol), 0.2 μCi of [^{14}C]-L-alanine (518 mCi/mmol), 0.2 μCi of [^{14}C]-L-leucine (10 mCi/mmol), 0.2 μCi of [^{14}C]-L-histidine (10 mCi/mmol), 0.25 μCi of [^{14}C]-L-serine (10 mCi/ mmol), 0.3 μCi of [^{14}C]-L-iosleucine (10 mCi/mmol), and 0.25 μCi of [^{14}C]-L-asparagine (10 μCi/mmol) at the different concentration of between from 0.05 mM to 2 mM. At 30 second intervals 1 mL of samples were removed by pipette and collected on cellulose filter (pore size 1.2 μm: Schleicher & Scheull, Dassel). Each sample was washed twice with 20 ml of ice cold buffer, and the filter and cells were transferred to a scintillation vial. The radioactivity of the samples was determined by counting in 5 mL of scintillation cocktail (Xylene 150 mL: Toluen 250 mL: PPO 5 g: POPPO 0.6 g) using a Beckman LS 9000 liquid scintillation counter.

To determine whether the uptake of a particular pair of amino acids used the same carrier system or different carrier systems, I performed the uptake under reciprocal competition conditions. The uptake of 0.1 mM [^{14}C]-labelled amino acids was measured in presence or absence of 1 mM non-labelled amino acids. In cases where the reciprocal competition, as measured by the reduction of uptake compared to control levels, was not reciprocal, it was measured competition using Lineweaver-Burke plots.

The K_m-value for each amino acid was determined by measuring the uptake between a concentration range from 0.05 mM to 1.0 mM.

Ion Flux Measurement

To measure the ion flux, 1 g of cells was suspended in 10 ml of 5 mM calcium chloride in a 25 ml flask. The net movement of ions was followed with electrodes inserted into the cells suspension with a rotary shaker. The output was amplified and continuously recorded. All experiments were followed by methods by Cho and Komor (1984).

RESULT AND DISCUSSION

Neutral Amino Acid Transport System

To the determination of the neutral amino acids transport systems in parenchym cells suspension culture, it has been performed the reciprocal inhibitory studies. In Table 1 was shown the influx of five labelled neutral amino acids (glycine, valine, alanine, leucine, and histidine) in presence and absence of 1 mM unlabelled neutral, basic, and acidic amino acids. The influx of glycine was strongly inhibited by most of the neutral amino acids and by the acidic amino acid, glutamate, but not by aspartate, and was not strongly inhibited by the basic amino acid, arginine. The reciprocal examination with the labelled amino acids, such as alanine, leucine, and valine was strongly inhibited by the unlabelled glycine. The influx of tested labelled neutral amino acids were inhibited by most unlabelled neutral amino acids. It was conformed that all neutral amino acids were taken up by the same transport system.

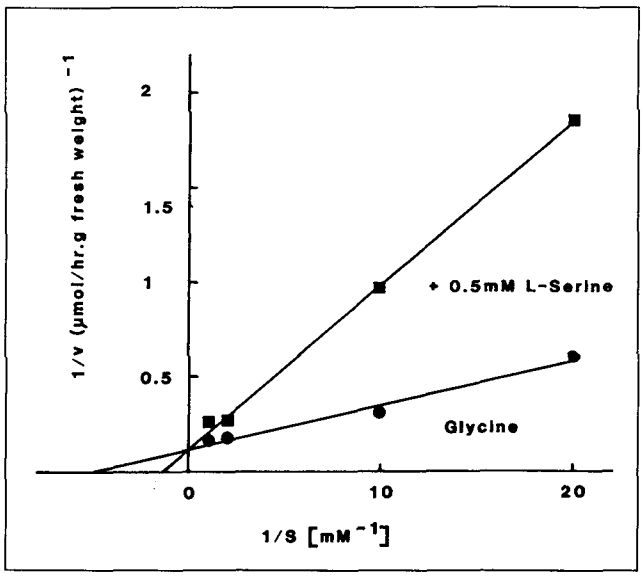

Figure 1a. Inhibition test during the uptake of glycine with the concentration of 0.5 mM L-serine.

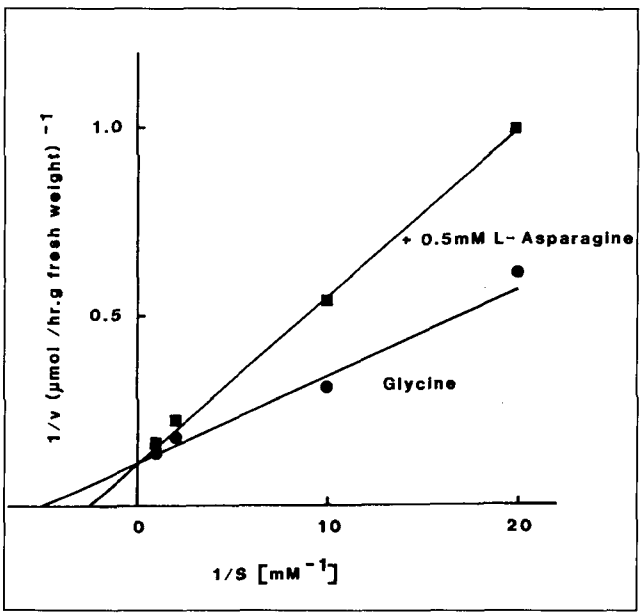

Figure 1b Inhibition test during the uptake of glycine with the concentration of 0.5 mM L-asparagine.

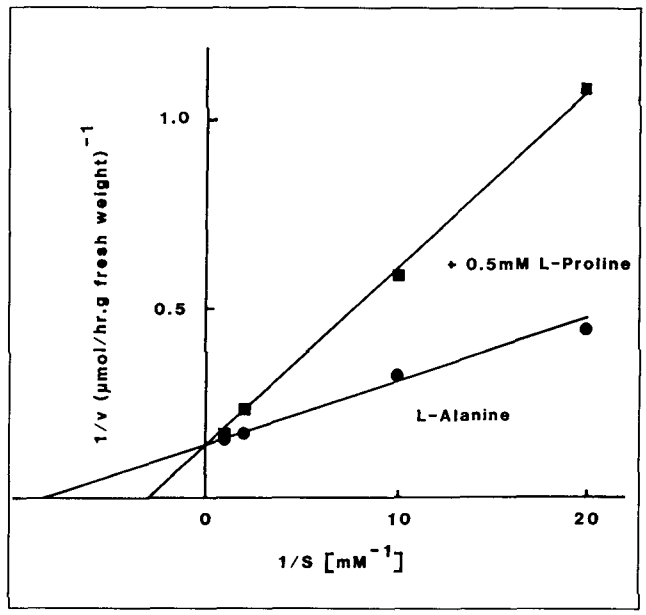

Figure 2a. Inhibition test during the uptake of L-alanine with the concentration of 0.5 mM L-proline.

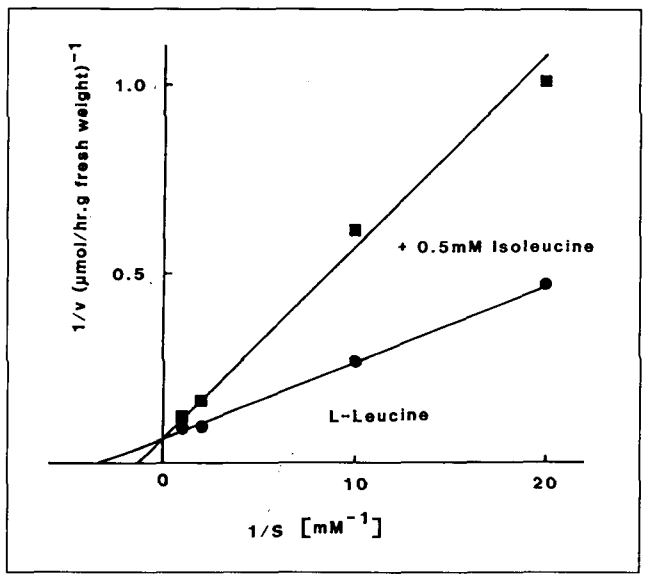

Figure 2b. Inhibition test during the uptake of L-leucine with the concentration of 0.5 mM L-isoleucine.

The influx of histidine was only inhibited by the neutral amino acids slightly, and not inhibited by the basic amino acids, such as arginie and lysine, and by the acidic amino acids, auch as aspartate and glutamate. To make sure, all the neutral amino acids including histidine belong to the same transport system, it was emploid the competitive inhibition studies with the various amino acids. The influx of glycine was competitively inhibited by 0.5 mM serine and asparagine (Fig. 1a, b). Km for glycine was 199 μM, and was 867 μM with the inhibitor, serine and 444 μM with the inhibitor, asparagine. As well as, the influx of alanine was inhibited by proline, of leucine was inhibited by isoleucine competitively (Fig. 2a, b). Km for alanine was 116 μM and Km for alanine with the inhibitor proline was 345 μM and Km for leucine was 303 μM and Km for leucine with the inhibitor isoleucine was 769 μM. This results are the same as the results in sugarcane cells (Wyse and Komor, 1984). This studies of competitive inhibition indicated clearly that the all neutral

Table 1. Inhibition of uptake of neutral amino acids by other amino acids.[a]

Labelled amino acid	Non-labelled amino acid	% Activity of control
glycine	-	100
	alanine	8
	proline	15
	valine	20
	leucine	15
	cysteine	12
	methionine	10
	phenylalanine	16
	asparagine	39
	histidine	24
	arginine	79
	glutamate	22
	aspartate	100
valine	-	100
	glycine	23
	leucine	25
	serine	20
	isoleucine	21
	tyrosine	21
	tryptophane	11
	glutamine	27
	glutamate	30
	aspartate	111
alanine	-	100
	glycine	42
	valine	46
	leucine	31
	methionine	30
	cysteine	45
	histidine	59
	glutamate	52
	aspartate	101
leucine	-	100
	valine	39
	proline	39
	glycine	41
	aspartate	118
histidine	-	100
	arginine	92
	lysine	82
	alanine	41
	glycine	47
	leucine	74
	valine	69
	glutamate	74
	aspartate	84

[a]The uptake of labelled amino acids was tested 0.1 mM in absence or presence of 1 mM non- labelled amino acids.

Table 2. Km-and Ki - value and Vmax of the neutral amino acids transport.[a]

Amino acid	Km and Ki (μM)	Vmax (μmol · h^{-1} · g fresh $weight^{-1}$)
Phenylalanine	58	3.21
methionine	63 (Ki)	-
alanine	116	7.14
serine	180	6.24
glycine	199	5.90
valine	208	15.11
threonine	281 (Ki)	-
leucine	303	14.70
isoleucine	319	9.17
proline	330 (Ki)	-
histidine	575	4.17
asparagine	640 (Ki)	-

[a]Ki was calculated by the equation $Ki = (Km+S)(V1/V2-1)$, whereas V1 = reaction rate without inhibitor, V2=maximal reaction rate, S = substrate concentration, Km = concentration of half-maximal reaction rate, I = inhibitor-concentration, and Ki = inhibitor concentration of half - maximal reaction rate.

amino acids belong to the same transport system (Table 1, Fig. 1 and b).

So far as concerning, the histidine molecule, of which in imidazole group possess the weakly basic charactor, therefore, at pH 6.0, histidine molecule has at least 50% protonated form of imidazole group. As shown in Table 1, histidine influx was not inhibited by arginine and lysine, and it concluded that histidine was not transported with charged form through the specific basic amino acids transport system (Cho, 1989). The influx of histidine was inhibited by 0.5 mM alanine and methionine comptitively (Fig. 3a, b). It means that histidine was transported only by neutral amino acid transport system as a neutral form.

As it was mentioned avobe in Table 1, the influx of glycine, valine and alanine was not inhibited by aspartate, it is possible that the acidic amino acids possess it's own transport system in the case of sugarcane suspension cells (Wyse and Komor, 1984). Furthermore, it will be tested whether the acidic amino acids were transported through the specific transport system or not (in prepare).

Kinetics of Neutral Amino Acid Transport System

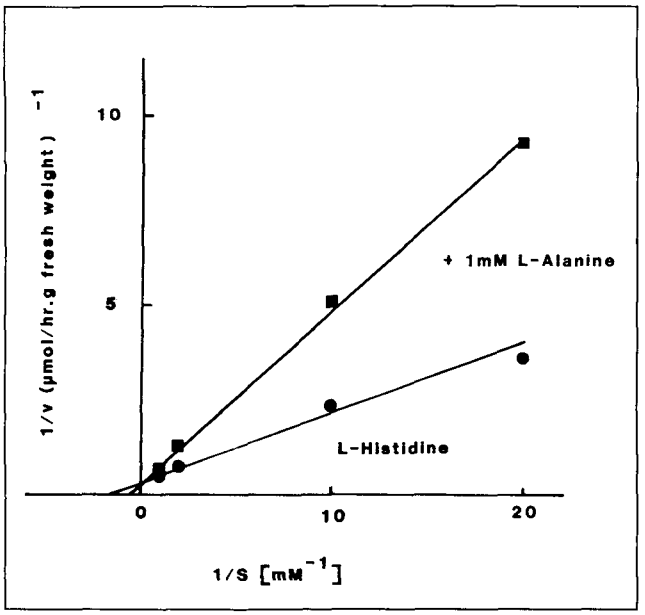

Figure 3a. Inhibition test during the uptake of L-histidine with the concentration of 1 mM L-alanine.

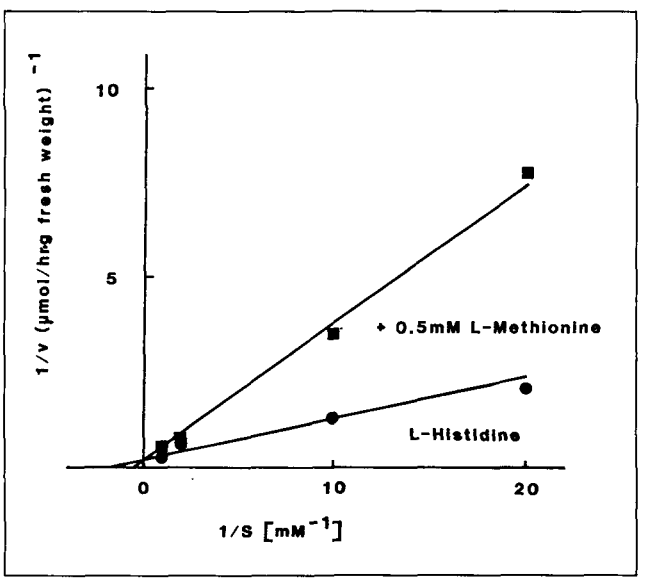

Figure 3b. Inhibition test during the uptake of L-histidine with the concentration of 0.5 mM L-methionine.

The Km values of neutral amino acids transport system have been divided into 3 different catagory on basis of the affinity to the carrier (Table 2). The Km value of phenylalanine and methionine was below 100 μM. The Km value of alanine, serine, valine, proline, leucine and isoleucine was between 100 μM - 500 μM. The Km value of histidine and asparagine was above 500 μM. The Vmax value of neutral amino acids was between 15.1 μmol · h^{-1} · g fresh weigh^{-1} and 3.12 μmol · h^{-1} · g fresh weight.$^{-1}$

Table 3. Stoichiometries of proton and potassium ion flux during amino acids uptake.[a]

Amino acid	H^+-influx (μmol h^{-1} g FW^{-1})	K^+-efflux (μmol · h^{-1} · g FW^{-1})
	Amino acid (μmol h^{-1} g FW^{-1})	Amino acid (μmol · h^{-1} · g FW^{-1})
glycine	0.62	0.90
valine	0.73	0.59
leucine	0.76	0.85
alanine	0.50	0.62
histidine	ND	1.06

[a]The concentration of amino acid used was 1 mM. FW = fresh weight, ND = not detectable initially, after proton efflux.

Stoichiometries of Neutral Amino Acids

The stoichiometries were found 0.62 influx of proton, 0.90 efflux of potassium ions for leucine (Table 3). The stoichiometry indicated that the neutral amino acids transported with one proton per one amino acid. During the influx of histidine didn't detactable any significant connection of proton influx, but the potassium ion efflux was detactable. The efflux of potassium ion during the histidine influx was stoichiometrically coupled one potassium ion per one histidine molecule after 1 minute. As it was mentioned avobe, the molecule of histidine transported as a neutral form through the transport system. Therefore, it was neccessary to release of proton in the medium to produce the neutral form of histidine molecules by the means of equlibrium constant during the influx. On the ground, one can't dectactable the proton-cotransport with histidine initially (Table 3). This results are the same as the results in *Ricinus* cotyledon (Robinson and Beevers, 1981), in sugarcane (Wyse and Komor, 1984), and bacteria (Gale and Liwellin, 1972), which the influx of the neutral amino acid was accompanied with two protons per one amino acid.

적 요

Glycine, valine, alanine 과 histidine의 수송은 모든 실험된 중성 아미노산에 의해 경쟁적인 방해를 당하였다. 그리고 reciprocal 연구 결과로 이들 중성 아미노산들은 서로 carrier의 활성부위를 점유하기위해 경쟁하므로 같은 운반자를 소유한다. Histidine은 전하를 띄우지 않은 상태로 중성 아미노산 운반자를 통해서 능동수송된다. 중성 운반자의 Km 값

은 아미노산이 운반자에 대한 친화성에 따라서 3가지로 분류하였다. 0.1 mM 보다 작은 값, 0.1 mM에서 0.5 mM 사이에 있는 값과 0.5 mM 보다 큰 값이다. V_{max} 는 3.12 μmol · h^{-1} · g fresh $weight^{-1}$ 과 15.1 μmole · h^{-1} · g fresh $weight^{-1}$ 사이에 있다. 중성 아미노산은 아미노산 한개당 수소이온 한개가 동반수송되고, K^+ 한개가 전하보상을 위해서 배출된다. Histidine 분자도 1 분자당 1개의 수소이온과 동반수송되나 전하를 띄운 histidine 분자로부터 수소이온 한개가 배지로 떨어져 나오므로 동반수송된 수소이온의 움직임을 일시적으로 측정 할 수 없다.

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