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Dynamic Aspects of Vacuolar and Cytosolic Amino Acid Pools of *Saccharomyces cerevisiae*

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By using the Cu^{2+} method (Y. Ohsumi, K. Kitamoto, and Y. Anraku, J. Bacteriol. 170:2676–2682, 1988) for differential extraction of the vacuolar and cytosolic amino acid pools from yeast cells, the amino acid compositions of the two pools extracted from *Saccharomyces cerevisiae* cells, grown in synthetic medium supplemented with various amino acids, were determined. Histidine and lysine in the medium expanded the vacuolar pool extremely. Glutamate also accumulated in the cells, but mainly in the cytosol. The composition of amino acids in the cytosolic pool was fairly constant, in contrast to that in the vacuolar pool. Cells grown in synthetic medium supplemented with 10 mM arginine accumulated arginine in the vacuoles at a concentration of about 430 mM. This large arginine pool was metabolically active and was effectively utilized during nitrogen starvation. Arginine efflux from the vacuoles was coupled with K^+ influx, with an arginine/K^+ exchange ratio of 1, as judged by the initial rate. The vacuolar arginine pool was exchangeable with lysine added to the medium and was decreased by treatment of the cells with the mating pheromone, α-factor.

Eucaryotic cells are highly integrated complexes with various membrane organelles. Yeast cells have an intracellular organization similar to that of higher plant cells, and their intracellular homeostasis of ions and nutrients is regulated by the interactions of the organelles. Their largest organelles are vacuoles, which are postulated to function in primary and secondary metabolism, turgor control, and vegetative growth (1).

Crabeel and Grenson (3) demonstrated the presence of more than one metabolic pool of basic amino acids in the cells. Wiemken and collaborators (6, 15, 16) discovered that the vacuoles in *Candida utilis* and *Saccharomyces cerevisiae* contain specific pools of basic amino acids, from which most acidic and neutral amino acids are excluded. These findings indicated that vacuoles are metabolically active organelles and must have differential sequestration systems for certain amino acids (2) and other metabolites (11).

Previous studies in our laboratory have shown that vacuolar membrane vesicles prepared from *S. cerevisiae* have seven independent transport systems for basic, aromatic, and neutral amino acids, which catalyze active transport of 10 amino acids into vesicles by amino acid/H^+ antiport systems (10). This finding strongly suggests that these transport systems play a major role in compartmentalization of amino acids in the cytoplasm, i.e., in differential formation of vacuolar and cytosolic amino acid pools. However, the mechanism of efflux of amino acids from the sequestered pool is poorly understood. Knowledge about the dynamics of compartmentation of amino acids is a prerequisite to understanding its regulation.

In the accompanying paper (9), we reported a simple method using Cu^{2+} for differential extraction of the vacuolar and cytosolic amino acid pools of *S. cerevisiae*. The present paper describes the changes of cytosolic and vacuolar constituents of yeast cells under several physiological conditions determined by the Cu^{2+} method.

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MATERIALS AND METHODS

Strains, culture conditions, and analytical methods are described in the accompanying paper (9). Mating pheromone, α-factor, was prepared by the method of Duntze et al. (4). The other chemicals used were commercial products of analytical grade.

RESULTS

Change of vacuolar amino acid pool during growth. Using the standard Cu^{2+} method (9), we examined how the vacuolar and cytosolic amino acid pools change during the growth phase. As shown in the accompanying paper, inclusion of glucose in the standard extraction medium enabled us to extract the two pools separately even from stationary-phase cells, which were resistant to polycationic polymers. Results showed that during growth, the total amino acid content per cell increased gradually up to the middle of the logarithmic phase (2×10^7 cells per ml) and decreased when the cells reached stationary phase. The relative concentrations of the 20 amino acids in the two pools did not change significantly during growth.

Effects of added amino acids on the vacuolar amino acid pool. To study the physiological role of vacuoles in amino acid metabolism and differential pool formation, we grew cells in YNBD medium supplemented with various amino acids and determined the contents of their vacuolar and cytosolic pools (Table 1).

On addition of lysine or histidine to the medium, the total cellular amino acid content almost doubled. The amounts of lysine and histidine in the vacuolar pool increased 27- and 42-fold, respectively, whereas their contents in the cytosolic pool did not change significantly. The doubling time did not alter appreciably in the presence of added lysine or histidine. On addition of arginine to the medium, the arginine content of the vacuolar pool increased sevenfold. Additions of aspartate, glutamate, methionine, and proline, respectively, resulted in five-, two-, five-, and fourfold increases in the contents of these amino acids in the cytosolic pool. However, the concentrations of all these amino acids, except

TABLE 1. Amino acid composition of cellular pools and distribution between pools[a]

Amino acid(s)	Amt of amino acid(s) with the following medium:																			
	YEPD		YNBD + Asp		YNBD + Glu		YNBD + Pro		YNBD + Gly		YNBD + Met		YNBD + His		YNBD + Lys		YNBD + Arg		YNBD	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Asp	39	11	49	9	4	1	12	74	13	42	11	14	9	48	22	40	19	33	11	22
Thr + Gln	38	40	49	49	128	60	69	37	36	73	16	34	19	54	19	48	32	42	94	64
Ser	16	23	11	39	16	58	7	63	30	73	4	46	10	43	12	46	4	42	10	47
Glu + Cit	144	14	130	19	323	34	126	24	76	55	69	15	93	61	148	47	104	32	144	34
Gly	42	21	16	42	16	51	9	46	255	46	7	47	16	34	13	48	5	37	11	48
Ala	42	23	36	40	50	52	37	42	32	62	24	18	29	42	30	37	40	35	49	42
Cys	4	21	6	48	9	47	7	49	9	62	7	53	7	53	10	62	4	53	6	51
Val	11	34	17	48	18	58	14	51	16	63	16	38	16	58	15	52	10	50	16	52
Met	5	23	5	50	4	46	6	49	1		34	27	6	45	5	45	3	53	7	52
Ile	9	36	6	50	5	59	5	53	5	52	4	56	7	57	6	55	4	67	6	54
Leu	15	32	5	65	5	70	4	63	3		6	55	6	60	7	66	3	77	4	63
Tyr	2	64	5	44	4	53	3	48	4	44	6	55	5	51	4	50	2	54	4	50
Phe	5		1						1		1		3		2		1		1	
Orn	7	99	12	76	42	84	17	81	57	94	31	84	19	76	12	72	24	80	16	90
Lys	106	89	24	77	38	85	26	83	20	81	35	82	43	66	660	96	16	61	30	79
NH_3	26	20	41	37	50	52	60	72	45	53	62	56	102	45	117	51	46	30	53	52
His	36	76	8	66	8	70	16	80	6	99	12	75	628	96	13	71	4	99	25	62
Arg	53	94	16	93	14	94	24	98	61	98	81	92	22	80	20	56	319	97	45	97
Pro	2		3				9								4		9		2	
Total	602	47	440	38	734	50	451	54	670	62	426	54	1,040	80	1,119	77	649	70	534	55

[a] *S. cerevisiae* X2180-1A cells grown in YEPD medium or YNBD medium supplemented with 10 mM concentrations of various amino acids were treated with $CuCl_2$ as described elsewhere (9), and their pools were analyzed with an amino acid analyzer. A, Total cellular amino acid content (nmol/10^8 cells); B, percentage of total in vacuolar extract. For the calculation of the concentration of amino acid in each pool, we assumed that the cell volume of the haploid cell was 29 μm^3 (5) and that the vacuoles account for approximately 25% of the cell volume (15). Total amino acid concentration (millimolar) was calculated as 0.345 × total cellular amino acid content; vacuolar concentration (millimolar) was calculated as 0.0138 × total cellular amino acid content × the percentage of the total in the vacuolar extract.

glutamate, were low, and consequently, the total amino acid pool size did not change much. Glutamate caused an increase in the total amino acid pool, but the distribution ratio of glutamate in the vacuolar pool was not increased. Upon addition of glycine to the medium, the total glycine pool increased 22-fold and glycine was found to be evenly distributed between the two compartments.

Arginine pool during nitrogen starvation. The results in Table 1 indicate that the cells accumulated arginine in the vacuoles at a concentration of about 430 mM. We examined how this large arginine pool changed during nitrogen starvation. The vacuolar arginine pool decreased rapidly when the cells were transferred to nitrogen-free YNBD medium (Fig. 1A).

Interestingly, during the course of nitrogen starvation, an unusual amino acid, γ-amino butyric acid (GABA) appeared within the first 1 h and decreased in parallel with arginine. GABA was localized exclusively in the vacuolar extract (about 10 mM in the cell). This observation means that arginine stored in the vacuoles was utilized for growth during nitrogen starvation. This removal of arginine from the vacuoles should cause a drastic change in vacuolar turgor pressure if arginine is present mainly in free form in the vacuoles. However, on microscopic examination, we could not detect any change in the size of vacuoles during nitrogen starvation. Therefore, we analyzed the change of K^+ concentration in vacuoles, since K^+ is the most abundant and osmotically active cation in yeast cells.

The K^+ ion content in the cytosolic pool was constant, but that in the vacuolar pool increased almost 10-fold during nitrogen starvation (Fig. 1B). In the first 2 h, the decrease of arginine corresponded well to the increase of K^+ ions. This suggests that arginine comes out from vacuoles preferentially with a stoichiometric exchange ratio of 1:1. Further starvation may mobilize other amino acids sequestered in the vacuoles, since K^+ increased more than arginine decreased.

Release of vacuolar arginine induced by lysine and α-factor. Whitney and Magasanik (14) reported that the addition of

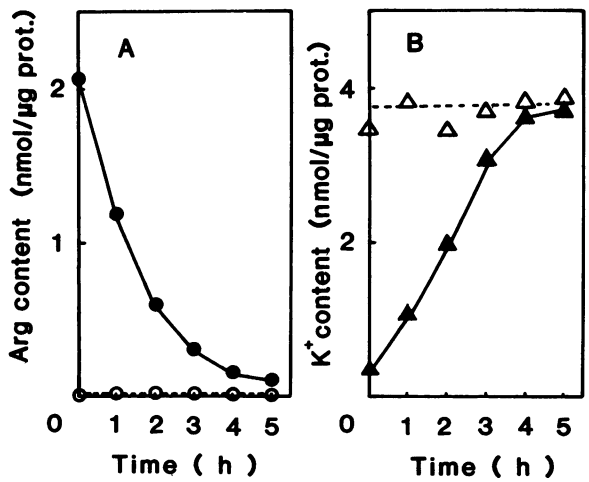

FIG. 1. Changes in arginine and K^+ contents during nitrogen starvation. *S. cerevisiae* X2180-1A cells grown in 25 ml of YNBD medium supplemented with 10 mM arginine were harvested, washed twice with 25 ml of nitrogen-free YNBD medium, and then transferred to 25 ml of nitrogen-free YNBD medium. At the indicated times after transfer, cytosolic (○, △) and vacuolar (●, ▲) pools were extracted by the standard Cu^{2+} method (9) and the arginine (A) and K^+ (B) contents were determined.

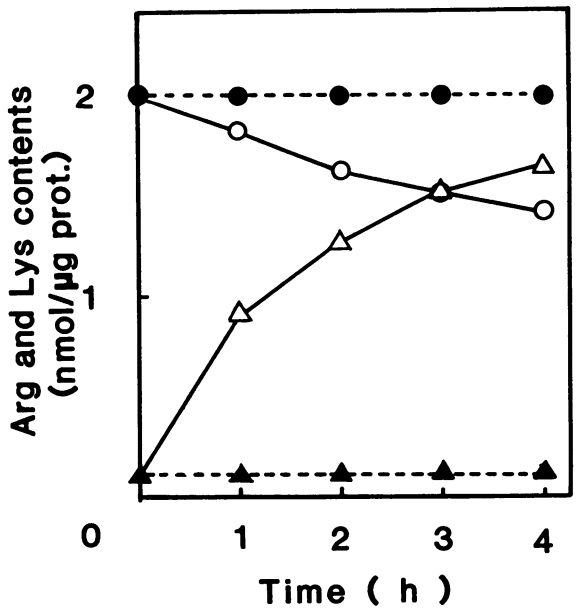

FIG. 2. Release of arginine from vacuoles by exchange with lysine. *S. cerevisiae* X2180-1A cells grown in 25 ml of YNBD medium supplemented with 10 mM arginine were harvested and transferred to YNBD medium supplemented with 10 mM lysine. The cytosolic and vacuolar pools were extracted by the standard Cu^{2+} method (9), and the arginine (○) and lysine (△) contents were determined. Control cells grown in YNBD medium supplemented with arginine were transferred to the same fresh medium, and their vacuolar arginine (●) and lysine (▲) contents were determined.

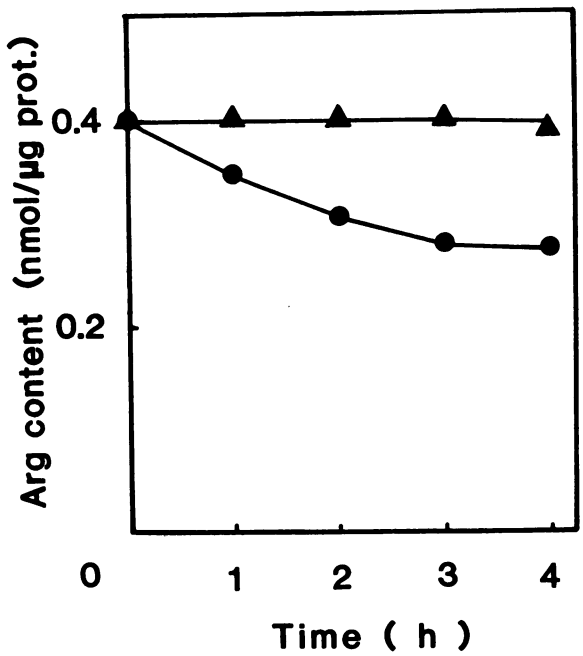

FIG. 3. Release of arginine from vacuoles on treatment with α-factor. *S. cerevisiae* X2180-1A cells were grown in YEPD medium, and α-factor was added to the cell suspension at a concentration of 50 U/ml. The cytosolic and vacuolar pools were extracted by the Cu^{2+} method (9). The arginine contents of vacuoles of cells incubated with (●) or without (▲) α-factor for the indicated times are shown. The glutamate content of the cytosol was not affected by the presence of α-factor.

lysine to the growth medium causes induction of arginase in yeast cells. They proposed that arginine first accumulated in the vacuoles and then exchanged with lysine in the cytosolic pool and that the arginine that was transferred to the cytosol induced arginase, the first enzyme of its degradation system. To test whether this explanation was physiologically tenable, we used the Cu^{2+} method to analyze the change in the vacuolar arginine pool on addition of lysine to the growth medium. Cells were grown in YNBD medium supplemented with 10 mM arginine and then transferred to YNBD medium supplemented with 10 mM lysine, and changes in the vacuolar arginine and lysine pools were monitored (Fig. 2). The results supported the explanation described above that lysine induced liberation of arginine from the vacuolar compartment. This interchange is consistent with the in situ operation of a counter-flow mechanism catalyzed by the arginine-lysine transport system of the vacuoles (10).

Sumrada and Cooper (12) observed that treatment of *MAT***a** cells with α-factor induces arginase and suggested that the pheromone may induce the release of vacuolar arginine into the cytosol by some unknown mechanism. We found, as they suggested, that on treatment of the cells with α-factor, the vacuolar arginine pool in fact decreased, but only a little, while the glutamate pool in the cytosol did not change (Fig. 3). We also detected induction of arginase activity after treatment of the cells with α-factor for 1 h. These results demonstrated that the standard Cu^{2+} method is useful for determining dynamic vacuolar functions in situ under various physiological conditions.

DISCUSSION

We demonstrated that the Cu^{2+} method (9) is useful for differential extractions of the cytosolic and vacuolar amino acid pools from yeast cells under various growth conditions. Generally, we observed that cells growing slowly were more resistant to surface-acting reagents, i.e., polycations such as cytochrome *c* and DEAE-dextran. However, under standard conditions, the Cu^{2+} method was applicable even to stationary-phase cells and to the various strains of yeast cells tested (data not shown). Thus, it must be the most reliable and widely applicable method for selective permeabilization of yeast cells.

Previously, Messenguy et al. (8) reported the compartmentation of amino acid pools of *S. cerevisiae* by using cytochrome *c* as a permeabilizing reagent. They showed that under certain conditions, more than 80% of amino acids and more than 40% of acidic amino acids are localized in the vacuoles. Our data (Table 1) are not consistent with their results, but this discrepancy might be due to the differences in the strains or methods used. Wiemken and Dürr (15) examined amino acid pools of yeast by analyzing the isolated vacuoles and showed that most acidic amino acids (85%) are localized in the cytoplasm. Our results (Table 1) confirmed their findings. Determination of the cytosolic pool by differential extraction depends on the completeness of permeabilization of the plasma membrane. We checked that Cu^{2+} permeabilized almost all cells in every experiment described in this paper.

We have reported that the vacuolar membrane has seven independent amino acid transport systems which are specific for arginine, arginine-lysine, histidine, phenylalanine-tryptophan, tyrosine, glutamine-asparagine, and isoleucine-leucine. By these systems, 10 amino acids are accumulated in the cell vacuoles by an H^+/amino acid antiport mechanism (10). Acidic amino acids, methionine, glycine, and proline were not taken up by the vacuolar membrane vesicles (10)

and may not be appreciably sequestered in the vacuoles in situ. The amino acid composition of the vacuolar compartment (Table 1) is consistent with the idea that the active transport systems detected in vacuolar membrane vesicles are related to the formation of the vacuolar amino acid pool.

We found that in cells grown in YNBD medium supplemented with various amino acids, the cytosolic amino acid pool was fairly constant (192 to 367 nmol/10^8 cells; Table 1) but the vacuolar amino acid pool changed very much (167 to 862 nmol/10^8 cells) and was enlarged, depending on the amino acid added to the medium. We showed that arginine accumulated in the vacuoles at a high concentration of about 430 mM and was used as a nitrogen source during nitrogen starvation. This observation strongly suggests that the vacuoles are a physiologically active compartment for certain amino acids and are potentially concerned with cellular nitrogen metabolism.

The pool extracted by the Cu^{2+} method as the vacuolar fraction must contain amino acids associated with other organelles such as mitochondria, where protein synthesis takes place and some amino acid biosynthetic reactions are localized. GABA appeared during nitrogen starvation of cells in which arginine had accumulated and may have been derived from arginine via ornithine. Since an in vitro study using the vacuolar membrane vesicles showed that GABA was not taken up into vacuoles in an ATP-dependent manner, it is probable that GABA is mainly localized not in vacuoles but in mitochondria.

Arginine is a good nitrogen source, and excess arginine in the cytosol is degraded by arginase, the first enzyme of arginine catabolism. It should be noted that *S. cerevisiae* has no system for the degradation of lysine or histidine (13). This may be one reason why yeast cells accumulated more lysine and histidine than arginine in the vacuoles when the cells were grown in medium supplemented with these amino acids (Table 1). Conceivably, if these in vivo sequestering systems are perturbed by some mutation, the resulting mutant will show loss of homeostatic control of the lysine or histidine pool in the cytosol, and this may result in growth deficiency in medium supplemented with a high concentration of that amino acid. This idea has been tested, and we have obtained mutants defective in vacuolar functions, as described in the accompanying paper (7).

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