Biochimica et Biophysica Acta, 595 (1980) 109—120

BBA 78586

AMINO ACID UPTAKE BY *SACCHAROMYCES CEREVISIAE* PLASMA MEMBRANE VESICLES

GLENN J. MERKEL [a], FRED NAIDER [b] and JEFFREY M. BECKER [a]

[a] *Department of Microbiology, The University of Tennessee, Knoxville, TN and*
[b] *Department of Chemistry, College of Staten Island CUNY, Staten Island, NY (U.S.A.)*

(Received April 17th, 1979)

Key words: Amino acid transport; Membrane vesicle; (Saccharomyces cerevisiae)

Summary

A procedure is described which allows for the efficient separation of *Saccharomyces cerevisiae* plasma membranes from other cellular membranes by discontinuous sucrose density gradient centrifugation. After vesiculization in an osmotic stabilization buffer the plasma membrane vesicles retain the ability to transport amino acids. Amino acid uptake was affected by the proton gradient dissipator *m*-chlorocarbonylcyanide phenylhydrazone and was dependent, in some cases, on the presence of sodium ion.

Introduction

Much effort has been directed towards the elucidation of amino acid transport processes in procaryotic and eucaryotic cells [1,2]. Considerable insight has been gained more recently by the study of these phenomena in plasma membrane vesicles [3—7], because the effects of cellular metabolism are eliminated. There are no reports, however, of amino acid transport studies by plasma membrane vesicles prepared from yeast cells, even though many studies have focused on amino acid transport in yeasts [8,9].

This communication is concerned with the preparation of plasma membrane vesicles from *Saccharomyces cerevisiae* and a preliminary examination of amino acid uptake by these vesicles.

Methods

Yeast strains and growth conditions. S. cerevisiae XS144-S19 (*a*-mating type) and *S. cerevisiae* XS144-S22 (α-mating type) received from The Yeast Genetics

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenolindophenol.

Stock Culture Collection, Berkely, CA, were used throughout this investigation. Both strains are auxotrophic for tyrosine, phenylalanine, leucine, tryptophan, methionine, lysine, and adenine. The cells were maintained in Bacto Yeast Nitrogen Base without amino acids (Difco Labs, Detroit, MI) with 2% glucose and the appropriate nutritional requirements added at a concentration of 40 μg/ml.

Chemicals and isotopes. Radioactive amino acids were purchased from Amersham, Arlington Heights, IL. All chemicals were reagent grade or the purest commercially available.

Preparation of plasma membrane vesicles. Cells were grown in a 14 l fermentor (purged with filter-sterilized compressed air) with Yeast Nitrogen Base, containing 2% glucose and the required nutritional supplements, to late log phase, collected by centrifugation and washed once with 0.9% NaCl. A cell yield of 50—70 g wet wt. was normally obtained by this procedure. Sphaeroplasts were prepared by procedures modified from Schwencke [10]. The washed cell pellet was resuspended in 100 ml of buffer, pH 8.9, containing 5 mM disodium EDTA, 5 mM dithiothreitol and 100 mM Tris-HCl and incubated with periodic mixing at 30°C for 15 min. The cells were collected by centrifugation, washed once with 0.9% NaCl, and resuspended in 100 ml of 0.6 M KCl. To this suspension was added 15 ml of glusulase (Endo Lab. Inc., Garden City, NY), and the suspension was incubated at 35°C on a rotary shaker at approx. 100 rev./min for 2—3 h. Sphaeroplasting was monitored by phase contrast microscopy.

The sphaeroplasts were collected by centrifugation at 2400 × *g*. The resulting pellet was resuspended in 100 mM Tris-HCl, pH 8.0, containing 5 mM EDTA (Tris/EDTA) and immediately frozen at −70°C. Just prior to the preparation of membrane vesicles, the frozen sphaeroplast preparation was defrosted and homogenized by 2—5 complete strokes with a tight-fitting Dounce homogenizer. The homogenate was centrifuged at 2400 × *g* for 10 min to remove whole cells. The pellet was discarded and the supernatant was divided into two equal portions. To one portion was added 2.5 μCi of [^{3}H]concanavalin A (56 Ci/mmol) (Amersham, Arlington Heights, IL) and the other portion was untreated. The [^{3}H]concanavalin A treated suspension was incubated at 37°C for 10 min with constant agitation. Both portions were then centrifuged separately at 70 000 × *g* for 30 min in a Beckman L5-75 ultra-centrifuge using a Beckman Model No. 42.1 fixed angle rotor. The pellets were washed one time with Tris/EDTA by centrifugation at 70 000 × *g* for 30 min and the final pellets were resuspended in 3 ml Tris/EDTA by dispersion with a 23 gauge needle. The suspensions were applied to discontinuous sucrose gradients composed of 4 ml each of 60%, 50%, 45%, and 40% (w/w) sucrose and 3 ml each 35%, 30%, 25%, 20%, 15%, and 10% (w/w) sucrose in Tris/EDTA. The gradients were centrifuged for 18 h at 53 000 × *g* in a Beckman Model Number SW27 swinging bucket rotor. The gradients were fractionated by collection from the bottom of the tubes in approx. 1.5-ml fractions and a portion of the [^{3}H]concanavalin A membrane fractions were suspended in Bray's Liquid Scintillation cocktail [11] and counted. The [^{3}H]concanavalin A treated and untreated fractions were diluted with an equal volume of Tris/EDTA and centrifuged at 70 000 × *g* for 30 min. The resulting pellets were resuspended in a small volume of stabilization buffer

(TM-buffer) consisting of 20 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, and 0.6 M mannitol, pH 6.5. These fractions were then stored at −70°C.

Enzymatic analysis of membrane fractions. Portions of the membrane fractions suspended in TM-buffer described in the previous section were centrifuged at 70 000 × *g* for 30 min and the resulting pellets resuspended in TM-buffer without mannitol, pH 6.5.

Chitin synthetase was measured by methods modified from Duran et al. [12]. The individual membrane fractions (500 μl suspension containing 50—100 μg of protein in TM-buffer without mannitol) were mixed with 50 μl of 0.5 M imidazole (pH 6.5), 50 μl of trypsin (Sigma Chem. Co., St. Louis, MO) (2 mg/ml in 0.05 M sodium phosphate buffer, pH 7.8), and incubated for 30 min at 37°C. An equal volume of soybean trypsin inhibitor (Sigma) (3 mg/ml in 0.05 M phosphate buffer, pH 7.8) was added followed by 10 μl of 0.8 M *N*-acetyl-D-glucosamine and 30 μl of UDP-*N*-[^{14}C]acetyl-D-glucosamine (Amersham) (0.07 μCi/30 μl from a 10 mM solution with a specific activity of 0.23 μCi/mmol). The suspensions were mixed and incubated at 37°C for 1 h. The reactions were stopped by the addition of 2—3 ml of ice-cold 66% ethanol and the resulting precipitate was collected by centrifugation at 2500 × *g* for 20 min. The supernatants were discarded and the pellets washed three consecutive times with 66% ethanol containing 0.1 M ammonium acetate and centrifuged at 2500 × *g* for 20 min. The final pellet was suspended in 0.5 ml of absolute ethanol, added to 5 ml of Bray's solution and counted.

Succinic dehydrogenase, used as a mitochondrial marker, was assayed as described by Kasahara [13]. Sodium succinate (to final concentration of 20 mM) was added to 500 μl of each membrane fraction suspended in TM-buffer without mannitol. After incubation for 5 min, 2,6-dichlorophenol-indophenol (DCIP) ($\epsilon = 2.2 \cdot 10^4$/cm per mol) was added to a final volume of 3 ml. The reduction of DCIP was measured by the decrease in absorbance at 600 nm in a Gilford (Model 250) recording spectrophotometer with a light path of 1 cm.

ATPase activity was measured by mixing 500 μl of the membrane fractions suspended in TM-buffer without mannitol, with 500 μl TM-buffer containing 10 mM ATP (Sigma). The suspensions were incubated at 30°C for 30 min and the reactions stopped by the addition of 1 ml of 10% trichloroacetic acid. The precipitate was removed by centrifugation and an aliquot of the supernatant was assayed for P_i by the method of Chen [14].

Protein concentration was estimated by the BioRad Protein Assay procedure (BioRad Lab., Richmond, CA) with bovine serum albumin as standard.

Measurement of amino acid uptake. The purified plasma membrane preparation, suspended in TM-buffer containing mannitol, was diluted with fresh buffer to a final concentration of 20—40 μg of protein in a volume of 0.5 ml. This suspension was passed two times through a 21 gauge needle and incubated at 30°C for 5 min. The amino acid to be analyzed for uptake was added in a volume of 50 μl of distilled water, the suspension mixed, and 100 μl portions removed at various intervals. The vesicles were collected on 0.1 μm Millipore filters (Millipore Corp., Bedford, MA) and the filter-bound vesicles were washed two times with 1 ml of ice-cold TM-buffer with mannitol. The filters were suspended in 5 ml of Bray's Liquid Scintillation cocktail and counted.

The effect of carbonylcyanide *m*-chlorophenylhydrazone (CCCP) on amino acid uptake was measured by adding CCCP, in 95% ETOH (usually 10 μl/550 μl uptake mixture), to a final concentration of 50 μM and allowing the vesicles to incubate for 5 min. Amino acid uptake was assayed as described above. No effect of the ethanol was found in control experiments and these data are not shown in subsequent figures.

Results

Purification of plasma membrane vesicles

Separation of the yeast plasma membranes of *a*-mating type cells by discontinuous sucrose density gradient centrifugation yielded three visible bands. The separation into three zones was not as distinct in the absence of EDTA in the separation medium. No differences occurred in the separation of concanavalin A-labeled and unlabeled membranes (which has been shown previously) [12].

It has been established that the maximum binding of concanavalin A to yeast plasma membranes in density gradients corresponds to the area of maximum chitin synthetase activity when sphaeroplasts are treated with labeled concanavalin A prior to membrane purification [12]. We observed no differ-

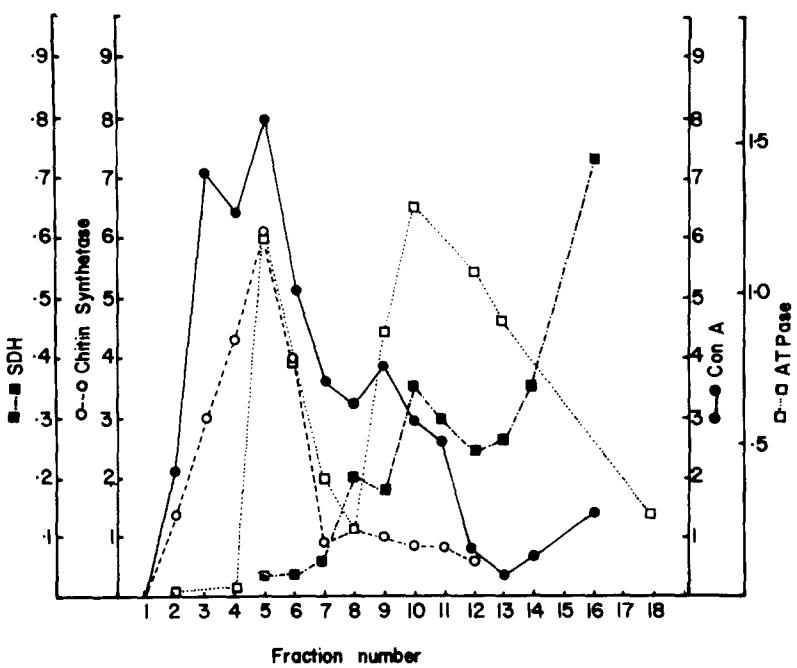


Fig. 1. Sucrose gradient centrifugation of total membranes of XS144-S19 *a* and the distribution in the gradient of the specific activities of Chitin synthetase, ATPase, succinic dehydrogenase (SDH), and concanavalin A binding. Succinic dehydrogenase activity is expressed as mol DCIP reduced per μg of membrane protein ($\times 10^{-8}$); Chitin synthetase is expressed as mmol *N*-[^{14}C]acetyl-D-glucoseamine incorporated per μg of membrane protein ($\times 10^{-11}$); concanavalin A-binding activity is expressed as mmol ^{3}H-concanavalin A (70 Ci/mmol) bound per μg membrane protein ($\times 10^{-14}$); and ATPase activity is expressed as μmol of phosphate released/h per μg of membrane protein ($\times 10^{-3}$). Fraction no. 1 corresponds to the bottom of the gradient.

ence in the specific binding activity of [^{3}H]concanavalin A to the plasma membranes isolated from the sucrose gradient whether the concanavalin A treatment occurred before or after the sphaeroplasts were lysed. This is exemplified by coinciding maximum specific chitin synthetase activity with maximum [^{3}H]concanavalin A binding as shown in Fig. 1. Maximum chitin synthetase activity was found in fraction 5, corresponding to the area around the original 40—45% sucrose interface. This band (fraction 5) was free of succinic dehydrogenase activity suggesting no mitochondrial membrane contamination. ATPase activity was also found in this fraction, but maximum ATPase activity coincided with one of the succinic dehydrogenase activity peaks as would be expected since the ATPase assay used would detect both mitochondrial and plasma membrane ATPase [15].

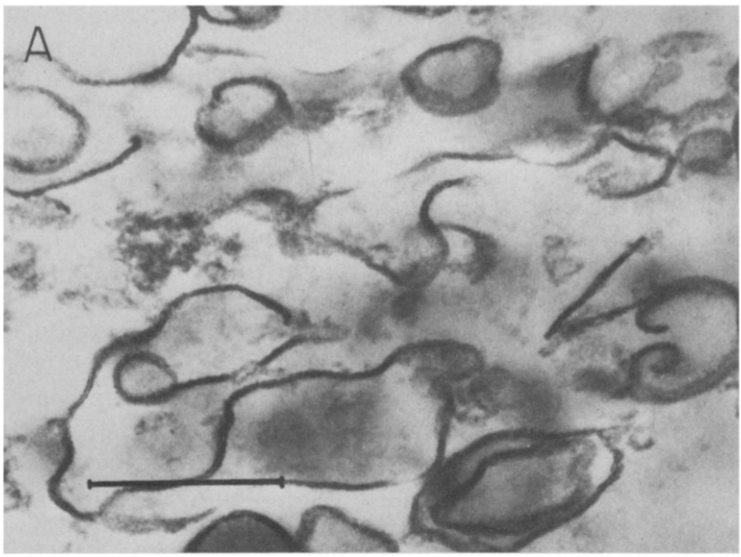
Electron micrographs of membranes from fraction 5, which were prepared directly from a Tris/EDTA suspension, showed typical double-track structures with very little contaminating debris visible (Fig. 2A). After centrifugation at 70 000 × *g*, resuspension of the pellet in TM-buffer with mannitol, and passage of the preparation through a 21 gauge needle, the majority of the membrane fragments form vesicles (Fig. 2B). The approximate diameter of the vesicles was between 0.2 and 0.5 μm.

Similar results for the purification of plasma membranes from the α-mating strain were obtained. The maximum specific [^{3}H]concanavalin A binding coincided with the maximum chitin synthetase activity. Similarly no succinic dehydrogenase activity was associated with the band containing predominantly plasma membranes. Plasma membranes from the α-strain were, however, considerably less dense than those from the *a*-strain. The α-plasma membrane markers were found in the equivalent of fraction 10 shown in Fig. 1. The differences in the membranes derived from *a*- and α-mating strains are being examined in greater detail.

Amino acid uptake in the plasma membrane vesicles

Leucine and glycine (both at a final concentration of $9.1 \cdot 10^{-7}$ M) were taken up by *a*-strain plasma membrane vesicles at an initial rate of $3.5 \cdot 10^{-4}$ and $1.4 \cdot 10^{-4}$ nmol/min per μg protein, respectively (Fig. 3). The rates appear linear for the first 2 min and then begin to level off. The addition of CCCP (50 μM final concentration) eliminated glycine uptake activity and reduced the initial rate of leucine uptake to $5.8 \cdot 10^{-5}$ nmol/min per μg protein. However, after 10 min the total accumulation of leucine in the vesicles was about 50% of the control value.

Substrate concentrations of $9.1 \cdot 10^{-6}$ M and $9.1 \cdot 10^{-8}$ M leucine yielded initial uptake rates of $2.1 \cdot 10^{-3}$ and $1.5 \cdot 10^{-5}$ nmol/min per μg protein, respectively, whereas $9.1 \cdot 10^{-6}$ M and $9.1 \cdot 10^{-8}$ M glycine resulted in initial uptake rates of $6.5 \cdot 10^{-4}$ and $3.8 \cdot 10^{-6}$ nmol/min per μg protein. At all substrate concentrations examined, the effect of CCCP on uptake was similar to that described previously. From the substrate concentrations examined thus far, it appears that for both leucine and glycine uptake, the K_m will be above $9.1 \cdot 10^{-6}$ M, assuming that the system is saturable. Initial rates of proline uptake by *a*-strain plasma membrane vesicles were similar to those determined for glycine uptake. L-[3,4-^{3}H]proline (60 Ci/nmol) was added to 500 μl of membrane



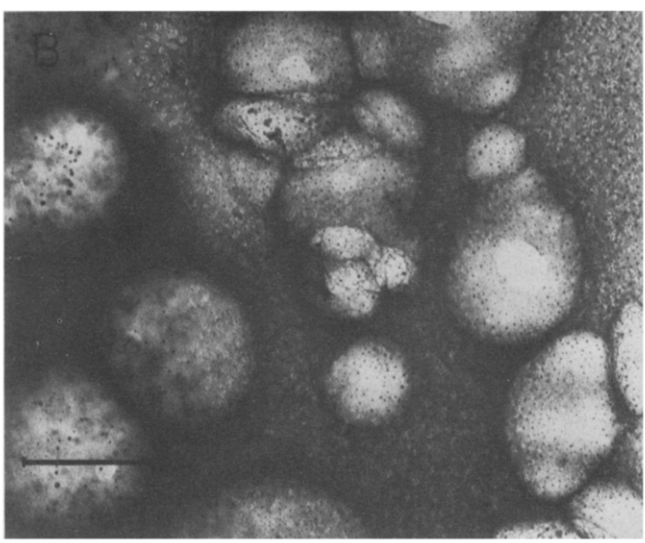


Fig. 2. Electron micrographs of plasma membranes in Fraction 5 (Fig. 1) isolated from sucrose density gradients. (A) Thin section of membranes from Fraction 5 before suspension in stabilization buffer. Membranes were fixed with 3% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h and post fixed with 2% osmium tetroxide for 1 h. The preparation was dehydrated with ethanol, infiltrated with propylene oxide, and embedded in Epon. Thin sections were stained with 4% uranyl acetate and poststained with lead citrate. Magnification ×75 900; Bar = 0.5 μm. (B) Negative stain (2% phosphotungstic acid, pH 7.0) of membranes from Fraction 5 after suspension in TM-buffer with mannitol. The presence of high concentrations of mannitol in the buffer result in the appearance of dark blotches in this preparation. Magnification ×58 000; Bar = 0.5 μm.

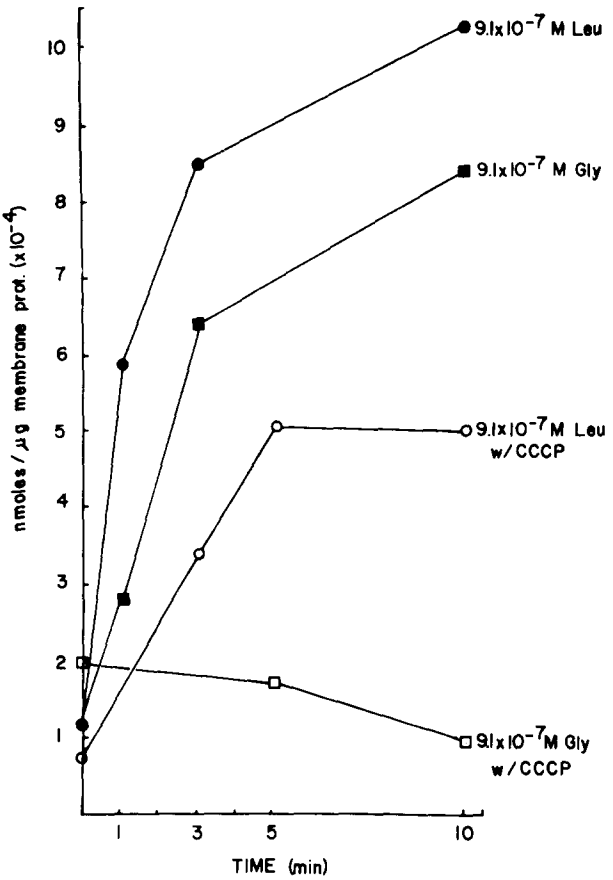


Fig. 3. Uptake of leucine (●——●, ○——○) and glycine (■——■, □——□) by XS144 S19 *a*-plasma membrane vesicles. L-[4,5-^{3}H]Leucine (105 Ci/mmol) or [2-^{3}H]glycine (23 Ci/mmol) were added to 500 µl of membrane suspension (47.5 µg protein/500 µl) to a final concentration of $9.1 \cdot 10^{-7}$ M.

suspension (47.5 µg protein/500 µl) and the uptake monitored as before. Substrate concentrations of $9.1 \cdot 10^{-6}$, $9.1 \cdot 10^{-7}$, and $9.1 \cdot 10^{-8}$ M proline yielded initial rates of $7.5 \cdot 10^{-4}$, $7.3 \cdot 10^{-5}$, and $8.4 \cdot 10^{-6}$ nmol/min per µg of protein, respectively. CCCP was found also to completely eliminate uptake of proline into these vesicles.

An experiment was designed to test the osmotic sensitivity of the plasma membrane vesicles. A suspension of membranes was divided into two equal portions and centrifuged at 70 000 × *g* for 30 min. One pellet was resuspended in TM-buffer with mannitol while the other pellet was resuspended in distilled water. Leucine ($9.1 \cdot 10^{-5}$ M) was used as the substrate to test the uptake capabilities of the distilled water suspension. Fig. 4 shows that the uptake of leucine was essentially equivalent in both preparations up to 3 min. After 3 min, the TM-buffer with mannitol-suspended vesicles continued to accumulate leucine while the distilled water-suspended vesicles began to lose counts and eventually returned to the background reading.

To test the dependence of amino acid uptake on sodium, equal volumes of

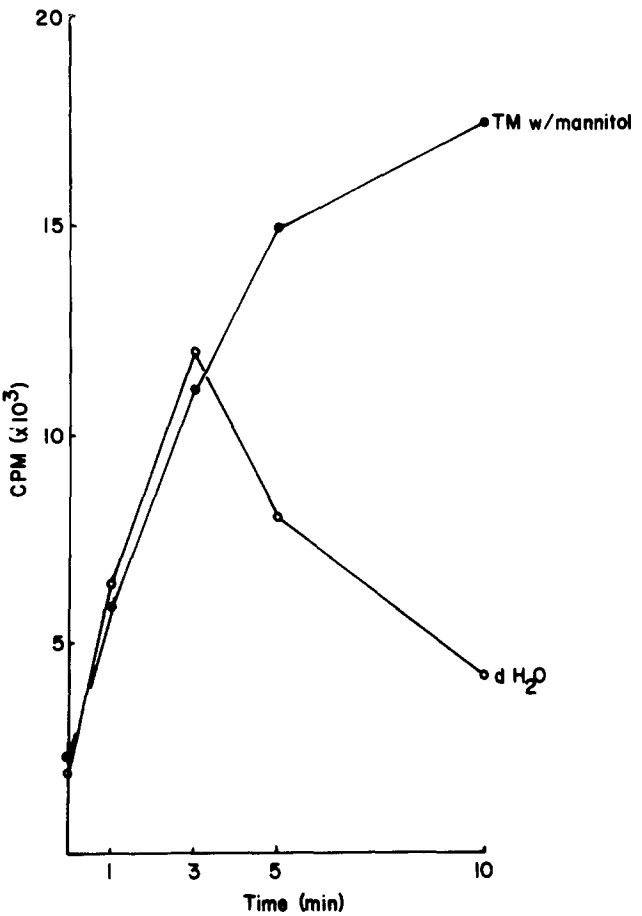


Fig. 4. Effect of distilled water on leucine uptake by XS144 S19 α-plasma membrane vesicles. L-[4,5-^{3}H]-Leucine (105 Ci/mmol) was added to either membrane vesicles suspended in TM-buffer with mannitol (●——●) or distilled water (○——○) to a final concentration of $9.1 \cdot 10^{-5}$ M. Amino acid uptake was assayed as described in the text.

membrane vesicles (containing equal amounts of membrane protein) suspended in TM-buffer with mannitol were centrifuged as described above and the pellets were resuspended in equal volumes of complete TM-buffer or TM-buffer without NaCl. The amino acid uptakes were run as before using a 100-fold lower substrate concentration of leucine and glycine ($9.1 \cdot 10^{-7}$ M) than described above. Removal of Na^+ from the TM-buffer with mannitol eliminates any measurable uptake of these two amino acids (Fig. 5).

Growth of the *a*- and *α*-strains of XS144 with either proline or ammonia as a nitrogen source made no difference in the initial rate for amino acid uptake in the plasma membrane vesicles. We have found, however, that membrane vesicles prepared from *a*-strain 2180 1A grown with ammonia as a nitrogen source do not take up leucine whereas vesicles prepared from cells grown with proline as a nitrogen source do take up leucine. The effect of nitrogen metabolism on the transport properties of plasma membrane vesicles is currently under further investigation.

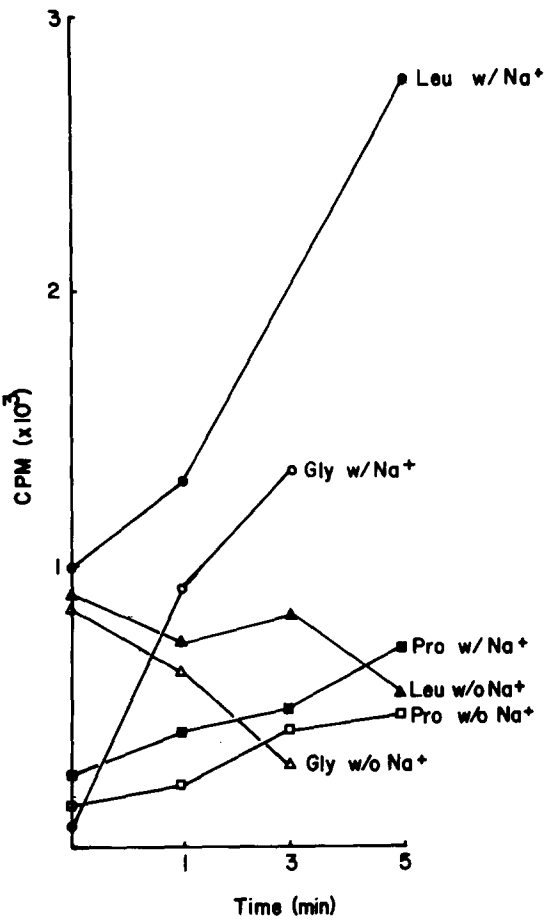


Fig. 5. Effect of sodium on leucine (●———●, ▲———▲), glycine (○———○, △———△) and proline (■———■, □———□) uptake by XS144 S19 *a*-plasma membrane vesicles. L-[4,5-^{3}H]Leucine (105 Ci/mmol), [2-^{3}H]glycine (23 Ci/mmol) and L-[3,4-^{3}H]proline (60 Ci/mmol) were added to the membrane suspensions to a final concentration of $9.1 \cdot 10^{-7}$ M.

Discussion

A variety of procedures are available for the preparation of yeast plasma membranes [12,15–28]. The only recent attempt at demonstrating amino acid uptake in purified plasma membrane vesicles was by Christensen and Cirillo [21]. They were unable, however, to show that their vesicles transported the amino acids tested. The procedure described in this communication allows for the efficient separation of plasma membranes from other cellular membranes; after vesiculization in the stabilization buffer, the plasma membrane vesicles retain amino acid uptake activity.

It has been shown recently that chitin synthetase is evenly distributed in (or on) the yeast plasma membrane and that this enzyme serves as a reliable marker for judging the purity of plasma membrane preparations [28]. It has been demonstrated by Cabib and co-workers [12] that if sphaeroplasts are treated

with [^{3}H]concanavalin A the location of highest binding activity in the density gradients coincides with the maximum chitin synthetase activity. We have extended these observations to show that concanavalin A binds selectively with maximum activity to the plasma membrane either before or after lysis of the sphaeroplasts.

ATPase has been found in numerous plasma membrane preparations from yeasts [15,18,20,29—34], and Slayman and co-workers have even purified the membrane-bound ATPase from *Neurospora* [29,30]. Our data indicate the presence of membrane-bound or associated ATPase in *a*-strain XS144 S19. Maximum ATPase activity was associated, however, with the membrane fraction showing high succinic dehydrogenase activity which probably indicates the location of mitochondrial membranes. We are uncertain whether the membrane-bound ATPase in our membrane preparations is oligomycin and ouabain resistant (as has been shown for other yeast plasma membrane ATPase), and this remains to be clarified through further analysis. However, the occurrence of ATPase activity in the region of the sucrose gradient containing maximum chitin synthetase and concanavalin A binding activity is good evidence for the presence of a plasma membrane associated ATPase in our preparations.

Our initial attempts at showing amino acid uptake in the plasma membrane vesicles were discouraging (data not shown). We surmised that divalent cations were necessary for vesiculization, however, our first buffer system did not contain an osmotic stabilizer and we continuously observed very rapid amino acid uptake for the first one or two minutes followed by equally as rapid loss of filter-associated counts. In many cases our data were similar to reported 'transport overshoot' described in certain mammalian plasma membrane vesicles [35,36]. The incorporation of mannitol in the uptake buffer allowed transport to proceed in the vesicles with no indication of leakage. Efflux of amino acids from the vesicles was encountered when the pH of our system was 5.5 or 7.5 or when we assayed other fractions which did not show maximum chitin synthetase or concanavalin A binding activity.

CCCP is known to dissipate electrical and pH gradients across the plasma membrane. We have shown that CCCP eliminates glycine and proline uptake in XS144 *a*-plasma membrane vesicles. These data support the generally accepted theory that an ATP-driven H^+ pump is primarily responsible for plasma membrane transport phenomena in yeast [30]. The assumption is that H^+ is pumped out of the cell causing the generation of an electrochemical gradient, and proton dependent co-transport systems for amino acids are coupled to the reabsorption of H^+. Unfortunately, reliable conclusions cannot yet be drawn as to the mechanism of amino acid transport in yeast plasma membrane vesicles based only on the effect of CCCP. It can only be concluded that either an electrical or pH gradient is required for proline, glycine, and leucine uptake. It is also important to mention that the addition of typical energy sources such as glucose, succinate, or lactate did not enhance amino acid uptake in the yeast plasma membrane vesicles. This can be expected since in yeasts the metabolizing enzymes for the substrates are not associated with the plasma membrane.

A sodium dependence has been shown for the uptake of many amino acids by plasma membrane vesicles [1,3,37—42]. At low substrate concentrations there is no apparent uptake of leucine or glycine by XS144 S19 *a*-membrane

vesicles when Na^+ is removed from the uptake buffer. Proline uptake did not appear to be affected (Fig. 5) although the rates of uptake were so low in the control that it is difficult to distinguish any true effects of sodium. The possibility exists that since the vesicles contain Na^+ initially the omission of Na^+ from the suspension could create an outwardly directed Na^+ gradient. If the membrane contains a Na^+/H^+ exchanger this could lead to the uptake of protons. Since Tris is a poor buffer at pH 6.5, the uptake of protons could result in an acidified suspension within the vesicle. It is, therefore, not possible to determine at this time whether the requirement for external Na^+ is a consequence of its presence within the vesicles or an indication of a direct interaction of sodium with the amino acid transport system.

Uptake of leucine (at a 100-fold higher concentration than used in Fig. 5) in distilled-water suspended vesicles proceeded up to a certain point after which the counts leaked out (Fig. 4). There are two possible explanations for this phenomenon. If a membrane gradient (either electro-chemical or pH) is required to keep the transported amino acid inside the vesicles, then the dissipation of a gradient caused by amino acid influx signals the onset of leakage from the vesicles. In fact, the data shown for the distilled water-suspended vesicles (Fig. 4) resembles the pattern of amino acid uptake when the buffer pH deviates from the optimum. The other explanation is that the amino acid is accumulated in the vesicles until a concentration equilibrium is reached with the surrounding medium. As uptake continues against the concentration gradient, the vesicles burst since no osmotic stabilizer is present in the uptake system. A similar experiment was conducted in TM-buffer (complete) without mannitol and equivalent results were obtained (data not shown).

No firm generalizations can be made at present concerning the mechanism of amino acid transport in yeast plasma membrane vesicles. We have shown, however, that the preparation of plasma membrane vesicles in which amino acid uptake can be demonstrated is possible from yeast cells. The elucidation of the molecular aspects of the amino acid transport phenomenon in vesicles awaits further investigation.

Acknowledgements

We acknowledge support for this work by Public Health Service grants GM 22086 and GM 22087 from the National Institute of General Medical Sciences and a grant from the Faculty Research Award Program of City University of New York. J.M.B. and F.N. are Public Health Service Research Career Development Awardees GM 00094 and GM 00025 from the National Institute of General Medical Sciences. We thank David Parker for doing the electron microscopy.

References

1 Wilson, D.B. (1978) Ann. Rev. Biochem. 47, 937—965
2 Guidotti, G.G., Borghetti, A.F. and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329—366
3 Lever, J.E. (1977) J. Supramol. Struct. 6, 103—124
4 Kaback, H.R. (1976) J. Cell. Physiol. 89, 575—594
5 Rosen, B.P. and Kashket, E.R. (1978) in Bacterial Transport (Rosen, B., ed.), Marcel Dekker, New York

6 Konings, W.N . (1977) Adv. Microb. Physiol. 15, 175—251
7 Kaback, H.R. (1974) in Perspectives in Membrane Biology (Estrada-O., S. and Gitter, C., eds.), pp. 213—227, Academic Press, New York
8 Grenson, M., Hou, C. and Crabell, M. (1970) J. Bacteriol. 103, 770—777
9 Stoppani, A.O.M. and Ramos, E.H. (1978) in Biochemistry and Genetics of Yeasts (Bacila, M., Horecker, B.L., Stoppani, A.O.M., eds.), pp. 171—196, Academic Press, New York
10 Schwencke, J., Magna-Schwencke, N. and Laporte, J. (1977) Ann. Microbiol. Paris 128, 3—17
11 Bray, G.A. (1960) Anal. Biochem. 1, 279—285
12 Duran, A., Bowers, B. and Cabib, E. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3952—3955
13 Kasahara, M. and Anraku, Y. (1974) J. Biochem. 76, 959—966
14 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756—1758
15 Fuhrmann, G.F., Wehrli, E. and Boehm, C. (1974) Biochim. Biophys. Acta 363, 295—319
16 Kramer, R., Kopp, F., Niedermeyer, W. and Fuhrmann, G.F. (1978) Biochim. Biophys. Acta 507, 369—380
17 Schibeci, A., Rattray, J.B.M. and Kidby, D.K. (1973) Biochim. Biophys. Acta 311, 15—25
18 Fuhrmann, G.F., Boehm, C. and Theuvenet, A.P.R. (1976) Biochim. Biophys. Acta 433, 583—596
19 Nurminen, T., Oura, E. and Suomalainin, H. (1970) Biochem. J. 116, 61—69
20 Matile, P. (1970) in Membrane Structure and Function (Villanueva, J.R. and Ponz, F., eds.), pp. 39—49, Fed. Eur. Biochim. Soc. Symp.
21 Christensen, M.S. and Cirillo, V.P. (1972) J. Bacteriol. 110, 1190—1250
22 Santos, E., Villanueva, J.R. and Sentandreu, R. (1978) Biochim. Biophys. Acta 508, 39—54
23 Lewis, M.J. and Patel, P.C. (1978) Appl. Environ. Microbiol. 36, 851—856
24 Longley, R.P., Rose, A.H. and Knights, B.A. (1968) Biochem. J. 108, 401—412
25 Garcia-Mendoza, C. and Villanueva, J.R. (1967) Biochim. Biophys. Acta 135, 189—195
26 Marriott, M.S. (1977) J. Gen. Microbiol. 103, 51—59
27 Marriott, M.S. (1975) J. Gen. Microbiol. 86, 115—132
28 Duran, A., Cabib, E. and Bowers, B. (1979) Science 203, 363—365
29 Bowman, B.J. and Slayman, C.W. (1977) J. Biol. Chem. 252, 3357—3363
30 Bowman, B.J., Mainzer, S.E., Allen, K.E. and Slayman, C.W. (1978) Biochim. Biophys. Acta 512, 13—88
31 Scarborough, G.A. (1975) J. Biol. Chem. 250, 1106—1111
32 Matile, P., Moor, M. and Muhlethaler, K. (1967) Arch. Mikrobiol. 58, 201—211
33 Nurminen, T., Taskinen, L. and Somalainen, H. (1975) Biochem. J. 154, 751—763
34 Delhez, J., Dufour, J.P., Thines, D. and Goffeau, A. (1977) Eur. J. Biochem. 79, 319—328
35 Parnes, J.R., Garvey, T.Q. and Isselbacher, K.J. (1976) J. Cell. Physiol. 89, 789—794
36 Quinlan, D.C., Parnes, J.R., Shalom, R., Garvey, T.Q., Isselbacher, K.J. and Hochstadt, J. (1976) Proc. Natl. Acad. Sci. U.S. 73, 1631—1635
37 Kahane, S., Marcus, M., Barash, H., Halpern, Y.S. and Kaback, H.R. (1975) Fed. Eur. Biochem. Soc. Lett. 56, 235—239
38 Halpern, Y.S., Barsh, H., Dover, S. and Druck, K. (1973) J. Bacteriol. 114, 53—58
39 Nishino, H., Christopher, C.W., Schiller, R.M., Gammon, M.T., Ullrey, D., Isselbacher, K.J. (1978) Proc. Natl. Acad. of Sci. U.S. 75, 5048—5051
40 Johnstone, R.M. (1978) Biochim. Biophys. Acta 512, 199—213
41 Kimmich, G.A. and Carter-Su, C. (1978) Am. J. Physiol. 235, C73—C81
42 Oxender, D.C. (1972) Ann. Rev. Biochem. 41, 777—814