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TRANSPORT OF 2-METHYLALANINE IN
AZOTOBACTER VINELANDII

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Allan E. Kaufman

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Harold L. Sadoff
Major professor

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TRANSPORT OF 2-METHYLALANINE IN
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BY

Allan E. Kaufman

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ABSTRACT

TRANSPORT OF 2-METHYLALANINE IN AZOTOBACTER VINELANDII

by

Allan E. Kaufman

The growth of Azotobacter vinelandii is impeded by 2-methylalanine (MA) when cultures are growing on glucose, but not when they are growing on fructose. Cells growing on fructose and cells resistant to MA take up less ^{14}C-labeled MA than do cells growing on glucose. The wide range of structurally dissimilar compounds that can counteract the effect of MA suggests that MA enters cells by a nonspecific transport mechanism that somehow depends upon glucose.

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INTRODUCTION

Azobacter vinelandii is a large, gram-negative rod shaped bacterium that is highly aerobic and found living freely in soil and water (11). It has been the object of scientific interest for several reasons, including:

a. its ability to convert atmospheric nitrogen to ammonia (40);

b. its unusual respiratory physiology (several uncommon respiratory proteins and electron carriers are present in significant amounts (40));

c. its unusual genetic properties and a recently discovered system for genetic exchange (27).

Relatively litten attention has been focused on how *A. vinelandii* transports substances from its environment across the cellular envelope and into the cytoplasm. The purpose of this review is to summarize what is known about transport processes in *A. vinelandii* and, where appropriate, supplement this information with studies which have been done with other organisms.

TRANSPORT MECHANISMS

Diffusion

Transport by diffusion is the result of random molecular motion and occurs without the intervention of specific membrane components (19). A passive process, diffusion is the result of concentration differences or gradients and generally is subject to such effects as solvent drag, membrane charge and hydrophobicity of the membrane (20). The membrane of *A. vinelandii*, however is thought to be generally impermeable to solutes (35) and as such diffusion is not a major mechanism by which substrates gain entry into the cell.

Facilitated Diffusion

A special case of diffusion, facilitated diffusion occurs when specific membrane components, or "carriers", are involved in transporting substances down concentration gradients (19). As in passive diffusion, the transported substance can not move against concentration gradients. No metabolic energy is expended during transport by this process.

Active Transport

By expending metabolic energy, a cell can accomplish the work of concentrating within the volume enclosed by its membrane substances in low abundance outside the cell. Active transport is the general term applied to a process that is initiated by the formation of a complex between a membrane or cell surface component and an external substrate; the process is completed when the substrate has been translocated into the cell, the "carrier" regenerated at the cell surface, and energy utilized in the process (16). *A. vinelandii* appears to depend on such processes to move most substances into or out of the cell (35). As is the case in many other bacterial species (19,20), the energy component of active transport resides in the proton gradient established across the cell membrane by respiration. "Symport" and "antiport" mechanisms couple the entry of protons to the entry of external substrates or exodus of substances from inside the cell, respectively.

Group Translocation

Although this mechanism for transport appears to be absent from *A. vinelandii* (34), its prevalence in so many other organisms warrants brief mention. In this process a covalent change is exerted upon the transported molecule such that the reaction itself is simultaneous with its entry into the cell (19,20). The best understood example of this mechanism is the hexose:phosphenolpyruvate (PEP) phosphotransferase system (13). Here PEP is used to phosphorylate glucose while also transporting it into the cell. Interestingly, both membrane-bound (intrinsic) as well as soluble (extrinsic) proteins participate in the reaction (13).

METHODS OF INVESTIGATING TRANSPORT PROCESSES

Whole Cells

Intact cells are at first glance the choice system for study because the convenience of working with them is combined with the advantage of being assured that the transport system(s) under investigation has not been altered or damaged by fractionation procedures. However the redundancy and complexity of individual transport systems as well as the rapidity with which substrates are assimilated or transformed by cells, contribute to the difficulties of investigators using such systems (37). Some techniques used to counter such problems have been: the use of starved cells, inhibitors and non-metabolizable substrates; the use of mutants blocked in transport; the use of rapid spectrophotometric assays or assays using radioactive materials; and the use of kinetic techniques such as stopped flow dialysis (15,35). Early studies of transport in *A. vinelandii* relied on indirect indications of transport in whole cells; uptake of oxygen due to respiration of substrates entering the cell (31); changes in packed cell volume when starved cells were exposed to various permeable or impermeable substances

(21) and changes in light scattering when spheroplasts were placed in solutions containing various electrolytes (38).

Cytoplasmic Membrane Vesicles

The development of the technique for forming small vesicles composed of cytoplasmic membrane and essentially devoid of cytoplasmic constituents revolutionized the study of transport phenomena (16). Vesicles with "right side out" orientation are produced when osmotically sensitized cells are lysed with EDTA, DNAase, and RNAase, then subjected to extensive homogenization, washing and differential centrifugation (19). Such vesicles made from *E. coli* membranes, when provided with an energy source such as lactate, will take up a wide variety of substrates including galactose, β-galactosides, arabinose, peptides, nucleosides and dicarboxylic acids (20). Vesicles with reversed or "inside-out" topology have been obtained by the use of various non-penetrating buffers and alternative techniques for breaking cells, especially the French pressure cell (15). Inverted vesicles allow the study *as uptake* of processes usually occurring as secretion or extrusion in whole cells. This is especially useful in the study of various membrane bound ATPases (5).

In addition to confirming the "basic assumption of the inherent impermeability of lipid bilayers to hydrophilic molecules" (39), experiments with vesicles have contributed greatly to an understanding of transport at a fundamental molecular level. The basic mode of action for many active transport systems is now thought to be "symport", the transport of a substrate linked to the movement of protons or sodium ions down a concentration gradient. Although very few intact transport systems have been isolated and characterized in vitro (16), it now seems unlikely that "carriers" shuttle across membranes. Rather transport proteins somehow span the lipid bilayer and function by some sort of conformational change or, possibly, by the association of oligomers which form channels or pores in the bilayer (39).

There are, however, questions raised by the work with membrane vesicles. For example, there seems to be no correlation between the oxidation of various energy substrates and their effectiveness in transport processes (20). In vesicles made from A. vinelandii membranes, lactate, malate and NADH are all oxidized at about the same rate. However, lactate serves as a poor energy source for supporting glucose uptake and as a good source for Ca^{++} uptake. Malate yields the opposite results. NADH, which is theoretically the best electron source, will not drive either process (1,8). Clearly there exist interactions between transport proteins, membrane dehydrogenases and electron transport proteins that are still poorly understood (16).

Membrane Binding Proteins

Oxender has shown that when gram-negative bacteria are subjected to mild proteolytic treatment or osmotic shock in the presence of EDTA, a group of low molecular weight proteins are removed from the periplasmic region of the cell envelope (26). Since cells so treated no longer transport various substrates across the cell membrane, these proteins are implicated as being involved in transport processes. Known as binding proteins, a number of these proteins have affinities for substrates not transported by membrane vesicle systems. A few substrates, especially amino acids, do interact with both types of transport systems. Such redundancy may be required to allow substrates to pass across the inner and outer membranes of the cell. Binding proteins themselves are not thought to cross the cell membrane. Rather they may serve to enhance the uptake of substances having extremely low external concentrations. Generally, the Km for binding protein systems is much lower than the Km for membrane vesicle systems (39). Another major difference in the two kinds of transport is that vesicles depend on proton gradients to "drive" transport whereas binding protein systems appear to use the hydrolysis of ATP (16). To date no studies involving binding proteins in *A. vinelandii* have been published.

TRANSPORT OF CARBOXYLIC ACIDS

The earliest work concerning transport in A. vinelandii arose from attempts to measure the level of Krebs-cycle enzymes in cells growing on various media (31). It was observed that merely exposing a culture to a particular substance ("adaptation") did not insure that an appropriate "permease" system would be synthesized and thereby allow the substance to enter the cell. By the judicious use of inhibitors of DNA, RNA and protein synthesis, it was shown that a culture fully "adapted" to malate or succinate had synthesized a specific "permease". In addition it was demonstrated that the presence of glucose in low concentrations and not serving as a carbon of energy source decreased the time required for the adaptation to occur.

Subsequent investigations of transport of di-and tricarboxylic acids in A. vinelandii were carried out by P.W. Postma and his associates at the University of Amsterdam (29,32,33). By measuring the levels of specific enzymes, the presence of four separate translocation systems was inferred having specificities for the following anions:

I. succinate, fumarate, malate, oxalacetate
II. α-ketoglutarate
III. citrate, isocitrate
IV. malonate

There was no cross specificity between groups although any of the group I acids would fully induce the translocator effective for the entire group (29). Pyruvate, lactate and acetate however, appeared to be transported regardless of which transport system was present and consequently it remains unclear by what means these compounds traverse the cell membrane. Although no specific inhibitors of transport were used, it was believed that all transport processes observed were by energy dependent mechanisms since anoxia led to their immediate cessation (29).

The role of glucose in accelerating transport of carboxylic acids was further investigated (32). At high concentrations of glucose (50-100mM) catabolite repression of the malate translocator was observed. However the enhancement of malate transport by low concentrations of glucose was not fully explained. This "glucose effect" could be accomplished, though with lesser efficiency, by replacing glucose with glutamate, casamino acids or α-ketoglutarate (33). The disappearance of ^{14}C-labeled glucose was the same regardless of whether or not induction of the translocators for malate or succinate occurred.

It was concluded that glucose was not serving as an energy source in stimulating carboxylic acid transport (33).

Induction of the malonate transport system simultaneously induces the appearance of enzymes concerned with its metabolism: Malonyl-CoA synthetase and malonyl-CoA decarboxylase (33). Cyclic AMP (3'5'cAMP) can derepress the synthesis of carboxylic acid translocators (32). Hence the regulation of these systems appears similar to the classic operon model in E. coli (24,28).

Greater understanding of the molecular basis of transport exists in E. coli than is the case in A. vinelandii at present. "Porin", an outer membrane protein of 36,500 daltons molecular weight, appears to be required for the energy dependent transport of some carboxylic acids in E. coli (6). Transport function is restored when this protein is added to cells blocked in transport by treatment with proteolytic enzymes or to transport mutants (7). It appears that multimeric aggregates of the protein form channels that allow carboxylic acid molecules to pass into the periplasmic space (24). However the energy dependence as well as the manner in which specificity is invoked are not yet understood.

TRANSPORT OF INORGANIC IONS

Studies of how inorganic ions enter cells have not, until recently, been in the forefront of membrane research. However, there has been a gradual confirmation of an underlying belief that separate, specific mechanisms exist for every inorganic cation or anion required for growth (36). Early studies of A. vinelandii using starved, washed cells in various salt solutions suggested that the cell membrane is generally impermeable to ions (22, 23). As the external concentration of LiCl, NaCl or KCl increased, the packed cell volume decreased as water was drawn out of the cell, presumably due to increased osmotic pressure. It was concluded that ions could not pass the membrane. Slow entry, however, was observed when NH_4Cl, NaCl and $CaCl_2$ were tested. When pulsed with an energy substrate, the cells swelled and consumed oxygen. Since these events could be prevented by addition of various inhibitors and especially uncouplers of oxidative phosphorylation, it was concluded that ion transport was an active, energy requiring, process (23).

Further studies using A. vinelandii protoplasts demonstrated that the membrane is impermeable to K^+, Na^+, NH_4^+, Cl^-, $PO_4^{\equiv}$ and $SO_4^{=}$ in the absence of energy sources (38). However, in contrast to findings in whole cells (29), protoplast membranes were permeable to potassium acetate but not ammonium acetate. Although a metabolic energy requirement was demonstrated, it was not clear whether transport was the result of a specific translocator or due to some kind of ion exchange process (30,38).

Another study of NH_4^+ transport was done by Kleiner (21) who measured NH_4^+ from cells grown with various amounts of ammonia as well as various counter ions. He concluded that several ammonium ion translocators exist because an adaptive process was required prior to ammonium entry when the anion in the medium was changed. Again, studies with inhibitors indicated that energy was required to maintain a one hundred-fold difference between external and internal ammonium ion concentrations.

Several studies of ion transport in membrane vesicles from _A_. _vinelandii_ have been carried out by E.M. Barnes and his associates (4,5,8,10). Previously, a membrane-associated, trypsin-activated ATPase had been described in _A_. _vinelandii_ (18). Barnes' treatment of membrane vesicles with trypsin followed by the addition of lactate led to the specific, ATP dependent uptake of Ca^{++} (4,8). Since the vesicles were prepared with inverted orientation (5) it was concluded that _A_. _vinelandii_ is similar to many other bacteria in actively extruding Ca^{++} from the cytosol despite its requirement for Ca^{++} as a growth factor.

Sodium ions also appear to be expelled from the cell in a energy dependent process (10). However, in contrast to Ca^{++}, the Na^+ exodus is independent of ATP hydrolysis and requires a proton gradient. Kinetic studies of Na^+ uptake by everted vesicles, as well as measurement of the appearance of Na^+ in the medium surrounding normally oriented vesicles, suggest a classic antiport mechanism is at work exchanging one Na^+ for each proton entering the cell (10).

Transport of Fe^{+3} in A. vinelandii has been inferred due to its production of a green, fluorescent pigment in response to iron deficiency (12,14). Although little is known about iron transport in A. vinelandii, the mechanism of iron transport is better understood from work in other species, especially from studies of mutants blocked in various aspects of the process (25,36). E. coli cells produce enterochelin, an iron chelator, in response to low iron stress. Iron-chelator complexes are then actively transported into the cell where hydrolytic enzymes release the iron and degrade the carrier molecule. Although receptors for the iron-chelator complex have been identified on the cell surface, the exact mode of transport through the membrane is unknown.

TRANSPORT OF GLUCOSE

Most of the information available about glucose uptake in A. vinelandii comes from experiments with membrane vesicles (1,2,3,9). Vesicles with normal orientation were shown to take up ^{14}C-labeled glucose when provided with malate as an energy source but not with lactate. It appears that glucose uptake in A. vinelandii is coupled to a flavin-linked malate dehydrogenase in the membrane (1). Difference spectra of various membrane preparations indicated that a second site for glucose uptake exists proximal to ubiquinone in the respiratory chain (3). Both sites function by active processes, i.e. as glucose-proton symports (9).

The glucose transport system in A. vinelandii appears to be inducible since vesicles from acetate grown cells take up less than 1% of the amount taken up by glucose grown cells (2). Although little is known about how glucose is transported through the membrane, a very high specificity for glucose can be demonstrated by the use of analogs. Hopefully, this might lead to ultimate isolation of at least the receptor component of this transport system (42).

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TRANSPORT OF 2-METHYLALANINE IN *AZOTOBACTER VINELANDII*

by

A. E. Kaufman and H. L. Sadoff

Department of Microbiology and Public Health
Michigan State University
East Lansing, Michigan 48824

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INTRODUCTION

When the non-metabolizable analog of alanine, 2-methylalanine (also known as α-amino isobutyric acid), is added to a culture of *Azotobacter vinelandii*, inhibition of growth is observed if glucose or maltose is the sole growth substrate in the medium (13). Providing a source of fixed nitrogen partially prevents 2-methylalanine (MA) induced inhibition, although MA and ammonia are thought to exert dissimilar effects upon the regulatory mechanism for nitrogen fixation (4). Inhibition of growth does not occur when MA is added to a culture growing on alternate substrates such as acetate, galactose, fructose or sucrose (13). The experiments described herein were undertaken to explain why the effects of 2-methylalanine upon *A. vinelandii* are limited to cells growing on glucose.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

A. vinelandii (ATCC 12837) was used throughout this study. A 2-methylalanine resistant strain (mar-25) was derived from the parental strain by mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine, according to the method of Sorger (16). Cell stocks were maintained on modified Burk nitrogen free medium (9) containing 0.8 mM $MgSO_4$, 0.58 mM $CaSO_4$, 18.0 μM $FeSO_4$, 1.0 μM $NaMoO_4$, 5.0 mM potassium phosphate buffer (pH7.4) and 1% glucose.

Growth Studies

Exponential phase cells were washed twice in glucose-free medium and suspended in 50 ml of medium containing an appropriate carbon source at a concentration of 50 mM. Cultures were incubated on a water shaker bath (New Brunswick Scientific, New Brunswick, N.J.) at 30°C, and monitored with Klett-Summerson Photo Colorimeter (Klett Mfg., New York, N.Y.) equipped with a #54 filter. When the cell density reached 50 Klett units, MA and/or other compounds were added to a final concentration of

20 mM (pH 7.0-7.4). One Klett unit represents a viable cell count of 2.2×10^6 cells/ml as determined by plating on Burk Nitrogen free medium.

Chemicals

Isobutyric acid, 2-methylalanine and α-hydroxyisobutyric acid were obtained from Sigma Chem. Co. (St. Louis, Mo.). Radiolabeled 2-methylalanine [α - (3-^{14}C) - amino isobutyric acid] was obtained from New England Nuclear Co. (Boston, MA); 2-deoxy-D- [1-^{14}C] glucose was obtained from Amersham Corp. (Arlington Heights, Ill.). All other materials were obtained from commercial sources and were of analytic or reagent quality.

Uptake of ^{14}C MA

Radioactive MA (0.1 μC/μmole(was added to 10 ml cultures (final concentration: 5μCi/ml) growing on glucose or fructose upon reaching a turbidity of 50 Klett Units. Samples (0.25 ml.) were removed in duplicate and filtered on a multisample vacuum manifold (Millipore Co., Bedford, MA) through glass microfibre filters (Whatman Ltd., Mailstrom, England). The filters were immediately rinsed with 5.0 ml of iced, glucose-free medium, dried for 2-4 hours at 80°C and placed in scintillation fluid. Radioactivity was measured with a Packard 3000 Tricarb liquid scintillation spectrometer. Protein was determined by the method of Lowry et al. (8) with bovine serum albumin used as a standard.

RESULTS

Inhibition of Growth

The effect of MA upon growing cells was most pronounced when it was introduced early in the exponential phase of growth (Fig. 1). Inhibition was marked when MA was added to cultures having a turbidity of 50 Klett units. The response of older cultures was not as great or undetectable. In addition, the effect of MA appeared to be reversible. Exponential phase cultures (turbidity of 50 Klett units) were exposed to MA for 1,2,4 or 6 hours. When washed twice and suspended in MA-free medium at their original turbidities, these cultures grew, without noticeable lag, to final turbidities equivalent to control cultures unexposed to MA (data not shown).

In order to ascertain whether MA interferes with the transport of glucose into cells, suspensions of glucose-grown cells were exposed to 2-deoxy-D-(1-^{14}C) glucose in the presence of MA (Table 1). The initial rate of uptake increased with exposure to greater amounts of MA. The data were in a range similar to those of Barnes (2) who measured the uptake of glucose and glucose analogs by membrane vesicles prepared form _A_. _vinelandii_, strain O.

Uptake of radiolabeled MA.

Inhibition of growth was observed when MA was added to cultures of wild type cells growing on glucose (Fig. 2A); however similar effects were absent in cultures of mar-25 or wild type cells growing on fructose. After 24 hours the inhibited (glucose + MA) cultures contained three times the levels of ^{14}C-MA observed in the growing cultures (Fig. 2B). In addition the amount of radioactivity associated with the non-growing cells increased nearly eight-fold over the course of the experiment; the increase in radioactivity in the other cultures was only about two-fold. Using a t-test (14) the regression slopes of the uptake of radioactivity were compared. A nonsignificant difference ($p<.001$) was found between them. Therefore it is reasonable to assume that the uptake of MA in mar-25 and the wild type cells growing on fructose were equal.

Prevention of MA effects

Structurally similar amino acids, such as alanine or serine have been shown to compete with MA for transport into cells in several bacterial species (3,5,14,17). Consequently, these as well as other compounds were tested for ability to block the inhibitory effect of MA on growth. Serine was capable of counteracting MA while isocitrate was not (Fig. 3). Neither serine nor isocitrate is capable of supporting growth of *A. vinelandii* independently. Additional substances that were tested are listed in Table 2.

Effects of Isobutyric Acid and α-Hydroxyisobutyric Acid

In order to determine whether the inhibitory properties of MA might be attributed to any particular chemical group on the molecule, isobutyric acid and α-hydroxyisobutyric acid were added to cultures growing on glucose or fructose (Fig. 4). Isobutyric acid did appear to transiently affect the growth of only the culture growing on glucose. In contrast α-hydroxyisobutyric acid immediately stopped the growth of both cultures. Microscopic examination of the culture revealed non-motile, refractile cells in large aggregates. When the cells were washed and plated on fresh medium not containing α-hydroxyisobutyric acid no growth was observed.

DISCUSSION

The cellular envelope of A. vinelandii has been shown to be selectively impermeable. Ions such as Ca^{++}, K^+, Cl^- and NH_4^- (6,7,18) as well as carboxylic acids (10) enter the cell only by means of highly specific, energy dependent transport systems. In the case of glucose, an inducible, malate dehydrogenase-linked, carrier-mediated system appears to be responsible for moving glucose across the cell membrane (6,16). To date no group translocation (phosphotransferase) system has been shown to operate in A. vinelandii (17). The observation that the inhibitory action of MA is limited to early exponential phase cells metabolizing glucose suggested that glucose transport might be the site of MA interference with growth. However, our finding that MA enhanced 2-deoxyglucose uptake is not consistent with such an hypothesis.

The significant increase in the amount of MA entering cells exposed to glucose is reminiscent of previous findings regarding carboxylic acid translocation systems in A. vinelandii (10,11). It was found that the presence of glucose facilitated the entry of succinate and malate prior to induction of the specific transport systems.

Our experiments also seem to indicate that glucose increases the permeability of A. vinelandii, although it is not clear whether MA is co-transported with glucose or is interacting with a polyfunctional transport component at the cell membrane. Support for the latter notion comes from the wide range of compounds that counteract the effect of MA. This is in contrast to the behavior of transport systems specific for alanine and serine, which are unaffected by molecules of dissimilar structure (5,15,17).

The action of isobutyric acid upon A. vinelandii is similar to that of MA in that both affect only glucose growing cells. However, the brevity of its effect on growth as well as the failure of MA resistant cells to resist isobutyric acid (data not shown) suggests that isobutyric acid is exerting a different effect than MA. Similarly, α-hydroxyisobutyric acid appears to be acting in a different manner than either MA or isobutyric acid in immediately killing both fructose and glucose grown cells. Since the cells tend to agglutinate as if their surface had become "sticky" the toxic effect of α-hydroxyisobutyrate may be due to some chemical action upon the cell surface rather than the result of entry into the cell.

Figure 1. Effect of MA depending on the age of the culture. Arrows and associated symbols indicate turbidity at the time of addition: ▲ - 50 Klett units; ○- 100 Klett units; ■- 150 Klett units; ●- 200 Klett units; □- control (no MA added).

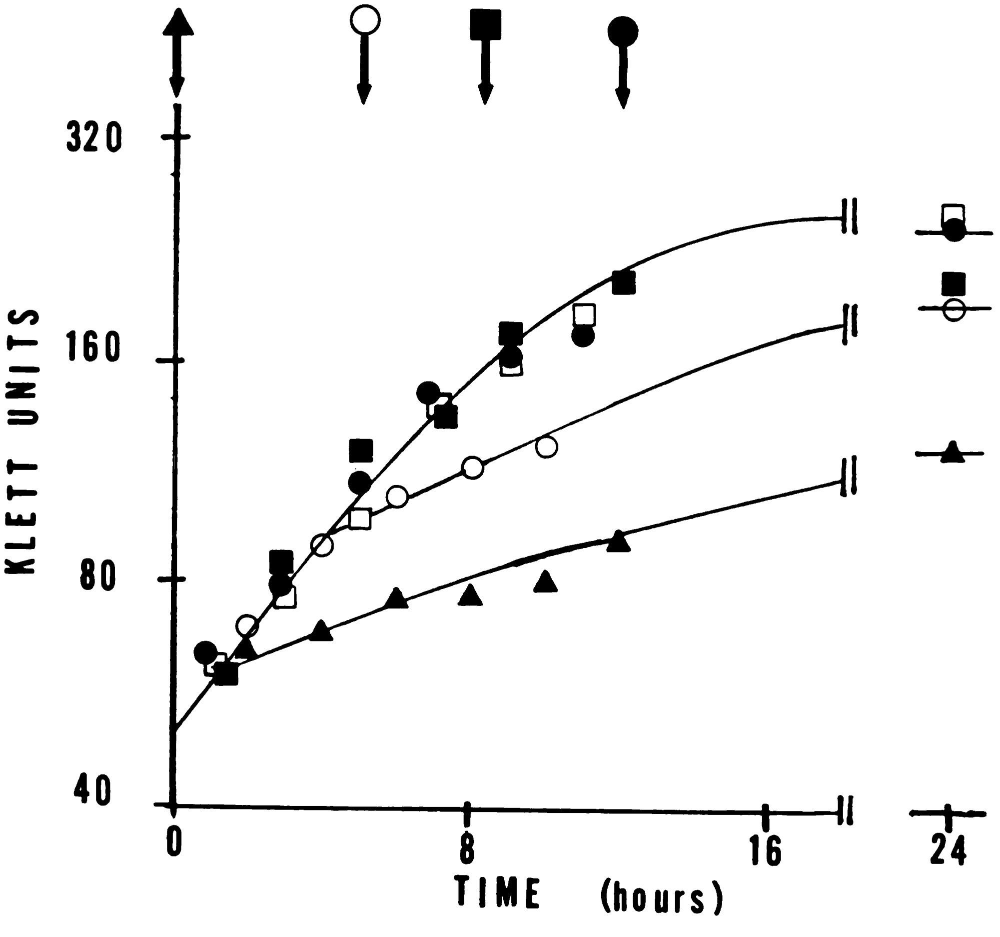
KLETT UNITS
320
160
80
40
0
8
16
24
TIME (hours)

Figure 2. Growth of cells (A) and uptake of ^{14}C-labled MA(B) in wild type cells growing on glucose (△), glucose + MA (▲), fructose (○), or fructose + MA (●) and in mar-25 cells growing on glucose (□) or on glucose + MA(■).

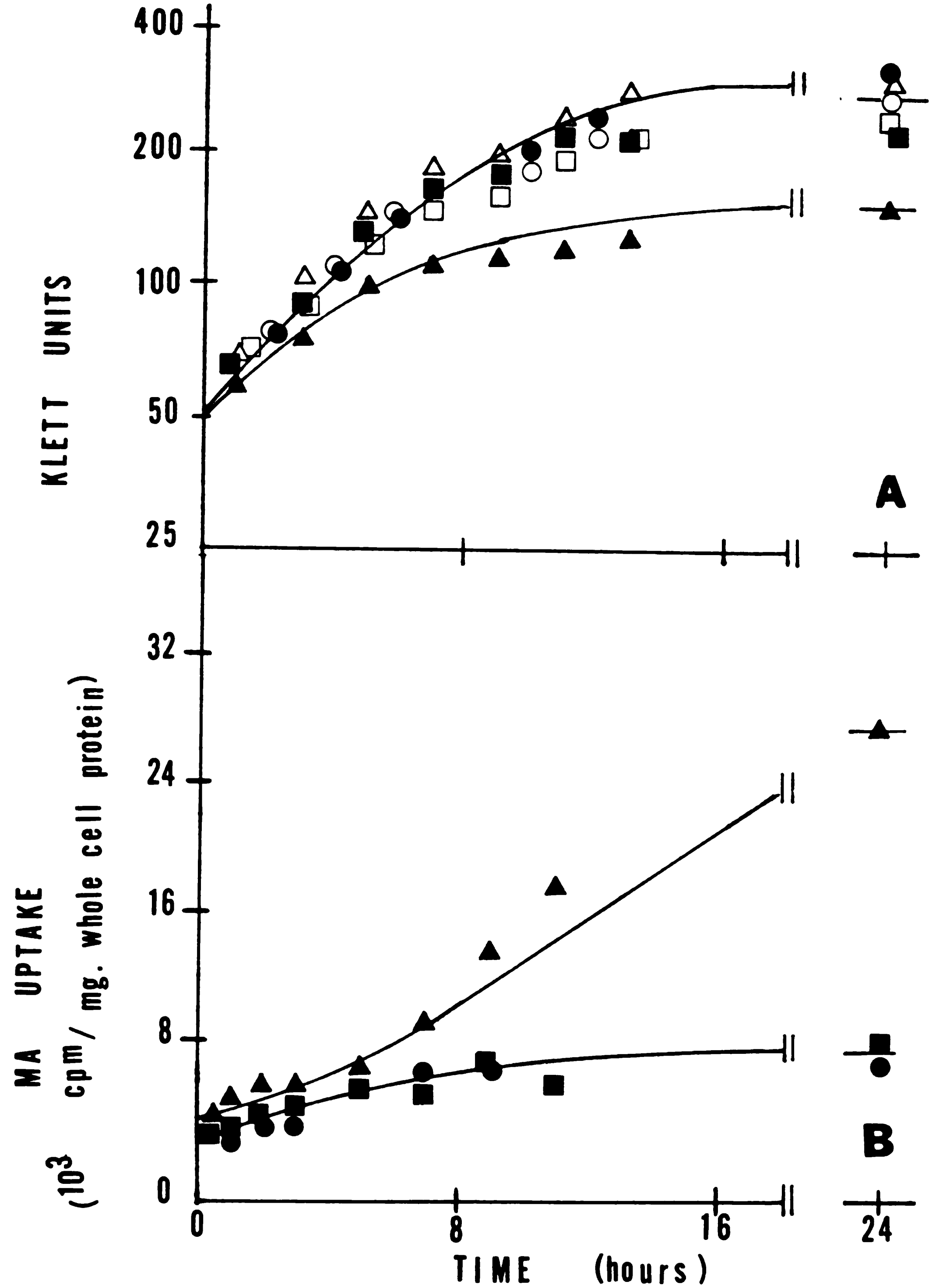
400
200
100
50
25
KLETT UNITS
A
32
24
16
8
0
MA UPTAKE
(10^3 cpm/mg. whole cell protein)
B
0
8
16
24
TIME (hours)

Figure 3. Growth of wild type cells on media containing: glucose (△), glucose + MA (▲), glucose + L-serine (□), glucose + L-serine + MA (■), glucose + isocitrate (○), glucose + isocitrate + MA (●), isocitrate (⬡) and L-serine (⬢).

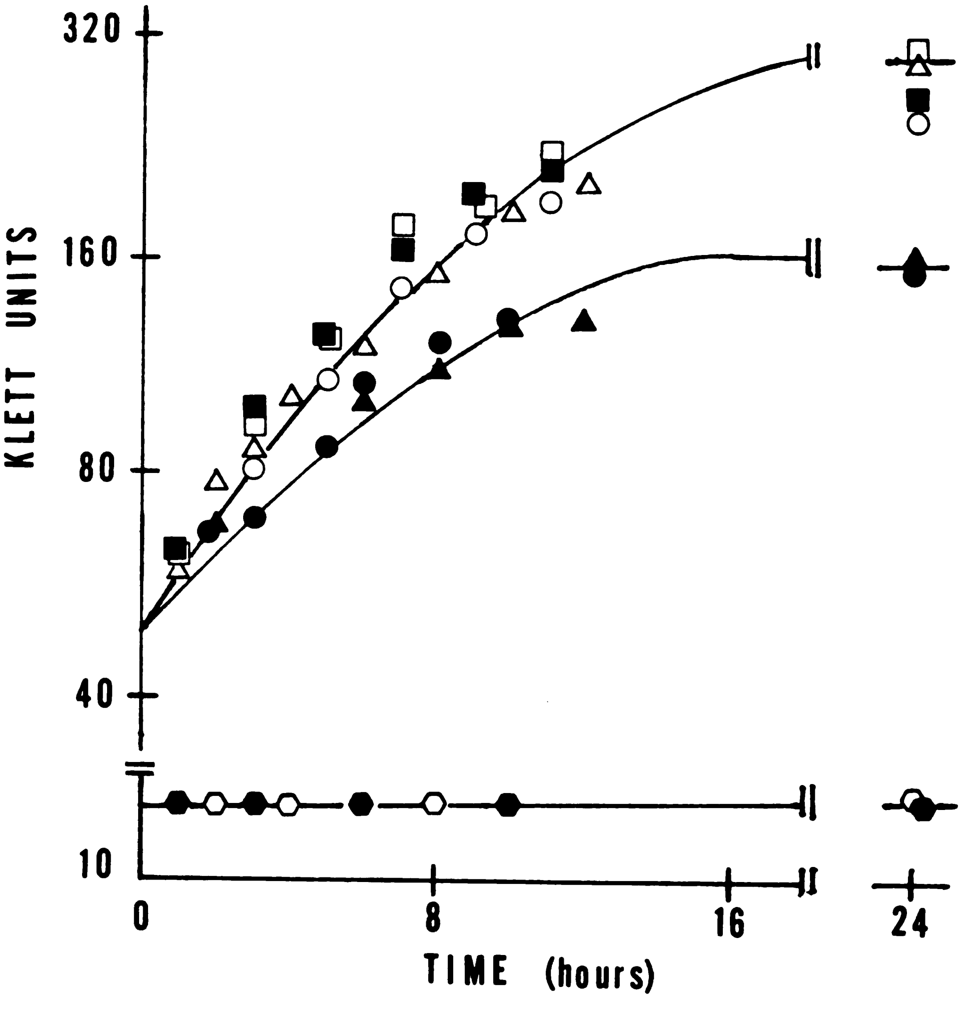
KLETT UNITS
320
160
80
40
10
0
8
16
24
TIME (hours)

Figure 4. Growth of wild type cells on media containing: glucose (△), glucose + isobutyric acid (▲), fructose (□), fructose + isobutyric acid (■), glucose + α-hydroxyisobutyric acid (●), and fructose + α-hydroxyisobutyric acid (○).

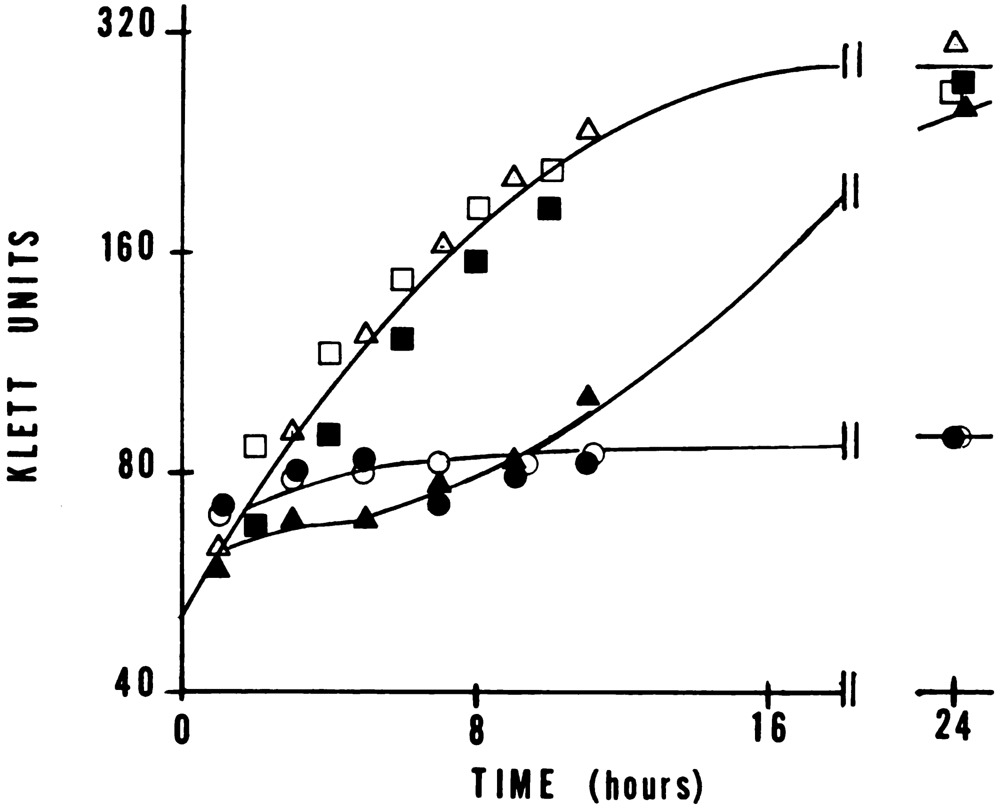
KLETT UNITS
320
160
80
40
0
8
16
24
TIME (hours)

Table 1

Initial rate of uptake of 2-deoxy-D-[1-^{14}C]glucose by washed suspensions of _A_. _vinelandii_ in the presence of 2-methylalanine.[a]

2-methylalanine: (mM)	2-deoxyglucose: (mM) 4	8	16
0	.02±.005	.02±.009	.09±.002
5	-[b]	.07±.001	.15±.001
10	-	.11±.040	.17±.006
20	-	.10±.013	.27±.242

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[a] 10 ml of washed cells in glucose-free Burk medium were incubated for 5 minutes in a shaker bath at 30°C with indicated amounts of MA and pulsed with indicated amounts of 2-deoxyglucose (0.15μCi/μmole). Values represent initial uptake rates at each concentration (3 replicates) and have units of m moles/min./mg whole cell protein.

[b] not determined

Table 2

Ability of compounds to counteract the effect of 2-methylalanine on *A. vinelandii* growing on glucose.

Compound	ability to serve as sole growth substrate	ability to prevent MA inhibition
acetate	+	+
ammonium chloride	-	±[a]
alanine	-	+
arginine	-	+
aspartate	-	+
benzoate	+	-
butyrate	+	+
citrate	+	+
gluconate	+	+
isocitrate	-	-
α-ketoglutarate	-	-
lactate	+	+
lysine	-	+
malate	+	+
oxalacetate	-	+
proline	-	+
pyruvate	+	+
serine	-	+
succinate	+	+

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[a] ± = partial prevention

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