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Choline Transport in *Saccharomyces cerevisiae*

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Choline transport of *Saccharomyces cerevisiae* was measured by the filtration method with the use of glass microfiber paper. The uptake was time and temperature dependent. The kinetics of choline transport showed Michaelis behavior; an apparent K_m for choline was 0.56 μM. *N*-Methylethanolamine, *N,N*-dimethylethanolamine, and β-methylcholine were competitive inhibitors of choline transport, with K_i values of 40.1, 3.1, and 6.9 μM, respectively. Ethanolamine, phosphorylcholine, and various amino acids examined had no effect. Choline transport required metabolic energy; removal of glucose resulted in a great loss of transport activity, and the remaining activity was abolished by 2,4-dinitrophenol, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone, arsenate, and cyanide. External Na^+ was not required, and the transport was not affected by ionophores, valinomycin, and gramicidin D. These results indicate that *S. cerevisiae* possesses an active choline transport system mediated by a specific carrier. This view is further supported by the isolation and characterization of a choline transport mutant. The choline transport activity in this mutant was very low, whereas the transport of L-leucine, L-methionine, D-glucose, and *myo*-inositol was normal. Together with the choline transport mutant, mutants defective in choline kinase were also isolated.

Phosphatidylcholine is the major phospholipid in most eucaryotic cells. It functions as a structural component of the cell membranes and also as a modulator of certain enzymes. Choline, a quarternary ammonium compound, constitutes the hydrophilic part of the molecule, and together with hydrophobic hydrocarbon chain of fatty acid it provides the amphipathic nature of this lipid. This amine is incorporated into phosphatidylcholine via the CDP-choline pathway (6).

It has been postulated that mammalian cells are largely dependent on extracellular choline for the supply of this amine. Therefore, the transport of extracellular choline into the cell is an obligatory process for phosphatidylcholine biosynthesis. The uptake of choline has been investigated with some excitable tissues of higher organisms which are capable of synthesizing phosphatidylcholine as well as acetylcholine (1, 4, 5, 8, 15) and also with nonexcitable tissues (9, 13). The present investigation is concerned with the characterization of the choline transport system of a yeast, *Saccharomyces cerevisiae,* which possesses enzymes of the CDP-choline pathway for the synthesis of phosphatidylcholine (12). From a conditional choline auxotroph, a mutant defective in choline transport was obtained and characterized. The results indicate the presence of an energy-requiring transport system specific for choline in this microorganism.

MATERIALS AND METHODS

Media and culture. The compositions of inositol-depleted minimum medium (M-i) and complex medium (WaD) were described previously (16). Inositol-supplemented minimum media (M 0.2i, M 2i, M 20i) were prepared by supplementing M-i medium with *myo*-inositol at concentrations of 0.2, 2, and 20 μg/ml, respectively. A nitrogen-free medium (M-N) and a nitrogen- and sodium-free medium (M-N-Na^+) were prepared by omitting ammonium sulfate and by omitting both ammonium sulfate and sodium chloride from medium M 2i, respectively. Agar plates contained 2.5% agar in addition to the components of the medium indicated.

Yeast cells were cultured aerobically at 30°C with shaking. Cell growth was followed by measuring the optical density at 550 nm in an 18-mm-diameter test tube with an Hitachi 101 spectrophotometer equipped with a test tube holder.

Yeast strains. *S. cerevisiae* X2180-1B, kindly supplied by the Yeast Genetic Center, the University of California, was used as a wild-type strain. A conditional choline auxotroph, 172α, was derived from X2180-1B as described elsewhere (16). This mutant grew at normal rates in the presence of low concentrations of *myo*-inositol, but at markedly reduced rates in the presence of high concentrations of *myo*-inositol. The inhibition of growth by *myo*-inositol was completely prevented by supplementation with choline. Therefore, 172α proliferated in media M-i, M 0.2i, and choline-supplemented M 20i, but not in medium M 20i.

Isolation of choline transport mutant 187α and its revertant. Strain 172α grown overnight in medium WaD was mutagenized with 3% ethyl methane sulfo-

nate for 50 min at 30°C. The survival rate was 13%. The mutant was enriched by two cycles of the nystatin treatment as described by Fink (3). The mutagenized cells were cultured in medium M 0.2i for 2 days and starved for nitrogen in medium M-N for 11.5 h. Then the cells were suspended in medium M 20i supplemented with 20 μg of choline per ml to an optical density of 0.3 at 550 nm and cultured for 4 h until the density increased 3%. Then nystatin was added to a concentration of 4 μg/ml, and the culture was shaken for 1 h at 30°C. The treated cells were washed with water and grown in medium M 0.2i for 2 days. The nystatin treatment was repeated again. Finally, cells were grown on M 0.2i agar and then replica plated onto both M 0.2i agar and M 20i agar supplemented with 20 μg of choline per ml. The cells that grew on medium M 0.2i but not on medium M 20i containing 20 μg of choline per ml were selected. Seven presumptive mutants were assayed for choline transport activity, and one designated 187α was found to have very low choline transport activity (less than 1% of that of the wild-type strain). Among the other six strains were found mutants which were defective in choline kinase (186α, 193α).

Spontaneous revertant strain 187α R32 was isolated as follows. The mutant 187α was grown for 2 days in medium M 0.2i. From this culture the cells that grew on M 20i agar containing 20 μg of choline per ml but not on M 20i agar were isolated by replica plating and tested for choline transport activity. The strain which showed recovered choline transport activity was saved. The revertant frequency was approximately 10^{-6}.

Choline transport assay. Strains were grown in medium M 2i or medium M 0.2i to an optical density of 0.5 to 0.8 at 550 nm in a Monoshin II shaker (Taiyo Kagaku, Tokyo). Cells were harvested by centrifugation at 4,000 × *g* for 10 min at 0°C, washed twice with cold saline, and suspended in medium M-N. The standard assay mixture contained medium M-N, 30 nmol of [*methyl*-^{14}C]choline (8,000 to 10,000 cpm/nmol), and yeast cells (10 to 50 μg of cell protein) in a final volume of 1 ml. A mixture (0.97 ml) containing all the ingredients except for choline was preincubated at 30°C for 5 min, and the transport was started by the addition of 0.03 ml of 1 mM [^{14}C]choline. After 2 min of incubation at 30°C with shaking, the transport was terminated by the addition of 5 ml of cold saline, followed by immediate filtration through Whatman GF/C glass microfiber paper. The filter paper was washed three times with 5 ml of cold saline and dried in a scintillation counting vial. The filter paper was incubated at 40°C for 5 min with 0.5 ml of Scintilamine-OH (Dojin Chemicals) in the vial and counted for radioactivity with Beckman LS7000 liquid scintillation spectrometer in 5 ml of scintillation liquid which contained 5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene per liter of toluene. The filtration-washing procedure was finished within 15 s. Under the conditions employed, almost all the cells of strains X2180-1B, 172α, 186α, 187α, 187α R32, and 193α were trapped by the GF/C filter.

Identification of the compounds derived from transported choline. The incubation was performed as described above except that the incorporation period was 1 min and that 290 μg of protein of X2180-1B cells grown in medium M 2i was used. After washing, the filter was immediately soaked in 3 ml of 10% cold perchloric acid, which contained 1 μmol each of choline, phosphorylcholine, and CDP-choline as carriers, and was sonicated in the presence of 2 g of glass beads (diameter, 1 mm) for 5 min on ice with a Ohtake 5202 sonicator. The sonicated sample was filtered through GF/C microfiber paper, and the paper was washed with 2 ml of 10% cold perchloric acid. The combined fluid (acid-soluble fraction) was neutralized with potassium hydroxide. After centrifugation at 1,300 × *g* for 10 min, the supernatant fluid was lyophilized. The lyophilized sample was dissolved in 1 ml of water, and analyzed for choline and other water-soluble compounds by thin-layer chromatography on a cellulose plate (Avicel SF; Funakoshi Pharmaceutical) with butanol–acetic acid–water (5:2:3, by volume) as developing solvent.

From the acid-insoluble fraction retained on the GF/C filter, lipids were extracted with 20 ml of chloroform-methanol (2:1, by volume), washed with 5 ml saline, and then twice with 5 ml of methanol-saline (1:1, by volume). The washed extract was evaporated to dryness and dissolved in 1 ml of chloroform-methanol (2:1, by volume). A 0.5-ml portion of the sample was analyzed by thin-layer chromatography on a silicic acid plate with chloroform-methanol-acetic acid-water (25:15:4:2, by volume) as developing solvent.

Enzyme assay. Yeast cells were cultured in 250 ml of medium M 0.2i with TAH-32 shaker (Thomas Scientific) to an early stationary phase. Cells were harvested by centrifugation at 3,800 × *g* for 10 min at 0°C, disrupted, and fractionated as described previously (16) except that the homogenate was centrifuged at 4,000 × *g* for 10 min and then at 100,000 × *g* for 60 min. The 4,000 × *g* supernatant, the 100,000 × *g* supernatant, and membrane fractions were assayed for cholinephosphate cytidyltransferase (EC 2.7.7.15), choline kinase (EC 2.7.1.32), and cholinephosphotransferase (EC 2.7.8.2), respectively. All enzyme reactions were carried out at 25°C with shaking. One unit is defined as that amount of the enzyme which catalyzes the conversion of 1 μmol of substrate per min. Choline kinase was assayed isotopically as described previously (16). The activities of the other enzyme were determined as follows.

Cholinephosphate cytidyltransferase was assayed by measuring the formation of [^{14}C]CDP-choline from [^{14}C]phosphorylcholine. The reaction mixture contained 10 μmol of Tris-hydrochloride (pH 8.0), 1 μmol of CTP, 2.5 μmol of $MgCl_2$, 0.8 μmol of phosphoryl [*methyl*-^{14}C]choline (550 cpm/nmol), and the enzyme (0.1 to 0.5 mg of protein) in a total volume of 0.2 ml. After a 20-min incubation, the reaction was terminated by the addition of 0.1 ml of cold 10% trichloroacetic acid. The [^{14}C]CDP-choline formed was separated by paper chromatography and counted as described by Feldman et al. (2) except that isopropanol–20% trichloroacetic acid–28% NH_4OH (75:25:0.3, by volume) was used as developing solvent.

Cholinephosphotransferase was assayed by a procedure modified from that described by Weiss et al. (14). The reaction mixture contained 12 μmol of Tris-hydrochloride (pH 8.0), 0.25 μmol of dithiothreitol, 100

μg of bovine serum albumin, 2.5 μmol of $MgCl_2$, 0.1 μmol of CDP-[*methyl*-^{14}C]choline (500 cpm/nmol), 1 μmol of 1,2-diacyl-*sn*-glycerol (added as a 20 mM emulsion in 0.1% Tween 20), and the enzyme (0.1 to 0.3 mg) in a total volume of 0.25 ml. After a 20-min incubation, the reaction was terminated by the addition of 2 ml of methanol. Then, 4 ml of chloroform and 1 ml of 1 M KCl were added, and the mixture was shaken vigorously with a Vortex mixer. After the phases separated, the upper phase was discarded. The lower phase was washed three times with 4 ml of methanol-1 M KCl (1:1, by volume). The washed extract was taken to dryness in a scintillation counting vial, and the radioactivity was determined in 7 ml of toluene–Triton X-100 scintillation liquid (10).

Protein determination. Protein was determined by the method of Lowry et al. (7) with bovine serum albumin as the standard. Membrane protein was solubilized with sodium deoxycholate before assay. When the protein of unbroken cells was determined, cells were digested with 0.1 ml of 2 N NaOH at 100°C for 10 min before assay. The protein concentration of the X2180-1B culture growing exponentially in medium M 2i was calculated routinely from the optical density at 550 nm with the use of a calibration curve prepared for that culture conditions of the cell.

Reagents. ATP, CTP, CDP-choline, valinomycin, and carbonyl cyanide *p*-trifluormethoxyphenyl hydrazone was obtained from Boehringer Mannheim. Gramicidin D was from P-L Biochemicals. 2-Deoxy-D-glucose, 2,4-dinitrophenol, *N,N*-dimethylethanolamine, betaine, DL-carnitine, and sarcosine were purchased from Wako Chemicals. Ethyl methane sulfonate, ethanolamine, *N*-methylethanolamine, and choline chloride were obtained from Nakarai Chemicals. β-Methylcholine and *myo*-inositol were products of Tokyo Kasei. Amino acids were products of Kyowa Hakko. Nystatin was provided by Japan Squibb. 1,2-Diacyl-*sn*-glycerol was prepared from egg yolk phosphatidylcholine by the action of *Clostridium welchii* phospholipase C (Sigma). L-[*U*-^{14}C]leucine, L-[*methyl*-^{14}C]methionine, D-[*U*-^{14}C]glucose, [*methyl*-^{14}C]choline, and CDP-[*methyl*-^{14}C]choline chloride were purchased from the Radiochemical Centre. Phosphoryl[*methyl*-^{14}C]choline and *myo*-[*U*-^{14}C]inositol were obtained from New England Nuclear. Other chemicals were commercial products of analytical grade.

RESULTS

Kinetics of choline transport. Choline transport of the yeast *S. cerevisiae* was examined with exponentially growing wild-type strain X2180-1B. The cells were incubated with [^{14}C]choline at 30°C, and the cells which incorporated radioactivity were collected by rapid filtration through a glass microfiber paper and counted. The incubation was performed in the nitrogen-depleted medium, M-N, since the cells exhibited the highest activity in this medium. The transport system was stable at 0°C for at least 6 h. In the experiment illustrated in Fig. 1, the transport was assayed at 30 and 0°C. The data show that an increasing amount of choline

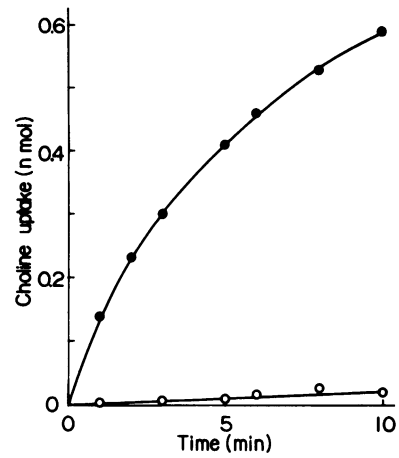

FIG. 1. *Time course of [^{14}C]choline uptake. Wild-type cells were grown in medium M 2i, and choline transport was measured with 21 μg of cell protein under the standard assay conditions as described in the text except that the incubation period and the temperature were varied as indicated. Symbols: ●, 30°C; ○, 0°C.*

accumulated at 30°C with time. In contrast, no choline uptake was observed at 0°C. These results suggest that the process is mediated by a specific carrier system. This concept is also supported by the kinetic behavior of the process. As depicted in Fig. 2A, plots of the initial rate of choline transport against choline concentration showed a hyperbolic curve approaching a maximum at which the rate was zero order with respect to choline concentration. The Lineweaver-Burk representation of this saturation curve gave a linear relationship over the choline concentration range examined (Fig. 2B). An apparent K_m for choline was determined to be 0.56 μM. These results suggest that the choline transport system contains a specific site to which choline binds reversibly when it is transported across the membrane.

Specificity of the transport system. In an attempt to elucidate the specificity of the choline transport system, the ability of various structural analogs of choline and amino acids to inhibit choline transport activity was examined. In the experiment represented in Table 1, transport activity was assayed in the presence of these compounds. The amount of the compounds added was 100 times as much as that of the substrate. The choline uptake was essentially not affected by ethanolamine, phosphorylcholine, sarcosine, and various amino acids examined. In agreement with these data, [^{14}C]phosphorylcholine was not transported into the cell when examined under the similar conditions used for [^{14}C]choline transport. Betaine and car-

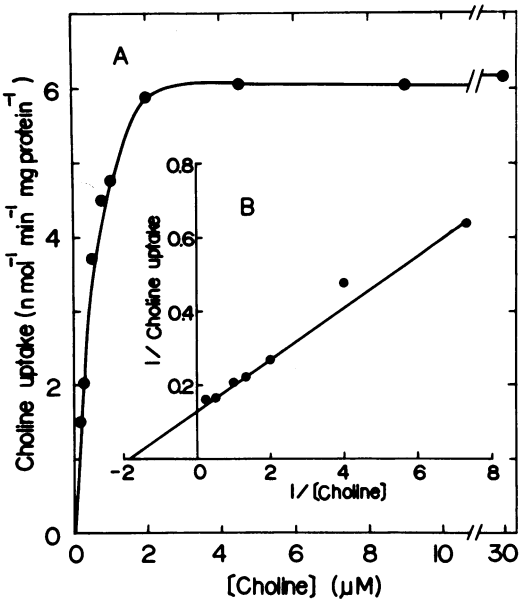

FIG. 2. *Effect of choline concentration on [^{14}C]-choline uptake (A) and Lineweaver-Burk plots (B). Wild-type cell was grown in medium M 2i, and choline transport was measured with 19 μg of cell protein under the standard assay conditions as described in the text except that the incubation period was 1 min and that the concentration of choline was varied as indicated.*

TABLE 1. *Effects of various choline analogs and amino acids on choline transport*[a]

Addition	Activity (%)[b]
None	100
Ethanolamine	97
N-Methylethanolamine	45
N,N-Dimethylethanolamine	5
β-Methylcholine	10
Phosphorylcholine	101
Betaine	69
DL-Carnitine	69
Sarcosine	93
L-Methionine	84
L-Arginine	78
L-Alanine	87
L-Leucine	91

[a] Wild-type cell was grown in medium M 2i, and choline transport was measured with 20 μg of cell protein under the standard assay conditions as described in the text except that various choline analogs and amino acids were added simultaneously with choline to yield a final concentration of 3 mM.

[b] One hundred percent activity corresponded to 5.93 nmol min^{-1} mg of protein^{-1}.

nitine slightly inhibited choline transport. *N*-Methylethanolamine affected the system to some extent. *N,N*-Dimethylethanolamine and β-methylcholine, which are closely related to choline in structure, caused a marked inhibition on choline transport at the concentration used. A Lineweaver-Burk plot analysis with fixed concentrations of the inhibitors and varying concentrations of choline showed that *N*-methylethanolamine, *N,N*-dimethylethanolamine, and β-methylcholine behaved as competitive inhibitors for the choline transport system. Apparent K_i values for *N*-methylethanolamine, *N,N*-dimethylethanolamine, and β-methylcholine were 40.1, 3.1, and 6.9 μM, respectively. The results of these competition experiments indicate a high specificity of the *S. cerevisiae* choline transport system. This view is also supported by the findings obtained with choline transport mutant 187α (see below).

Energy requirement for choline transport. It is of interest to explore the dependence of the choline transport process on metabolic energy. For this purpose, the effect of depletion of glucose as well as that of addition of some metabolic inhibitors was examined. As shown in Table 2, removal of glucose from the assay mixture resulted in a great loss of choline transport activity. The addition of 2-deoxyglucose in place of glucose was completely ineffective. Moreover, residual activities exhibited in the absence of glucose were further diminished by the addition of inhibitors of energy metabolism such as 2,4-dinitrophenol, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone, sodium arsenate, and potassium cyanide. These results indicate that choline transport is an active transport process.

Effects of Na^+ and ionophores on choline transport. External Na^+ is a common requirement for the transport of many substances (11). Choline transport into synaptosomes has also been reported to require Na^+ (4, 8, 15). There-

TABLE 2. *Energy requirement of [^{14}C]choline uptake*[a]

Assay mixture	Activity (nmol min^{-1} mg of protein^{-1})
Complete	4.2
−Glucose	0.85
−Glucose + 2% 2-deoxyglucose	0.45
−Glucose + 1 mM 2,4-dinitrophenol	0.27
−Glucose + 2 μM carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone	0.32
−Glucose + 5 mM Na_2AsO_4	0.44
−Glucose + 1 mM KCN	0.42

[a] Wild-type cell was grown in medium M 2i, and choline transport was measured with 38 μg of cell protein under the standard assay conditions as described in the text except that the assay mixture was modified as indicated. Inhibitors were added at the start of preincubation.

fore, we examined the effects of Na^+ and various ionophores on choline transport in *S. cerevisiae*. Wild-type cells grown in medium M 2i to exponential growth phase were washed with water, suspended in nitrogen-depleted minimum medium containing varying concentrations of Na^+, and determined for choline transport activity. The activities assayed in M-N-Na^+, M-N-Na^+-50 mM NaCl, M-N-Na^+-150 mM NaCl, and M-N-Na^+-300 mM NaCl were 5.56, 5.48, 5.67, and 5.76 nmol min^{-1} mg of $protein^{-1}$, respectively. Moreover, valinomycin and gramicidin D, when examined up to the concentrations of 10 and 20 μg/ml, respectively, did not affect the transport activity under the standard assay conditions. These data show that external Na^+ is not required for choline transport in *S. cerevisiae*.

Fate of [^{14}C]choline after transport into the cell. [^{14}C]choline was transported for 1 min into the wild-type cells, and the accumulated radioactivity was identified by thin-layer chromatography. Upon entry into the cell, 19% of the radioactivity could be recovered as unaltered choline. A large portion (72%) of the transported choline was phosphorylated to phosphorylcholine. CDP-choline constituted approximately 2%. An additional 6% was identified as phosphatidylcholine.

Mutant defective in choline transport. Since the above-mentioned results show the presence of a specific carrier system for choline in *S. cerevisiae*, an attempt was undertaken to isolate a mutant with a lesion in choline transport activity. Conditional choline auxotroph 172α was a suitable parental strain for the isolation of such mutant. This strain required choline for its growth in the presence of 20 μg of *myo*-inositol per ml, but not in its absence. After the mutagenesis of 172α with ethyl methane sulfonate and the nystatin enrichment procedure, the cells which can grow in low *myo*-inositol medium but not in high *myo*-inositol medium supplemented with choline were selected. Among these cells was found a mutant which was specifically defective in choline transport. As shown in Table 3, the transport activity in this mutant (187α) was 0.9% of that in the parental strain 172α. The activities of choline kinase, cholinephosphate cytidyltransferase, and cholinephosphotransferase were normal. It should be noted that the isolation procedure was also effective for the isolation of choline kinaseless mutants. Two mutants were obtained. The choline kinase activities of these strains, 186α and 193α, were 0.7 and 1.3% of that of the wild-type strain X2180-1B, respectively. These mutants also showed decreased choline transport activities. The reason for the low transport activity in choline kinaseless mutants is not clear. Figure 3 compares the choline uptake of the parental, mutant, and revertant cells. The mutant 187α was unable to transport choline almost entirely, whereas the parent 172α and the revertant 187α R32 were able to transport choline at the rates comparable to those of wild-type cells. Since the results of the above-mentioned inhibition studies indicate high specificity of choline transport system in *S. cerevisiae*, it is of interest to examine the uptake of various substances into choline transport mutant 187α. In the experiment represented in Table 4, the activities of the mutant to transport L-leucine, L-methionine, D-glucose, and *myo*-inositol were compared with those of the parental strain. The data clearly demonstrate that the transport of the compounds other than choline was not altered by the mutation.

TABLE 3. *Levels of choline transport, choline kinase, cholinephosphate cytidyltransferase, and cholinephosphotransferase activities in various strains*

Strain	Choline transport (nmol min^{-1} mg of $protein^{-1}$)	Choline kinase (mU/mg of protein)	Cholinephosphate cytidyltransferase (mU/mg of protein)	Cholinephosphotransferase (mU/mg of protein)
Expt 1				
172α	4.28	9.3	0.94	3.7
187α	0.04	13.0	0.89	3.3
Exp 2				
X2180-1B	7.85	20.8	0.68	4.3
186α	0.80	0.14	1.64	3.8
193α	0.82	0.27	1.08	3.3

[a] Cells were grown in medium M 0.2i, and the activities were determined as described in the text.

DISCUSSION

The findings reported here provide evidence for the existence of a specific choline transport system in *S. cerevisiae*. The transport system of this yeast differed from that of mammalian cells; the K_m value for choline was very low (1, 9, 13). Moreover, the transport was independent of Na^+ (4, 8, 15). The high affinity for choline explains well the fact that choline auxotrophs isolated from this microorganism attained maximum growth at a very low concentration of choline (below 10 μM) (16). Because of the rapid turnover of choline after entry into the cell, direct evidence was not obtained indicating that choline was incorporated against the concentration gradient. However, the strict energy requirement indicates that choline is transported by an active transport process.

Inhibition studies with various choline analogs and amino acids indicate that the transport system is specific for choline. The results can be

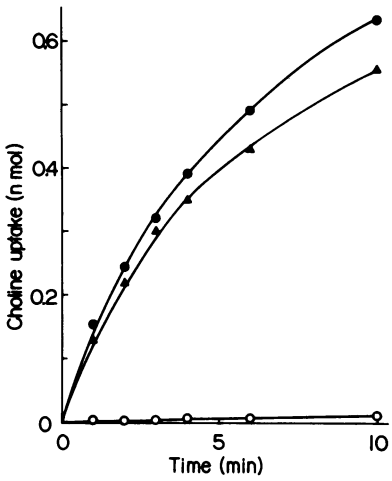

FIG. 3. *[^{14}C]choline transport of the parental strain 172α, the mutant 187α, and its revertant 187α R32. Strains 172α, 187α, and 187α R33 were cultured in medium M 0.2i, and choline transport was measured under the standard assay conditions as described in the text except that the incubation period was varied as indicated. Symbols: ●, 172α (16.4 μg of cell protein); ○, 187α (15 μg of cell protein); ▲, 187α R32 (20 μg of cell protein).*

TABLE 4. *Transport of choline, L-leucine, L-methionine, D-glucose, and myo-inositol into 172α and 187α cells*[a]

Strain	Activity (nmol min^{-1} mg of protein^{-1})				
	Choline	L-Leucine	L-Methionine	D-Glucose	*myo*-Inositol
172α	4.7	58.9	138	8.8	18.9
187α	0.04	53.9	128	9.4	25.4

[a] Cells were grown in medium M 0.2i, and choline transport was assayed as described in the text. The transport of the compounds other than choline was assayed in a similar manner except that instead of [^{14}C]choline, L-[*U*-^{14}C]leucine (1,330 cpm/nmol), L-[*methyl*-^{14}C]methionine (830 cpm/nmol), D-[*U*-^{14}C]-glucose (1,650 cpm/nmol), and *myo*-[*U*-^{14}C]inositol (740 cpm/nmol) were used in final concentrations of 0.125, 0.05, 1.5, and 0.18 mM, respectively.

summarized as follows: (i) substitution of the nitrogen atom of ethanolamine with an increasing number of methyl groups was attended by an enhanced inhibition, and (ii) carnitine and betaine were only slightly inhibitory, and phosphorylcholine had no effect, although the three compounds have the same trimethylammonium moiety as choline. The K_i value for β-methylcholine, which also had the trimethylammonium moiety, was about 10 times as high as the K_m value for choline. These results suggest that the transport system recognizes both trimethyl ammonium and hydroxymethyl moieties of choline.

In addition, the isolation and characterization of a mutant strain with a lesion specific in choline transport gives further support to the concept that *S. cerevisiae* possesses a specific transport system for choline.

Finally, it is noteworthy that the method employed for the isolation of a choline transportless mutant is also useful for the isolation of mutants which are defective in enzymes of CDP-choline pathway. So far, we have been able to isolate choline kinaseless mutants by this procedure. The mutant of CDP-choline pathway as well as of phosphatidylethanolamine methylation pathway (16) will provide a useful system to study the regulation of phosphatidylcholine biosynthesis.

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