Diversity of Oligopeptide Transport Specificity in Lactococcus lactis Species

A TOOL TO UNRAVEL THE ROLE OF OppA IN UPTAKE SPECIFICITY*

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The specific oligopeptide transport system Opp is essential for growth of Lactococcus lactis in milk. We examined the biodiversity of oligopeptide transport specificity in the L. lactis species. Six strains were tested for (i) consumption of peptides during growth in a chemically defined medium and (ii) their ability to transport these peptides. Each strain demonstrated some specific preferences for peptide utilization, which matched the specificity of peptide transport. Sequencing of the binding protein OppA in some strains revealed minor differences at the amino acid level. The differences in specificity were used as a tool to unravel the role of the binding protein in transport specificity. The genes encoding OppA in four strains were cloned and expressed in L. lactis MG1363 deleted for its oppA gene. The substrate specificity of these engineered strains was found to be similar to that of the L. lactis MG1363 parental strain, whichever oppA gene was expressed. In situ binding experiments demonstrated the ability of OppA to interact with non-transported peptides. Taken together, these results provide evidence for a new concept. Despite that fact that OppA is essential for peptide transport, it is not the (main) determinant of peptide transport specificity in L. lactis.

The oligopeptide transport system Opp has been described in many bacteria. This transport system may be involved in (i) nutrient acquisition in Lactococcus lactis (1, 2) or Streptococcus thermophilus (3); (ii) recycling of cell wall peptides for peptidoglycan synthesis in Salmonella typhimurium and Escherichia coli (4); (iii) sensing of extracellular signaling molecules (pheromones) required for the initiation of competence and sporulation in Bacillus subtilis (5–8), for the induction of conjugation in Enterococcus faecalis (9, 10), and for the induction of virulence in several pathogenic bacteria (11–14); and (iv) growth at low temperatures and intracellular survival in macrophages of Listeria monocytogenes (15).

Opp is a member of a superfamily of highly conserved ATP-binding cassette transporters. In Gram-negative bacteria, the transporter comprises a periplasmic solute-binding protein (OppA) and a translocon consisting of two integral membrane proteins (OppB and OppC) and two membrane-bound cytoplasmic ATP-binding proteins (OppD and OppF). In Gram-positive bacteria, OppA proteins are lipoproteins anchored to the cell membrane by their N-terminal lipid moiety. Although several copies of the gene encoding the binding protein might be present in Gram-positive bacteria (3, 16), only one seems to be functional in L. lactis (17, 18). OppA serves as an initial receptor. It binds the substrate and delivers it to the transmembrane complex. It is generally considered as the specificity determinant of the system, whereas the rate of peptide transport is imposed by the rate of peptide donation from OppA to the OppBC complex (19). The ATP-binding proteins couple ATP hydrolysis to the transport process.

The substrate specificity of Opp from S. typhimurium has been well established. S. typhimurium Opp transports peptides from two to five amino acids with a broad range of sequences (20). S. typhimurium OppA has a higher affinity for tripeptides than for dipeptides (21). The Gram-positive bacterium L. lactis shows significant differences in peptide uptake and affinity compared with S. typhimurium. First of all, L. lactis MG1363 is able to transport oligopeptides containing up to 18 amino acids (22). Its binding protein preferentially interacts with nonameric peptides, but is able to bind peptides containing up to 35 amino acid residues (23). Nevertheless, the strain preferentially uses hydrophobic basic peptides with molecular masses ranging between 600 and 1100 Da (24), whereas di- and tripeptides are not transported by the L. lactis Opp system (16, 25).

In this work, we compared the ability of different strains of L. lactis to transport oligopeptides. In the L. lactis species, we demonstrate the existence of variability in both the specificity of peptide transport and the amino acid sequence of L. lactis OppA (OppA<sub>Ll</sub>).<sup>1</sup> In an attempt to correlate these diversities, we expressed the different OppA<sub>Ll</sub> proteins in an oppA mutant with its native oppDFBC operon still functional. The ability of these engineered strains to transport peptides was compared with that of the corresponding wild-type strains. We reveal that, although OppA<sub>Ll</sub> was essential for peptide transport function, there was no correlation between the sequence of the binding protein and the specificity of peptide transport. These results suggest a role for the OppBCDF component in imposing the specificity of peptide transport in L. lactis.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Growth Conditions—The bacterial strains used in this study are listed in Table I. L. lactis strains were...

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1 The abbreviations used are: OppA<sub>Ll</sub>, L. lactis OppA; CDM, chemically defined medium; HPLC, high pressure liquid chromatography.
was isolated from a 2-ml culture grown overnight in M17 broth. Cells of *E. coli* lacking lactose.

(Bio-Tek Instruments, Inc., Winooski, VT) using 96-well sterile microplates. The same restriction enzymes. The resulting hybrid plasmids (pLEM1 to pLEM7) were confirmed by restriction digestion and DNA sequencing. After extraction from *E. coli* (34), the pLEM derivatives were transferred into *L. lactis* SL5145 by electroporation as previously described (35). Transformsants were selected on M17 agar medium supplemented with 0.5% (w/v) lactose, 5 mg/liter erythromycin, and 5 mg/liter chloramphenicol.

**Purification of *L. lactis* Wg2 OppA-His<sub>6</sub> Recombinant Protein and of OppA-His<sub>6</sub> Control of Lactococcal Lac Promoter.—Ery<sub>β</sub> expressed in *E. coli* was induced with 0.5 mM IPTG for 3 hours. The inclusion bodies were purified by ammonium sulfate precipitation at 30% saturation followed by gel filtration on a Sephadex G-25 column.

**Expression of the OppA-His<sub>6</sub> protein was carried out in 500 ml of ice-cold ethanol and finally resuspended in 200 μl of 10 mM Tris buffer.

- **OppA** genes from several lactococcal strains (17) were cloned into expression vector pQEW, which was then introduced into *E. coli* NM522, yielding ** Table I**

**Strains and plasmids used in this study**

| Strain/plasmid | Characteristics | Source/Ref. |
|---------------|-----------------|-------------|
| **E. coli** | | |
| NM522 | *supE thi-1 (lac-proAB) Δ(mcrB-hsdSM) 5(r<sup>−</sup> m<sup>−</sup>) F<sup>−</sup> proAB, lacIqZΔM15* | Amersham Biosciences |
| 8163 | NM522/pQEW | This work |
| MC1022 | araD1398Δ(araE Lav) 7697Δ(lacZΔ14)M15 galU galV StrA | This work |
| 8159 | MC1022pLM1 | This work |
| 8170 | MC1022pLM2 | This work |
| 8179 | MC1022pLM3 | This work |
| 8180 | MC1022pLM4 | This work |
| **L. lactis** | | |
| MG1363 | Plasmid-free, Lac<sup>+</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | Ref. 27 |
| Wg2 | Wild-type, Lac<sup>+</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| IL1403 | Plasmid-free, Lac<sup>+</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | Ref. 17 |
| CNRZ437 | Wild-type, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | INRA-URLGA |
| CNRZ226 | Wild-type, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | NIZO* |
| E8 | Wild-type, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | NZDRI |
| MG3<sup>+</sup> | MG1363/pILpOL, pMG820, pLET5, Lac<sup>+</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| AMP15 | MG1363, oppAα, Lac<sup>−</sup>, Prt<sup>−</sup> | Ref. 28 |
| SL5145 | AMP15/pILpOL, pMG820, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| SL5146 | AMP15/pILpOL, pMG820, pLET5, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| SL5147 | AMP15/pILpOL, pMG820, pLEM1, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| SL5152 | AMP15/pILpOL, pMG820, pLEM2, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| SL5174 | AMP15/pILpOL, pMG820, pLEM3, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| SL5175 | AMP15/pILpOL, pMG820, pLEM4, Lac<sup>−</sup>, Prt<sup>−</sup>, Opp<sup>−</sup> | This work |
| **Plasmid** | | |
| pQE30 | Vector of His<sub>6</sub>-tagged protein expression | QIAGEN S. A. |
| pQEW | pQE30 derivative carrying oppA gene from *L. lactis* Wg2 | This work |
| pLET5 | E. coli-L. lactis expression vector; Cm<sup>R</sup> | Ref. 29 |
| pLEM1 | pLET5 derivative carrying oppA gene from *L. lactis* MG1363 | Ref. 27 |
| pLEM2 | pLET5 derivative oppA gene from *L. lactis* Wg2 | This work |
| pLEM3 | pLET5 derivative oppA gene from *L. lactis* IL1403 | This work |
| pLEM4 | pLET5 derivative oppA gene from *L. lactis* CNRZ437 | This work |
| pLPOL | 8.3-kb pAM61 derivative carrying gene coding for T7 RNA polymerase under control of lactococcal lac promoter; Ery<sub>β</sub> | Ref. 29 |
| pMG820 | 23.7-kb derivative of pLP712 containing lac genes | Ref. 30 |

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urea according to the manufacturer’s instructions. The OppA-His6 containing fractions, as determined by SDS-PAGE analysis, were dialyzed against sterile water and lyophilized.

Anti-OppA antibodies were purified from the serum of an immunized rabbit (Valbiex-Université Claude Bernard Lyon I, Villeurbanne, France) as described above, except that the resin was additionally washed with 40 ml of 50 mmol/liter Tris and 150 mmol/liter NaCl (pH 7.4) and then with 40 ml of 50 mmol/liter Tris and 1 mol/liter MgCl2. The elution fractions were collected, dialyzed against sterile water and lyophilized.

Immunoblotting—L. lactis protein extracts were first separated by SDS-12% polyacrylamide gel electrophoresis and then electrotransferred onto nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The OppA protein was detected by the method of Harlow and Lane (36) using anti-OppA polyclonal antibodies (diluted 1:220), peroxidase-conjugated anti-rabbit IgG (diluted 1:4000; Sigma, Saint-Louis, MO), and Lane (36) using anti-OppA polyclonal antibodies (diluted 1:220), peroxidase-conjugated anti-rabbit IgG (diluted 1:4000; Sigma, Saint-Louis, MO), and visualized using chemiluminescence detection substrate kit (Roche Molecular Biochemicals, Meylan, France).

**Milk Peptide Purification**—Milk proteins were precipitated with 1% (v/v) trifluoroacetic acid. After removal of the proteins by centrifugation at 10,000 × g for 10 min at 4 °C, the supernatant was ultrafiltered through a 3000-Da cutoff membrane (YM3, Amicon, Inc., Beverly, MA). The peptides were isolated by solid-phase extraction using reverse-phase cartridges (Sep-Pak C18, Waters Associates, Milford, MA). The peptides were separated at 40 °C by HPLC on a reverse-phase C18 column (Nucleosil (250 × 4.6 mm), Colochrom, Gagny, France) at a flow rate of 1 ml/min. Solvents A and B were 0.115% (v/v) trifluoroacetic acid and 0.1% (v/v) trifluoroacetic acid and 60% (v/v) acetonitrile in MilliQ water, respectively. A 5-min isocratic phase in solvent A was followed by a linear gradient of solvent B (0–60% within 40 min). The collected fractions were submitted to a second separation using 5 mmol/liter KH2PO4/K2HPO4 (pH 6.9) and then de-energized for 30 min at 30 °C. The OppA-His6 containing fractions, as determined by SDS-PAGE analysis, were dialyzed against sterile water and lyophilized.

**Results**—The ability of lactococcal strains to bind peptide VGDG was estimated at 30 °C as follows. Concentrated de-energized cells (A650 – 15) were incubated for 2 min (23) in 50 mmol/liter KH2PO4/K2HPO4 (pH 6.5) containing 500 μmol/liter VGDG. Cells were collected on a 0.22-μm pore size filter (Schleicher & Schull) and washed three times with ice-cold potassium phosphate buffer. The filter was then coated for 2 min with a solution of peptide YGGFL (500 μmol/liter) in potassium phosphate buffer. After removing cells by filtration, the peptides contained in the buffer were concentrated by solid-phase extraction using an anion cartridge exchanger (Accell Plus QMA, Waters Associates) and analyzed by HPLC as described above.

**Peptide Utilization by Different Strains of L. lactis**—Six strains of L. lactis were grown in CDM lacking an essential amino acid in the free form and supplied by a pure peptide. None of the strains was able to grow when the omitted amino acid was not replaced by a peptide. Twenty-five different peptides were selected on the basis of their various biochemical characteristics and their origin (Table II). Six of them were purified from milk. They were initially chosen because of their

**RESULTS**

**Peptide Utilization by Different Strains of L. lactis**—Six strains of L. lactis were grown in CDM lacking an essential amino acid in the free form and supplied by a pure peptide. None of the strains was able to grow when the omitted amino acid was not replaced by a peptide. Twenty-five different peptides were selected on the basis of their various biochemical characteristics and their origin (Table II). Six of them were purified from milk. They were initially chosen because of their
disappearance from milk after growth of some of the strains (data not shown).

Most peptides (18 of 25) were able to sustain growth of the six strains at a maximal rate in CDM deprived of one essential amino acid in the free form and provided in peptide form (Fig. 1). Growth systematically corresponded to consumption of the peptide, as revealed by HPLC analysis of the culture medium (data not shown). Nevertheless, except for CNRZ261, none of the strains was able to use all the tested peptides as a source of amino acids. Five peptides (VGDE, DRVYIHPFHL, RPKPQQFFGLM, ISQRYQK, and LPQY) were differently consumed by the six \textit{L. lactis} strains, suggesting that the strains under study do not share the same preferences for peptide utilization. Moreover, \textit{L. lactis} Wg2 grew very poorly in the presence of the basic heptapeptide ISQRYQK as the source of Gln or Ile, whereas this strain grew at a maximal rate in the presence of the basic heptapeptide YPFPGPI (source of Ile) or TVYHQHK (source of Gln). This indicates that previous observations made with \textit{L. lactis} MG1363, which indicated a preference for peptide utilization related to both the mass and the charge of the peptide (24), cannot be extended to the \textit{L. lactis} species.

Variability in Peptide Utilization Corresponds to Variability in Peptide Transport — The peptides used for growth experiments were incubated with cell-free extracts. They were all cleaved at the amino acid level. The lack of growth of some strains in CDM was therefore not due to an inability of the cells to cleave the peptide intracellularly. The peptides were also incubated either in the presence of the P$_1$-type proteinase PrtP released from \textit{L. lactis} E8, CNRZ261, and Wg2 by incubation in a Ca$^{2+}$-free buffer (39) or in the presence of P$_{III}$-type PrtP anchored to resting \textit{L. lactis} CNRZ437 cells (note that autolysin of P$_{III}$-type PrtP affects its specificity) (40). Only one of them (RPKPQQFFGLM) was cleaved by PrtP. Other peptides were not hydrolyzed. This suggests that most (if not all) of the differences observed during growth experiments were not due to a difference in extracellular cleavage of the peptide by PrtP. Consequently, the most convenient hypothesis to explain the differences in growth is that the strains under study do not have identical oligopeptide transport capabilities.

To ascertain this hypothesis, we tested the ability of four of
the strains to transport three peptides that revealed a difference in preferences for peptide utilization between strains (VGDE, DRVYIHPFHL, and RPKPQQFFGLM). To prevent extracellular cleavage of RPKPQQFFGLM by PrtP, uptake was performed in the presence of 1 mM 4-(2-aminoethyl)benzene-sulfonyl fluoride. As expected, the ability of the strains to grow
correlated with their ability to transport the peptides (Table III). By analyzing the presence of free Val or free Met in the external medium, we have experimentally excluded the possibility that differences in peptide uptake rates could be due to differences in amino acid efflux rates between strains. Despite the fact that VGDE ensured a maximal growth rate of *L. lactis* Wg2 or CNRZ437, a large difference in the initial rate of uptake was observed between the two strains. On the other hand, IL1403 did not grow at a maximal rate in the presence of VGDE, although its initial rate of VGDE uptake was higher than that of Wg2. This indicates that the rate of peptide transport did not determine the growth rate, at least under our experimental conditions.

**The Binding Protein OppA Is Not Responsible for Variability in Transport Specificity**—The binding protein is generally considered to be responsible for the specificity of peptide transport in ATP-binding cassette transporters (19). The oppA genes from *L. lactis* strains MG1363, Wg2, and CNRZ437 were therefore cloned and sequenced. Comparison of the deduced amino acid sequences, including those available in the GenBank™/EBI Data Bank (SSL135, IL1403, and SK11), revealed some differences at the amino acid level (Fig. 2). The OppA sequence of MG1363 was identical to that of SSL135, but differed from those of Wg2, CNRZ437, IL1403, and SK11 by 9, 10, 72, and 2 residues, respectively.

In an attempt to assign the differences in peptide transport pattern to the variations in the OppA amino acid sequence, the oppA genes from four different strains were introduced into the OppA-defective strain AMP15 (28). The oppA genes were cloned under the control of the T7 promoter into plasmid pLET5 (29). To ensure the expression of oppA, the two plasmids pILpOL and pMG820 were also introduced in the recipient strain, yielding *L. lactis* strains SL5147, SL5152, SL5174, and SL5175, carrying the oppA genes from MG1363, Wg2, IL1403, and CNRZ437, respectively (Table I). As controls, plasmid pLET5 free of cloned oppA plus pILpOL and pMG820 were introduced in MG1363 and AMP15, yielding MG3° and SL5146, respectively. The growth rates and maximal bacterial populations in milk of strains MG1363, MG3°, and SL5147 were similar (1.6 ± 0.1 h⁻¹ and (1.0 ± 0.1) × 10⁸ colony-forming units/ml, respectively). The amounts of OppA expressed by SL5147, SL5152, SL5174, and SL5175 grown in the appropriate medium (CDM containing 5 g/liter glucose as the carbon source, 2.5 g/liter lactose as the inducer (29), and 2.5 mg/liter each erythromycin and chloramphenicol) were in the same range and slightly lower in each case than that produced by *L. lactis* MG1363 as revealed by Western blot analyses (Fig. 3). As a last control, no significant differences in the uptake of the control peptide YGGFL by MG1363 and MG3° or SL5147 could be detected, whereas SL5146 was unable to transport peptides.

As expected, the properties of peptide transport by SL5147 exactly matched those of MG1363. Surprisingly, *L. lactis* SL5152, SL5174, and SL5175, carrying the oppA genes from Wg2, IL1403, and CNRZ437, respectively, had the same preferences for peptide utilization as MG1363 (Fig. 4). For example, strains SL5152, SL5174, and SL5175 were unable to grow in CDM containing VGDE as the source of Val, whereas the corresponding *L. lactis* wild-type strains Wg2, IL1403, and CNRZ437, respectively, grew. Moreover, the growth rates of SL5152, SL5174, SL5175, and MG1363 in CDM containing RPKQQFFGLM as the source of Met were comparable, although Wg2, IL1403, and CNRZ437 were unable to use this peptide as the source of Met (Fig. 1). It is worth mentioning that *L. lactis* strains SL5152, SL5147, SL5174, and SL5175 are Prt° strains and therefore unable to cleave peptides in the external medium prior to transport.

Uptake experiments confirmed that *L. lactis* strains SL5152, SL5174, and SL5175 transported the same peptides despite their expression of different OppA proteins (Table IV). The ability of the engineered strains to transport specific peptides was identical to that of MG1363. Initial rates of uptake were slightly lower than those obtained with the wild-type strain. This was presumably due to a lower expression level of the binding protein in the engineered strains compared with MG1363 (Fig. 3). The complementation of MG1633oppA with the binding protein isolated from *L. lactis* strain Wg2, IL1403, or CNRZ437 restored the substrate specificity of *L. lactis* MG1363 rather than that of the OppA donor strain.

**OppA₄₅ Is Able to Bind a Non-transported Peptide**—The ability of the binding protein OppA from *L. lactis* strain MG1363 to bind *in situ* non-transported peptides was estimated. De-energized cells were first incubated in the presence of the non-transported peptide VGDE (loading step). After extensive washing, cells were then incubated in the presence of YGGFL (chase step). The removal of VGDE from OppA by YGGFL was estimated by submitting the chase buffer to HPLC analysis after concentrating its peptide content by solid-phase extraction. No VGDE could be detected when YGGFL was omitted from the chase buffer. Similarly, only traces of VGDE were detected when using *L. lactis* AMP15, indicating that this strain did not bind a significant amount of VGDE during the loading step. In contrast, *L. lactis* MG1363 released ~0.35 nmol of VGDE/mg of protein in the presence of YGGFL (mean of three determinations, S.D. = 0.02) (Fig. 5). These results demonstrate the ability of *L. lactis* MG1363 OppA to interact *in situ* with a non-transported peptide.

**DISCUSSION**

Previous studies of substrate specificity in peptide transport and/or utilization by *L. lactis* were performed using only one strain, MG1363 (22, 24). The results demonstrated that the substrate specificity of *L. lactis* strain MG1363 is rather atypical compared with other model Gram-negative bacteria such as *E. coli* and *S. typhimurium* (20, 21). The present study provides evidence for variability in the ability of *L. lactis* strains to consume peptides as a source of amino acids during growth. For instance, MG1363 was unable to grow using the tetrapeptide VGDE as the source of Val. This result is in agreement with the established preferences for peptide utilization by this strain, i.e., rejection of acidic peptides with low molecular mass (24). However, this peptide was utilized by all the other strains under study. Consequently, the MG1363 preferences are not representative of the genus *Lactococcus*. We were able to distinguish four strains on the basis of their capabilities to use several peptides. This variability in peptide...
consumption resulted from a difference in the ability of the strains to transport peptides. The specificity of peptide transport by L. lactis strain MG1363 did not mirror that of all the lactococcal strains. Our first original conclusion is that the L. lactis genus shows a biodiversity in oligopeptide transport specificity.

Oligopeptide utilization by L. lactis requires the presence of the functional oligopeptide transport system Opp. Previous
studies indicated that the binding protein OppA dictates the specificity of the Opp system (28, 41). As proof, a ΔoppA mutant was shown to be unable to transport peptides, and mutagenesis of OppA affected the overall specificity of the protein for peptides (28).

The biodiversity in peptide transport specificity in lactococci was used as a tool to unravel the role of the binding protein OppAII in determining the substrate specificity of L. lactis. Comparison of the OppAII sequences, including those of the four strains studied, showed some amino acid substitutions. To determine to what extent these substitutions were responsible for the specificity, we cloned and expressed the oppA genes of the four strains in the recipient strain L. lactis MG1363ΔoppA (strain AMP15) (28). Complementation by different oppA genes restored all functional Opp hybrid transporters. This suggests that none of the substitutions in the different OppAII proteins impaired donation of the substrate from the binding protein to the transmembrane channel of MG1363. The substrate specificity of the four engineered strains was found to be identical to that of L. lactis strain MG1363. The substrate specificity of the complemented L. lactis strains SL5152, SL5174, and SL5175 did not match that of the corresponding wild-type strains (Wg2, IL1403, and CNRZ437, respectively).

These intriguing results question the role of OppAII in the specificity of peptide transport. If a peptide is not bound by OppAII, it will not be transported by the Opp system. In this respect, OppAII might be considered as one determinant of peptide transport specificity. Nevertheless, the specificity of peptide binding by OppAII is very broad (23). The use of recombinant strains allowed us to establish that the inability of lactococci to transport specific peptides was not due to peptide exclusion by OppAII. In other words, the specificity of peptide transport (i.e., the ability to ultimately transport specific peptides or not) is not solely dependent on OppAII. The broad binding specificity of OppAII excludes it from being the major specificity determinant of oligopeptide transport. Our second original conclusion is that, although OppAII is absolutely necessary for peptide transport, it does not (exclusively) determine its specificity.

Complementary results corroborate this interesting observation. Binding experiments indicated that the tetrapeptide VGDE was able to interact in situ with the oligopeptide-binding protein of L. lactis MG1363. This observation was in agreement with the reported competitive inhibition exerted by VGDE on the transport of a reporter peptide by L. lactis MG1363.2 This indicates that a peptide could bind to OppAII even if it is not transported by the Opp system. These results further support our conclusion, i.e., the specificity of peptide uptake by L. lactis is not exclusively dictated by OppAII.

The proposed model for oligopeptide transport by L. lactis is a four-step process: (i) reversible binding of the substrate to the open form of the binding protein; (ii) conformational change of the binding protein, resulting in the partial entrapment of the substrate; (iii) transfer of the partially entrapped substrate from the binding protein to the transmembrane complex; and (iv) translocation of the substrate across the membrane (23, 43). The peptide VGDE was translocated by the three L. lactis strains Wg2, IL1403, and CNRZ437 at different rates (Table III), although its K_m value was in the same range for the three strains (250 μmol/liter). This result indicates that the first step of transport, peptide binding by OppA, is not a limiting step for the transport process. It is in agreement with previous kinetic analyses that demonstrated that the rate of transport is determined by the kinetics of peptide donation from the binding protein to the translocator complex (19). In this kinetic model, the binding protein would act as a plug that blocks the ligand from returning to the external medium (44). A consequence of the peptide binding would be to transmit a signal via the transmembrane complex to the ATP subunits that results in an increase in the transporter affinity for ATP and subsequently leads to the opening of the translocation pore and the concomitant release of the substrate from the binding protein.

Transmembrane proteins OppB and OppC have been described as important actors in several bacterial phenomena. For example, bacterial adherence can be affected by mutations in the binding protein and other domains of the permease complex (42, 45). Indeed, the adherence of Streptococcus gordonii is affected by mutating either the binding protein (SarA) or OppC (42). One possibility is that the transmembrane complex OppBC acts as a filter. It is worth mentioning that the three available lactococcal opp sequences display variability in the OppB and OppC sequences (16–18). This sequence variability might explain the transport variability among L. lactis strains. If this hypothesis is correct, it remains to be shown how the transmembrane complex participates in the specificity of the oligopeptide transport process (e.g., exclusion of specific peptides from the channel or impairment of the interaction of the transmembrane complex with the binding protein liganded with specific peptides).

Our results show that the specificity of oligopeptide transport in L. lactis is determined by at least two successive filters. The first filter, OppA, captures oligopeptides and initiates the transport process, but is rather aspecific. Our further work will focus on the second filter, presumably the transmembrane channel, and will aim at identifying the ensemble of determinants involved in oligopeptide transport specificity in L. lactis.

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Diversity of Oligopeptide Transport Specificity in Lactococcus lactis Species: A TOOL TO UNRAVEL THE ROLE OF OppA IN UPTAKE SPECIFICITY
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