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Specificity of Peptide Transport Systems in *Lactococcus lactis*: Evidence for a Third System Which Transports Hydrophobic Di- and Tripeptides

CATHERINE FOUCAUD,[1]* EDMUND R. S. KUNJI,[2] ANJA HAGTING,[2] JEAN RICHARD,[1] WIL N. KONINGS,[2] MICHEL DESMAZEAUD,[1] AND BERT POOLMAN[2]

Station de Recherches Laitières, Institut National de la Recherche Agronomique, 78350 Jouy-en-Josas, France,[1] *and Department of Microbiology, University of Groningen, 9751 NN Haren, The Netherlands*[2]

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A proton motive force-driven di-tripeptide carrier protein (DtpT) and an ATP-dependent oligopeptide transport system (Opp) have been described for *Lactococcus lactis* MG1363. Using genetically well-defined mutants in which *dtpT* and/or *opp* were inactivated, we have now established the presence of a third peptide transport system (DtpP) in *L. lactis*. The specificity of DtpP partially overlaps that of DtpT. DtpP transports preferentially di- and tripeptides that are composed of hydrophobic (branched-chain amino acid) residues, whereas DtpT has a higher specificity for more-hydrophilic and charged peptides. The toxic dipeptide L-phenylalanyl-β-chloro-L-alanine has been used to select for a di-tripeptide transport-negative mutant with the Δ*dtpT* strain as a genetic background. This mutant is unable to transport di- and tripeptides but still shows uptake of amino acids and oligopeptides. The DtpP system is induced in the presence of di- and tripeptides containing branched-chain amino acids. The use of ionophores and metabolic inhibitors suggests that, similar to Opp, DtpP-mediated peptide transport is driven by ATP or a related energy-rich phosphorylated intermediate.

For optimal growth in milk, lactococci depend on the presence of a proteolytic system which consists of a cell envelope-located proteinase, several peptidases, and amino acid and peptide transport systems (20). At present, two peptide transport systems have been characterized for *Lactococcus lactis* (6, 10, 11, 29). The oligopeptide transport system (Opp) mediates the ATP-driven transport of peptides with four to at least eight residues. It plays a central role in the proteolytic pathway of *L. lactis*, as it is essential for the accumulation of all β-casein-derived amino acids (10). The level of activity in the Opp system is sufficiently high to support maximal growth rates on β-casein, provided that leucine and histidine are present in the medium as free amino acids. The di- and tripeptide transport system (DtpT) is unique among bacterial peptide transporters, as it is encoded by a single gene and uses the proton motive force to drive the transport of relatively hydrophilic di- and tripeptides (6). Spontaneous mutations which inactivate the *dtpT* gene lead to defective growth of *L. lactis* in chemically defined medium (CDM) supplemented with a mixture of caseins as the sole source of nitrogen (24).

On the basis of the observation that the transport of some di- and tripeptides is totally abolished in alanyl-β-chloroalanine-resistant mutants of *L. lactis* whereas other peptides are still taken up at significant rates (26), the utilization of di- and tripeptides in mutants lacking either Opp or DtpT or both peptide transport systems was investigated. In this report, we present evidence for a third peptide transport system in *L. lactis* with specificity for relatively hydrophobic di- and tripeptides.

* Corresponding author. Mailing address: INRA-SRL, F-78352 Jouy-en-Josas CEDEX, France. Phone: 33 1 34 65 20 67. Fax: 33 1 34 65 20 65.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and growth media. *L. lactis* subsp. *lactis* MG1363 wild-type and isogenic mutants, i.e., the di- and tripeptide transport mutant AG300, the oligopeptide transport mutant VS772, and the peptide transport double mutant CV4, have been described previously (6, 11, 29) and are presented in Table 1. Strains were grown at 30°C in M17 broth (27) or in a CDM (19) at pH 6.8 supplemented with 0.5% (wt/vol) glucose or 0.5% (wt/vol) maltose in combination with 25 mM arginine to induce the arginine deiminase pathway (17). Recombinant strains carrying replicating plasmids were grown in the presence of erythromycin (5 mg/liter). The strains were maintained in CDM containing 10% glycerol and stored at −80°C. Growth of *L. lactis* on peptides was tested in CDM containing all amino acids except for one essential amino acid, which was supplied in the form of a di- or a tripeptide. The influence of growth conditions on peptide uptake was tested in CDM in which Leu was supplied in the form of Leu (3.6 mM), Leu-Leu (1.8 mM), or Leu-Leu-Leu (1.2 mM).

Isolation of mutants resistant to toxic peptide analogs. Toxic-peptide-resistant mutants were isolated from *L. lactis* AG300 according to the method of Smid et al. (26). Approximately 10^8 CFU of an exponentially growing CDM culture were spread on a 1.2% (wt/vol) agar plate containing a complete amino acid mixture. After plating was performed, a single crystal of the toxic peptide analog L-phenylalanyl-β-chloro-L-alanine (FCA) (13) was put in the center of each plate. Growth inhibition was observed after 48 h of incubation at 30°C. Spontaneous FCA-resistant mutants were isolated from the inhibition zones and maintained in CDM plus 0.25 mM FCA.

The peptidase patterns of the AG300 FCA^r mutants were compared with those of *L. lactis* MG1363 and AG300 by using enzymatic test strips containing 60 different substrates (AP I to VI; Bio-Mérieux, Marcy l'Etoile, France).

Transport assays. Cells were harvested by centrifugation in the exponential phase of growth (A_{650} of approximately 0.6), washed twice, and resuspended to a final A_{650} of approximately 25 in 50 mM potassium phosphate (pH 6.5)–2 mM $MgSO_4$. The cells were de-energized with 10 mM 2-deoxy-D-glucose for 30 min at 30°C (28), which results in the depletion of intracellular amino acid pools. De-energized cells were washed twice with 50 mM potassium phosphate (pH 6.5)–2 mM $MgSO_4$. In experiments in which ionophores and metabolic inhibitors were used, the buffer was either 50 mM potassium phosphate (pH 6.5 or 7.5)–2 mM $MgSO_4$ or 50 mM sodium piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) (pH 6.5)–2 mM $MgSO_4$, as specified in the text. The pH dependency of transport was determined in 20 mM potassium succinate–20 mM potassium phosphate–20 mM Tris–2 mM $MgSO_4$. The buffers were adjusted to the desired pHs with KOH. All transport assays were performed at 30°C. The uptake of peptides was monitored by determining the intracellular concentrations of the corresponding amino acids by means of reversed-phase high performance liquid chromatography (HPLC) analysis as described previously (11). The dansylated amino acids were separated by HPLC on a C_{18} column (Novapak C_{18}, 3.9 × 150

TABLE 1. Bacterial strains used in this study

L. lactis subsp. *lactis* strain	Relevant characteristic(s)	Reference
MG1363	Lac$^-$ Prt$^-$ Opp$^+$ DtpT$^+$ DtpP$^+$, plasmid-free derivative of NCDO712	4
AG300	MG1363Δ*dtpT*	6
VS772	MG1363*oppA*::pLS19A, Em^r[a]	29
CV4	MG1363Δ*dtpT oppA*::pLS19A, Em^r	10
CF310	AG300, FCA^r	This work

[a] Em^r, resistance to erythromycin.

mm; Millipore Corporation, Milford, Mass.). The dansylated amino acids were eluted with a linear gradient (0 to 75% [vol/vol] acetonitrile [in 10 mM sodium citrate, pH 6.2]) in 30 min.

Miscellaneous. Protein measurement was done by the method of Lowry et al. (12), with bovine serum albumin as the standard. Growth experiments were performed with enzyme-linked immunosorbent assay plates. Changes in absorption were measured at 620 nm in a Ceres 900 spectrophotometer (Bio-Tek Instruments Inc., Winooski, Vt.). To prevent evaporation during the incubations at 30°C, the incubation mixtures (200 μl) were covered with 100 μl of paraffin oil. A specific internal volume of 3.6 μl/mg of protein was used to calculate the intracellular concentrations (22).

Chemicals. All chemicals were of reagent grade and were obtained from commercial sources. All peptides used were in the L configuration and were obtained from Bachem AG, Bubendorf, Switzerland. FCA was a generous gift of R. Plapp, University of Kaiserslautern, Kaiserslautern, Germany.

RESULTS

Utilization of peptides by *L. lactis* strains during growth. The utilization of essential amino acids containing di- and tripeptides was analyzed for *L. lactis* MG1363 and peptide transport mutant strains growing in CDM (Table 2). As expected *L. lactis* MG1363 and VS772 (OppA$^-$) can satisfy their requirements for essential amino acids by utilization of all di- and tripeptides except for Ile-Arg (26) and Gly-His-Gly. Surprisingly, *L. lactis* AG300, which lacks the di- and tripeptide transport system DtpT, can utilize the same set of peptides except for Gly-Leu. This pattern of growth was also observed with the double mutant CV4. These results suggest the presence of at least one other peptide transport system which facilitates the transport of dipeptides such as Leu-Leu, Val-Leu, Leu-Met, Phe-Val, His-Leu, Met-Met and Ile-Ile and of tripeptides such as Leu-Leu-Leu, His-Pro-Val, His-Gly-Gly, Ala-His-Ala, and Gly-Leu-Tyr. The external breakdown of these di- and tripeptides, as a result of cell lysis, and the subsequent transport of the corresponding amino acids are unlikely, since Ile-Arg, Gly-Leu, and Gly-His-Gly were not hydrolyzed. These nontransported peptides as well as others are rapidly hydrolyzed upon permeabilization of the cells (results not shown).

Evidence for a second di- and tripeptide transport system. The presence of a second, previously undiscovered, di- and tripeptide transport system was studied further in glycolyzing *L. lactis* CV4 cells (Fig. 1). The uptake of Leu-Val, Met-Met, and His-Gly-Gly was observed for *L. lactis* CV4, provided that an energy source such as glucose was present. The uptake of these peptides was inhibited (competitively) by a fivefold excess of one of the other peptides; the inhibiting peptide itself accumulated (as amino acids) inside the cell. The pattern of inhibition suggests that the affinity of the transporter for Leu-Val exceeds that of Met-Met and His-Gly-Gly. Free amino acids such as Leu and Gly and the oligopeptide Tyr-Gly-Gly-Phe-Leu did not affect the transport of the di- and tripeptides (data not shown). Free peptides could not be detected in the cell, which indicates that peptide uptake is accompanied by rapid intracellular hydrolysis (data not shown). Assuming that the intracellular peptidase activity does not limit the peptide utilization rate, the uptake rate of a given di- or tripeptide can be estimated from the accumulation rates of the amino acids and the frequency of a particular amino acid residue in the peptide. The rates of uptake of Met-Met*, Leu-Val*, and His-Gly-Gly* were 11, 19, and 21 nmol/min/mg of protein, respectively (the amino acid considered is marked with an asterisk). No amino acids could be detected in the external medium, which confirms that the external breakdown of peptides followed by transport of the amino acids does not occur.

Isolation and characterization of FCA-resistant mutants. The toxicity of the peptide analog L-alanyl-β-chloro-L-alanine has previously been used to isolate peptide transport mutants with DtpT$^-$ phenotypes (26). Since the putative second di- and tripeptide transport system has a specificity for more-hydrophobic peptides, we tested the toxicity of the peptide analog FCA on confluently plated *L. lactis* AG300 (Δ*dtpT*) cultures as described in Materials and Methods. Spontaneous FCA-resistant mutants (Table 1, AG300, FCA^r) were isolated with a mutation frequency of about 5×10^{-6}. As the resistance towards the toxic dipeptide can be the result of an inactive peptide transport system(s) and/or intracellular peptidase(s), the peptidase and transport activities in *L. lactis* MG1363, AG300, and one of the FCA-resistant mutants (designated CF310) were compared. The peptidase pattern of *L. lactis* CF310 was similar to those of the wild-type *L. lactis* MG1363 and *L. lactis* AG300 (data not shown). Furthermore, the uptake of Tyr-Gly-Gly-Phe-Leu and Ala were the same for *L. lactis* CF310, MG1363, and AG300. On the contrary, the uptake of Leu-Leu-Leu, Met-Met, Met-Leu, Phe-Ala, Leu-Val, Val-Leu, and His-Gly-Gly was not detectable in *L. lactis* CF310 (Table 3), indicating that a di- and tripeptide transport system was specifically inactivated in this mutant. The inactivation of the di- and tripeptide transport system (designated DtpP) in *L. lactis* CF310 was confirmed by growth experiments, since CF310 could not use any of these di- and tripeptides as a source of essential amino acids (data not shown). It was not possible to isolate FCA^r mutants with MG1363 or VS772 as

TABLE 2. Growth of *L. lactis* strains on various media[a]

Medium	Growth of *L. lactis* strain:			
	MG1363 (wild type)	AG300 (Δ*dtpT*)	VS772 (*oppA*::pLS19A)	CV4 (Δ*dtpT oppA*:: pLS19A)
CDM	+	+	+	+
Leu*-Leu*	+	+	+	+
Val-Leu*	+	+	+	+
Leu*-Met	+	+	+	+
Phe-Val*	+	+	+	+
His-Leu*	+	+	+	+
Met*-Met*	+	+	+	+
Ile*-Ile*	+	+	+	+
Leu*-Leu*-Leu*	+	+	+	+
His-Pro-Val*	+	+	+	+
His*-Gly-Gly	+	+	+	+
Ala-His*-Ala	+	+	+	+
Gly-Leu*-Tyr	+	+	+	+
Ile*-Arg	−	−	−	−
Gly-Leu*	+	−	+	−
Gly-His*-Gly	−	−	−	−

[a] Wild-type MG1363 and peptide transport mutants of *L. lactis* were grown in CDM or CDM lacking single essential amino acids (indicated with asterisks) and supplemented with the peptides indicated.

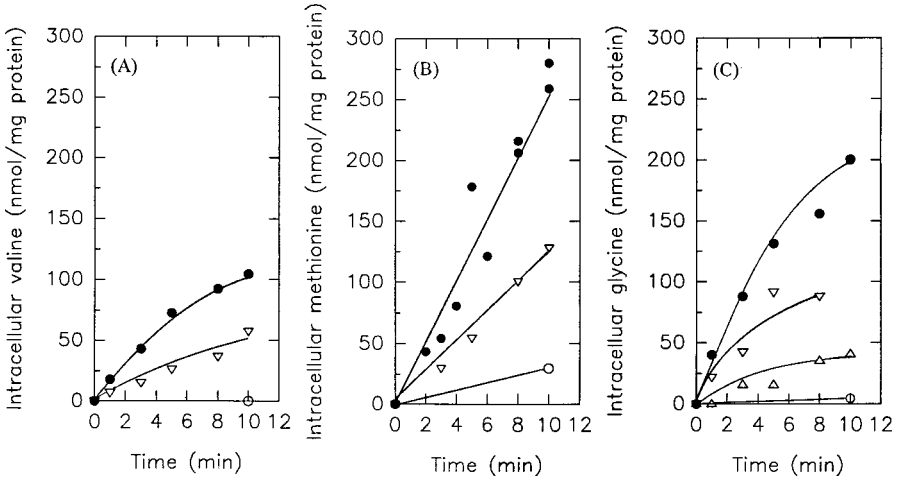

FIG. 1. Peptide uptake in glucose-metabolizing *L. lactis* CV4 cells. (A) Uptake of Leu-Val (1 mM) in the absence (●) or presence (▽) of 5 mM His-Gly-Gly; (B) uptake of Met-Met (1 mM) in the absence (●) or presence (▽) of 5 mM His-Gly-Gly; (C) uptake of His-Gly-Gly (1 mM) in the absence (●) or presence of 5 mM Leu-Val (△) or 5 mM Met-Met (▽). Uptake in the absence of glucose (○) is also shown.

the parent strain, most likely because FCA is transported by DtpT and DtpP.

Substrate specificity. The uptake of peptides by *L. lactis* MG1363 was compared with those by the transport mutants AG300, CV4, and VS772 (Table 3). The dipeptide Pro-Gly and the pentapeptide Tyr-Gly-Gly-Phe-Leu have previously been shown to be specific for DtpT and Opp, respectively (6, 10). Indeed, Pro-Gly requires a functional DtpT, whereas Opp activity is needed for the uptake of Tyr-Gly-Gly-Phe-Leu (Table 3). Peptides containing acidic amino acids, i.e., Glu-Val, Gly-Asp, or Met-Asp, or those containing basic amino acids, i.e., Lys-Leu, Arg-Gly, or His-Gly, were taken up by *L. lactis* MG1363. The uptake of both types of peptides was severely reduced or could not be detected in the double mutant *L. lactis* CV4. The positions of the acidic residues in the dipeptide was not crucial. Comparable findings were made with neutral glycine-containing peptides, i.e., X-Gly and Gly-X. Since the oligopeptide transport system is specific for peptides containing at least four residues (29), the defect in the uptake of charged and Gly-containing neutral dipeptides in the double mutant must be the result of the *dtpT* mutation. On the other hand, Phe-containing peptides were transported by *L. lactis* CV4, albeit at reduced rates compared with that for the wild-type MG1363. Also the hydrophobic peptides (containing Met, Leu, and/or Val) were taken up by *L. lactis* CV4. The transport of these peptides was reduced in the Δ*dtpT* mutant AG300 but was unaffected by the *opp* mutation (VS772). The net accumulation rate of Leu was 4 to 18 times higher with Leu-Leu as substrate compared with the rate obtained with Met-Leu, Val-Leu, or Leu-Val as substrates (Table 3). The accumulation rates for Leu and Met were similar at initial substrate concentrations of the dipeptides Leu-Val and Met-Met of 0.5 or 1 mM (5 and 7 nmol/min/mg of protein for Leu-Val; 32 and 25 nmol/min/mg of protein for Met-Met) (Fig. 1 and Table 3; also results not shown), suggesting that the transport reaction occurs at or near saturation conditions. *L. lactis* MG1363 transports the tripeptide Leu-Leu-Leu at a higher rate than it does His-Gly-Gly. The di-, tri-, and oligopeptide-deficient transport mutant CV4 displayed reduced uptake of both peptides, but the effect was most pronounced for Leu-Leu-Leu. The net accumulation rate of Leu was lower with Leu-Leu-Leu as substrate than it was with the dipeptide Leu-Leu as substrate (Table 3).

In summary, the rates of di- and tripeptide transport in the di- and tripeptide transport-deficient mutant AG300 and the double mutant CV4 varied between <1 to 48% of the rate observed for the wild-type strain MG1363 (and the oligopeptide transport mutant VS772). These results strongly suggest that the uptake of di- and tripeptides is mediated by at least two distinct transport systems with overlapping specificities. Since the transport of the di- and tripeptides was completely abolished in the Δ*dtpT* FCA^r mutant CF310 (Table 3), we propose that FCA resistance is due to a mutation in a second di- and tripeptide transport locus, designated *dtpP*.

TABLE 3. Uptake of di-, tri-, and oligopeptides by glycolyzing *L. lactis* cells[a]

Peptide	Amino acid accumulation rate (nmol/min/mg of protein)				
	MG1363 (wild type)	CV4 (Δ*dtpT oppA*:: pLS19A)	AG300 (Δ*dtpT*)	VS772 (*oppA*:: pLS19A)	CF310 (Δ*dtpT dtpP*$^-$)
Glu-Val*	84	<0.1			
Gly*-Asp	24	<0.1			
Met*-Asp	50	<0.1			
Lys-Leu*	424	<0.1			
Arg-Gly*	89	10			
His-Gly*	160	10			
Pro-Gly*	24	<0.1	<0.1	20	<0.1
Ala-Gly*	67	<0.1	<0.1	61	<0.1
Phe-Ala*	15	4			<0.1
Phe-Val*	15	4			
Leu*-Val	148	5	9	169	<0.1
Val-Leu*	284	18			<0.1
Leu*-Leu*	491	178	139	436	
Met-Leu*	50	24	21	60	<0.1
Met*-Met*	190	32	29	159	<0.1
His-Gly*-Gly*	89	43			<0.1
Leu*-Leu*-Leu*	216	24			<0.1
Tyr-$(Gly)_2$*-Phe-Leu	94	<0.1	80	<0.1	87

[a] Peptide uptake rates were determined from the time-dependent increase of the intracellular amino acid pools as described in Materials and Methods. The amino acids marked with asterisks were used to estimate the accumulation rates. Each peptide was present at a concentration of 0.5 mM. Blanks indicate data that were not determined.

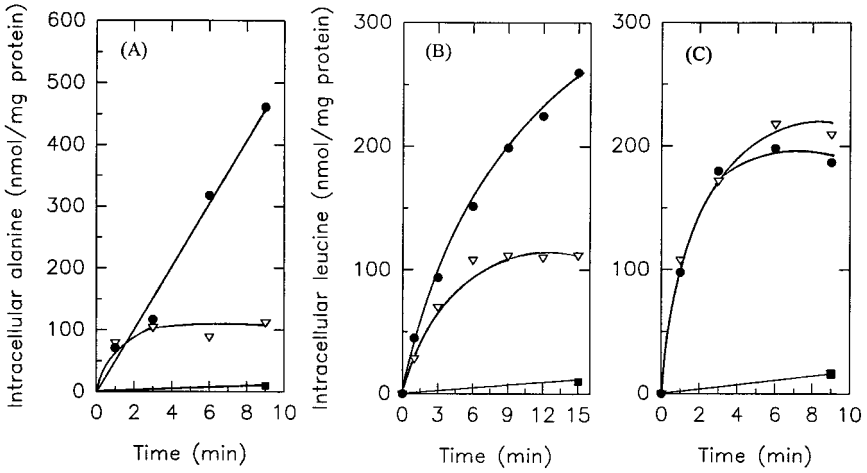

FIG. 2. Effects of *o*-vanadate on uptake of tetra-alanine (A), tri-leucine (B), and leucine (C) in arginine-energized *L. lactis* AG300 cells. Cells were grown in CDM supplemented with 0.5% maltose and 25 mM arginine to induce the arginine deiminase pathway. Prior to the transport assays, cells were de-energized as described in Materials and Methods. The cell suspensions were energized for 15 min with 25 mM arginine in 50 mM Na–PIPES (pH 6.5)–2 mM $MgSO_4$ (●) before the uptake assay was started by addition of the substrate at 1 mM (A and C) or 0.5 mM (B). *o*-Vanadate (▽) was added at a final concentration of 0.5 mM at the beginning of the preenergization period. Uptake in the absence of an energy source (■) is also shown.

Energetics of the second di- and tripeptide transport system. To characterize the transport of di- and tripeptides by the putative DtpP system further, Leu-Leu-Leu was chosen as substrate for uptake assays with *L. lactis* AG300. In the absence of metabolic energy, no significant uptake of the peptide was detected. Addition of a fermentable sugar (glucose) resulted in a high rate of tri-leucine uptake. At pH 7.5, when the external and internal pH values are similar and the proton motive force is composed of $\Delta\Psi$ only (16), Leu-Leu-Leu uptake was not significantly affected by the addition of either valinomycin, a potassium ionophore which dissipates the membrane potential in the presence of K^+ (data not shown), or nigericin, a potassium proton ionophore. However, the addition of valinomycin plus nigericin inhibited Leu-Leu-Leu uptake, whereas the uncoupler carbonyl cyanide–*m*-chlorophenylhydrazone (1 μM) did not. Monensin, which converts the ΔpH into a chemical sodium gradient, did not significantly lower tri-leucine transport at pH 6.5 and in the presence of 5 mM sodium ions (data not shown). These results are at variance with the involvement of a proton or sodium motive force as the driving force for the DtpP system.

To investigate further the nature of the driving force of di- and tripeptide transport by DtpP, the effect of the ATPase inhibitor *o*-vanadate was studied. Since ATP production in *L. lactis* by the glycolytic pathway is affected by *o*-vanadate (11), the arginine deiminase pathway was used for the generation of metabolic energy. The addition of arginine led to a rapid uptake of tetra-alanine, tri-leucine, and leucine (Fig. 2A to C, respectively) in *L. lactis* AG300 cells. The addition of *o*-vanadate did not influence leucine uptake (Fig. 2C), which was expected since leucine transport is driven by the proton motive force (2). In contrast, arginine-energized tri-leucine uptake (Fig. 2B) and tetra-alanine uptake (Fig. 2A) were partly inhibited by *o*-vanadate. This finding suggests that tri-leucine uptake via DtpP, as tetra-alanine uptake via Opp (11), is most likely driven by ATP or a related energy-rich phosphorylated intermediate.

Expression of DtpP in *L. lactis* CV4. The activity of the second di- and tripeptide transport system (DtpP) varied with the growth conditions. The rate of Leu-Leu-Leu uptake increased two- to threefold when CDM was used instead of the complex medium M17 to culture the cells (Fig. 3). A further two- to threefold increase in the transport rate was observed when Leu-Leu or Leu-Leu-Leu was present in CDM, suggesting that these peptides serve as inducers of the DtpP system. Similar results were obtained with Leu-Leu as the substrate of the transport system (data not shown).

pH dependence of DtpP-mediated transport. The external pH dependence of Met-Met uptake was measured in glycolyzing *L. lactis* CV4 cells (Fig. 4). Met-Met uptake showed a broad optimum range from pH 5.5 to 7.5. Below pH 4, no significant Met-Met uptake was detected. The highest rates of Met-Met uptake were observed between pHs 6 and 7. The broad optimum pH range for DtpP indicates that this system is operative in the physiological pH range as observed for the DtpT and Opp transport systems (24).

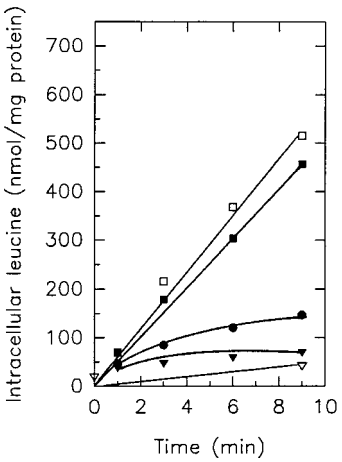

FIG. 3. Leu-Leu-Leu uptake in glucose-metabolizing *L. lactis* CV4 cells. Cells were grown in M17 complex medium (▼) or in CDM containing 3.6 mM Leu (●), 1.8 mM Leu-Leu (■), or 1.2 mM Leu-Leu-Leu (□). Transport assays were started by the addition of 0.5 mM Leu-Leu-Leu. Uptake in the absence of glucose (△) is also shown.

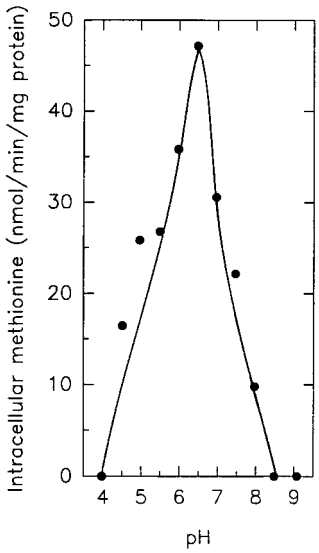

FIG. 4. Effect of external pH on Met-Met uptake (0.5 mM) in glucose-metabolizing *L. lactis* CV4 cells. Prior to the transport assays, *L. lactis* cells were de-energized as described in Materials and Methods. For the transport assays, cells were incubated in a solution containing 20 mM potassium succinate–20 mM potassium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–20 mM Tris–2 mM $MgSO_4$ (pH 4 to 9).

DISCUSSION

The presence of a second di- and tripeptide transport system in *L. lactis* is indicated by the residual di- and tripeptide uptake in strains lacking DtpT (with or without functional Opp) and the complete loss of di- and tripeptide transport activity in the Δ*dtpT* FCA^r mutant. The accumulation of the amino acids present in the peptides was clearly not due to external breakdown of the peptides followed by transport of the amino acids since (i) no free amino acids were detected in the external medium, (ii) the accumulation of amino acids was observed only with transportable peptides, the nontransported peptides were hydrolyzed upon permeabilization of the cells, and (iii) the di- and tripeptide transport activity was completely lost in the Δ*dtpT* FCA^r mutant, the peptidase activities in the wild-type strain and in the Δ*dtpT* FCA^r mutant were similar. DtpT and the newly described DtpP system have overlapping specificities for di- and tripeptides but do not transport amino acids or oligopeptides (Table 2 and results not shown) (11, 25, 26). The DtpT carrier transports relatively hydrophilic di- and tripeptides such as Glu-Val, Gly-Asp, Met-Asp, Ala-Gly, and Pro-Gly (6, 26; also this study), whereas DtpP transports a variety of structurally different di- and tripeptides, with an apparent preference for hydrophobic (branched-chain) residues.

The presence of two di- and tripeptide systems and one oligopeptide transport system, as observed in *L. lactis*, is also observed in the enteric bacteria (for a review, see reference 15). The oligopeptide permeases (Opp) of *Escherichia coli* and *Salmonella typhimurium* handle any peptide containing two to six amino acids, whereas the Opp system of *L. lactis* transports peptides of four to at least eight amino acid residues (29). The dipeptide permeases (Dpp) of *E. coli* and *S. typhimurium* are relatively specific for dipeptides, whereas the tripeptide permeases (Tpp) possess the highest affinity for hydrophobic tripeptides. The specificity of Tpp mimics that of DtpP in *L. lactis*. The peptide transport systems of enteric bacteria belong to the family of binding-protein-dependent transport systems that are composed of multiple subunits and use (most likely) ATP to drive transport (7). The Opp system of *L. lactis* also belongs to this family of transporters (29). On the contrary, the di- and tripeptide carrier (DtpT) of *L. lactis* is a secondary transport system which uses the proton motive force to accumulate peptides (6, 25). The DtpP system described in this study resembles the ATP-dependent peptide transporter of *E. coli* and *S. typhimurium* as well as the Opp system of *L. lactis* with regard to energy coupling to transport. For instance, transport is inhibited by *o*-vanadate and not by valinomycin under conditions in which Δp is composed solely of ΔΨ. The inhibition of peptide transport by nigericin plus valinomycin is most likely caused by effects other than the dissipation of the Δp, as the combination of both ionophores also affects the intracellular concentration of potassium ions, the turgor pressure, the ATP pool, the internal pH, and other physiologically relevant parameters (11, 14, 18, 21). Furthermore, dissipation of Δp will result in a rapid efflux of amino acids and, thus, in a lower level of accumulation of amino acids following peptide uptake (data not shown). The inhibition of DtpP, and likewise of Opp, by *o*-vanadate required preincubation of arginine-metabolizing cells with the inhibitor for at least 15 min (accumulation of the inhibitor by the phosphate uptake system). Even then, the inhibition of DtpP and Opp was incomplete, most likely as a result of competition between *o*-vanadate and the intracellular phosphate pool (ca. 80 mM [22]) and/or reducing activities in the cell which lower the concentration of the active oxidized vanadyl ion (3).

DtpP transport activity is higher when di- or tripeptides, i.e., substrates of the transport system, are present in the culture medium instead of amino acids or a complex nitrogen source. The regulation of peptide transport in bacteria has been addressed in a number of reports, and most transport systems appear to be regulated and expressed maximally when substrate is present (1, 8). The role of Opp in the uptake of cell wall peptides in *S. typhimurium* provides an explanation for the constitutive expression of the oligopeptide permease, since cell wall peptide substrates need to be recycled under all conditions of growth (5, 8). The central role of the oligopeptide transport system in the proteolytic pathway of *L. lactis* has recently been demonstrated (10). The need for di- and tripeptide transport systems in *L. lactis* is less apparent, since di- and tripeptides are not released from β-casein by the proteinase PrtP (9). However, growth of *L. lactis* in medium containing a mixture of caseins requires the presence of a functional di- and tripeptide transport system(s) (26). Although small peptides have not been detected in the degradation of β-casein by PrtP (9), it has been suggested that at least one essential amino acid is taken up in the form of a di- or a tripeptide which could be released from casein species other than the β form (e.g., κ-casein) (20, 23).

In *L. lactis*, peptide uptake is accompanied by rapid intracellular hydrolysis and the selective exodus of amino acids when the pools become excessively high. The high level of hydrophobicity of some amino acids can lead to passive leakage from the cell (2, 19). In addition, if the concentration of an amino acid exceeds the driving force imposed by the carrier mechanism, the amino acid may also leave the cell by facilitated diffusion. Therefore, the estimated uptake rates based on the concentrations of intracellular amino acids will always be underestimates of the actual peptide uptake rate (29). It is unlikely that amino acid loss as a result of protein synthesis affects the estimation of the peptide uptake rates, since amino acid incorporation into trichloroacetic acid-precipitable material is hardly or not at all observed under these conditions (15a). Furthermore the rates of peptide uptake are similar in the presence and absence of chloramphenicol.

In conclusion, the presence of a third lactococcal peptide transport system was demonstrated with the use of single and double transport mutants of *L. lactis*. The newly described transporter DtpP is specific for di- and tripeptides that are composed of amino acids with relatively hydrophobic side chains and requires ATP or a related energy-rich, phosphorylated intermediate to drive the peptide uptake.

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