# N-Acyl-(α,γ Diaminobutyric Acid)$_n$ Hydrazide as an Efficient Gene Transfer Vector in Mammalian Cells in Culture

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***Purpose.*** This study investigates the structure/activity relationship of a series of N-acyl-peptides (lipopeptides) for the transfection of mammalian cells.

***Methods.*** Lipopeptides comprising 1 to 3 basic amino-acids and a single fatty acid chain were synthesized. Transfecting complexes between lipopeptide, plasmid DNA and dioleoyl phosphatidylethanolamine were prepared and applied on cells in culture. Transfection efficiency was evaluated by measuring β-galactosidase activity 48 h post-transfection. Lipopeptide-DNA binding was also investigated by physical means and molecular modelling.

***Results.*** Besides the length of the fatty acid chain, the nature of the basic amino-acid and the C-terminal group were crucial parameters for high transfection efficiency. The N-acyl-(diaminobutyric acid)n derivatives were the most potent transfecting agents among those tested and induced a β-galactosidase activity 2 to 20 times higher than the N-acyl-lysine, -ornithine or -diaminopropionic acid derivatives. Furthermore, a hydrazide C-terminal modification greatly enhanced transfection efficiency for all compounds tested. The reason why α, γ-diaminobutyric acid hydrazide-based lipopeptides were the most potent in transfection is not fully understood but could be related to their high DNA binding.

***Conclusions.*** Poly- or oligo-diaminobutyric acid containing or not a hydrazide C-terminus could advantageously be used in peptide-based gene delivery systems.

**KEY WORDS:** transfection; gene therapy; peptide; diaminobutyric acid; hydrazide; DNA.

## INTRODUCTION

One of the central challenge in gene therapy is to find safe vectors capable of transporting genes efficiently into the target cells. Among non-viral vectors, cationic amphiphiles are widely used to deliver plasmid DNA into mammalian cells both *in vitro* and *in vivo* (1,2). Upon interaction with a cationic amphiphile, plasmid DNA is coated by a cationic layer which subsequently interacts with the anionic surface of the cell, followed by the internalization of the transfecting complex (2–5). This concept of transfection was first introduced by Felgner et al., who developed the dioleoyloxypropyl trimethylammonium chloride (DOTMA) (3). Since then, many other cationic compounds have been synthesized to deliver DNA into cells (1,2). Cationic amphiphiles are usually composed of two distinct moieties: an hydrophilic, cationic moiety which interacts with DNA primarily by charge interactions and a lipophilic moiety (1,2,6). The cationic moiety is usually a single or multiple amine group with different degrees of substitution. Whether a mono- or a polycationic compound is preferable is still unclear but seems to vary with the nature of the positively charged moiety as well as the type of cell to be transfected. The fatty moiety is usually a single or double fatty acid chain, comprising between 12 and 18 carbons each, saturated or not, or a cholesterol derivative (1,2,6). Nevertheless, the structure/activity relationship of cationic amphiphiles for gene transfer is still poorly understood.

In the aim of designing simple peptide-based gene delivery systems and exploring the features needed for a transfection-competent molecule, a series of lipophilic short cationic peptides has been investigated for gene transfer. The schematic structure of these compounds, referred as lipopeptides, is:

$$R_1\text{-NH-(amino-acid)n-CO-}R_2$$

where $R_1$ is a single fatty acid chain, $R_2$ an alkoxy or hydrazine substitution of the carboxyl group of the terminal amino-acid and n indicates the number of amino-acid residues (1, 2 or 3). Originally designed as biodegradable, non-toxic antibiotics, molecules of this type contain at least one basic amino-acid: lysine, ornithine, α,γ-diaminobutyric acid (Dab) or α,β-diaminopropionic acid (Dap) (7). We found that these molecules, in conjunction with a helper lipid and complexed with plasmid DNA could efficiently transfect mammalian cells in culture. However, large differences in transfection efficiency were observed between the various compounds tested. Beside the length of the fatty acid chain, the nature of the basic amino-acid and the C-terminal group were crucial parameters for transfection efficiency. The N-acyl-Dab derivatives were the most potent transfecting agents among those tested and showed a transfection efficiency 2 to 20 times higher than the N-acyl-lysine, -ornithine or -Dap derivatives. Furthermore, a hydrazide C-terminal modification greatly enhanced transfection efficiency for all compounds tested.

## MATERIALS AND METHODS

### Synthesis of the Lipopeptides

The lipopeptides were synthesized mainly as previously described (7). For example, Nα-palmitoyl-(L,Dab-L,Dab) hydrazide was synthesized as follows: the dipeptide construct was prepared by solid phase peptide synthesis using a polystyrene resin, functionalized with a hydroxymethylphenoxyacetic acid linker (8). The base-labile 9-fluorenylmethoxycarbonyl (Fmoc) group was used for temporary protection of the α-amino group (9). The side chain function of α,γ-Dab was blocked by the (tert-butoxy)carbonyl (Boc) protecting group. For activation of the amino-acid derivative, 1,1,3,3-tetramethyl-2-(2-oxo)-1(2H)-pyridyl-uronium tetrafluoroborate (TPTU) was used (10). Diisopropylcarbodiimide and dimethylamino-

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**ABBREVIATIONS:** CMC, critical micelle concentration; Dab, α,γ diaminobutyric acid; Dap, α,β diaminopropionic acid; DOTAP, dioleoyloxy-trimethylammonium propane.



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pyridine were used for the esterification of Fmoc-Dab (Boc)-OH to the Wang resin. Palmitoic acid was then coupled to the resin bound dipeptide. The palmitoyl peptide was cleaved in the protected form by hydrazinolysis and deprotected with 4N HCl in 1,4-dioxane. The compound was finally precipitated with ether, washed and dried. Ion spray mass spectrometry measurements were performed to ensure the identity and the purity of the compound.

### Preparation of the Transfecting Complex

Plasmid DNA, pCH110 (Promega) containing the β-galactosidase reporter gene under the control of the SV40 promoter was grown using standard techniques and purified by column chromatography (Qiagen, Germany). Plasmid concentration was measured by UV absorption at 260 nm. Phospholipids were obtained from Avanti Polar Lipids Inc. (Alabaster, USA). Cholesterol hemisuccinate (CHEMS) was purchased from Sigma. Dioleoyl phosphatidylethanolamine (DOPE), dioleoyl phosphatidylcholine (DOPC), DOPE:CHEMS 2:1 mol/mol and DOPE:dicapryloyl phosphatidylcholine 3:1 mol/mol dispersions were prepared by drying the lipids from chloroform stock solutions and then rehydrating the film with 30 mM Tris Cl pH 8.5. The final lipid concentration was 1 mM. Five μg of plasmid DNA were diluted in 100 μl of sterile distilled water in a polystyrene tube and 5–8 μl of the freshly-prepared lipopeptide aqueous solution (2 to 5 mM) were added. Then, the lipid dispersion was slowly added and the final complex was gently mixed. A typical complex corresponded to a 2/1 lipopeptide/DNA +/− charge ratio and a 5/1 DOPE/lipopeptide molar ratio. 1,2-bis(oleoyloxy)-3-(trimethylammonio) propane (DOTAP, Avanti Polar Lipids Inc.) liposomes were prepared as previously described (11,12) and used at a 2/1 +/− charge ratio with plasmid DNA. Complexes were prepared just prior to transfection experiments.

### Transfection Experiments

COS-1 (monkey kidney fibroblasts) cells were plated at a density of about $2 \times 10^4$ cells per well in 96-well plates and grown 24 h in 10% fetal calf serum (FCS, Gibco BRL) containing medium. Transfection took place in FCS free Dulbeco's modified Eagle medium (DMEM) and 5 h later medium was removed and replaced by 10% FCS containing medium. 0.2 or 1 μg of plasmid DNA was applied per well. Forty-eight h later, β-galactosidase activity was measured. After medium removal, 50 μl of 250 mM Tris Cl buffer pH 8 containing 0.5% of Triton X-100 were added per well. Cells were frozen at −70°C, then thawed at 37°C and 50 μl of phosphate buffer saline were added per well. Finally, 150 μl of a 2 mg/ml solution of o-nitrophenyl galactopyranoside (Sigma) in 60 mM $Na_2HPO_4$, 1 mM $MgSO_4$, 10 mM KCl and 50 mM β-mercaptoethanol were added per well. Optical density at 405 nm was measured after incubating the plate anywhere between 20 min and 1 h at 37°C, depending on the β-galactosidase activity. Values were computed from a β-galactosidase (Fluka) standard curve. Day-to-day β-galactosidase activity values usually varied by about 2 fold depending upon cell density and condition of the cells. Sensitivity of the assay was 50 μUnits of β-galactosidase per well of COS-1 cells.

### Physico-Chemical Characterization of Lipopeptides

pKa values of lipopeptides were measured by potentiometric titration in water containing 0.1 M $KNO_3$ as background electrolyte. Critical micelle concentration (CMC) of lipopeptides in water was determined by fluorescent dye solubilization (12) using nile red as the fluorescent probe.

### Ethidium Bromide Displacement Assay

The binding between lipopeptides and DNA was estimated by ethidium bromide (EtBr) displacement assay (14). Twenty μg of pCH110 plasmid were mixed with 1.5 μg of EtBr in 2 ml of water. Then, increasing amounts of lipopeptides were added in order to displace EtBr. The EtBr displacement was monitored by recording the decrease of EtBr fluorescence (λexc = 475 nm; λem = 595 nm). Fluorescence of EtBr alone was substracted from each fluorescence value and results are displayed as the percentage of the maximum (fluorescence of EtBr/DNA complex). At the concentrations used in the experiment, lipopeptides alone had no influence on EtBr fluorescence.

### Molecular Modelling

The evaluation of possible DNA-lipopeptide interactions was also done with our in-house molecular modelling software package MOLOC (15) on a Silicon Graphics Indy workstation. Reference structures were obtained from the nucleic acid database at Rutgers University (16).

## RESULTS

### Transfection of COS-1 Cells Using N-acyl-(lysine)n Lipopeptides

Since lysine is a commonly used amino-acid in peptide-based gene delivery systems, lysine-containing lipopeptides were first studied for transfection. In the absence of a helper lipid, N-acyl-(L,Lys)n/DNA complexes did not mediate any detectable transfection of COS-1 cells (data not shown). When DOPE was added to the lipopeptide/DNA complex, the resulting transfecting particles induced detectable β-galactosidase activity 48 h after incubation. However, the level of transfection was strongly dependent upon the structure of the lipopeptide.

The influence of the fatty acid chain on transfection was first studied and either a palmitoyl ($C_{16}$) or a capryloyl ($C_8$) or a stearoyl ($C_{20}$) chain was used to synthesize the lipopeptides. The Nα-stearoyl-(L,Lys-L,Lys) methyl ester mediated a similar transfection as the Nα-palmitoyl-(L,Lys-L,Lys) methyl ester, whereas the Nα-capryloyl-(L,Lys-L,Lys) methyl ester did not transfect cells (data not shown). Then, the role of the number of amino-acids forming the lipopeptide was investigated. At a 2/1 lipopeptide/DNA +/− charge ratio and a 5/1 DOPE/lipopeptide molar ratio, Nα-palmitoyl peptide derivatives containing 1, 2 or 3 lysine residues induced a similar β-galactosidase activity (Figure 1). In contrast, the C-terminal modification had a strong influence on the gene transfer efficiency of the lipopeptide. The presence of a hydrazide C-terminal modification enhanced transfection efficiency 4 fold as compared to the free, the amidified or the esterified carboxyl end group (Figure 2).

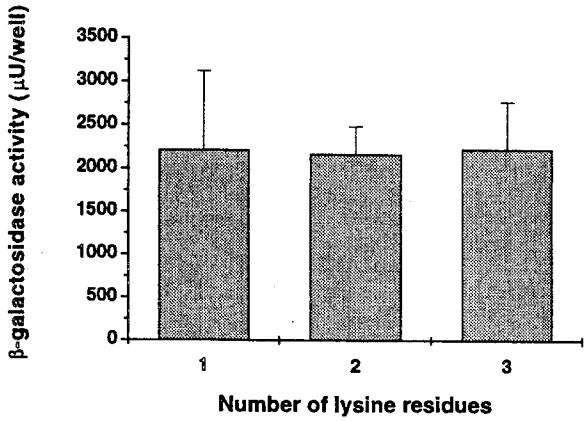



**Fig. 1.** Influence of the number of lysine residues forming the lipopeptide on transfection efficiency. Nα-palmitoyl-(L,Lys)n hydrazide lipopeptides (with n = 1, 2 or 3) were complexed with pCH110 plasmid and DOPE was added to the various complexes. Transfecting complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 DOPE/lipopeptide molar ratio. One μg of DNA was applied per well of COS-1 cells. Results are the mean ± SD of 12 transfecting wells in 3 separate experiments.

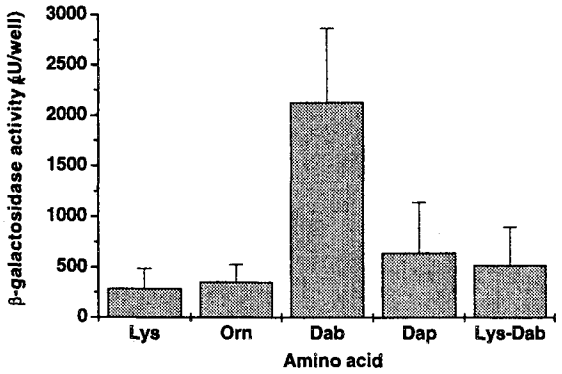



**Fig. 3.** Influence of the nature of the amino-acid on transfection efficiency. Nα-palmitoyl-(L,amino-acid-L,amino-acid) methyl ester lipopeptides were complexed with pCH110 plasmid and DOPE was added to the various complexes. Transfecting complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 DOPE/lipopeptide molar ratio. One μg of DNA was applied per well of COS-1 cells. Results are the mean ± SD of 8 transfecting wells in 2 separate experiments.

## Transfection of COS-1 Cells with Other Basic Amino-Acid-Based Lipopeptides

In addition to lysine-based lipopeptides, other Nα-palmitoyl-(L,amino-acid-L,amino-acid) lipopeptides containing either ornithine or Dab or Dap were investigated for transfection. These amino-acids only differ by the number of carbons of the amino side-chain. Lysine, ornithine, Dab and Dap have a 4, 3, 2 and 1 carbon long amino side-chain, respectively. Unexpectedly, Nα-palmitoyl-(L,Dab-L,Dab) methyl ester mediated the highest transfection among the compounds tested (Figure 3). Methyl ester derivatives of Nα-palmitoyl-(L,Lys-L,Lys), Nα-palmitoyl-(L,Orn-L,Orn) and Nα-palmitoyl-(L,Dap-L,Dap) mediated transfection levels 4 to 8 times lower than the one induced by Nα-palmitoyl-(L,Dab-L,Dab) methyl ester. When the Nα-palmitoyl-(L,Dab-L,Lys) methyl ester was used for transfection, β-galactosidase activity was again reduced as compared to the palmitoyl-(L,Dab-L,Dab) derivative. A non-basic amino-acid-based lipopeptide, the Nα-palmitoyl-(L,Glu-L,Glu) methyl ester did not lead to any detectable transfection (data not shown).

Furthermore, the higher transfection efficiency of Dab-based lipopeptides was also observed when a hydrazide C-terminal modification was present (Figure 4). When 1 μg of DNA was applied per well, Nα-palmitoyl-(L,Dab-L,Dab) was 7 and 4 fold more efficient than the Nα-palmitoyl-(L,Lys-L,Lys) for the methyl ester and the hydrazide derivatives, respectively. At a low amount of DNA (0.2 μg per well) only the Nα-palmitoyl-(L,Dab-L,Dab) hydrazide was able to mediate high transfection.

The superior transfection activity of Nα-palmitoyl-(L,Dab-L,Dab) hydrazide/DNA/DOPE complexes was also verified for a wide variety of formulations. At any amino-acid/DNA base/DOPE molar ratio tested, Nα-palmitoyl-(L,Dab-L,Dab) hydrazide-based complexes mediated a higher transfection than the complexes containing Nα-palmitoyl-(L,Lys-L,Lys) hydrazide (Figure 5).

Transfection efficiencies of Nα-palmitoyl- or Nα-oleoyl-(L,Dab-L,Dab) hydrazide/DNA/DOPE complex and DOTAP/DNA complex were compared in COS-1 cells. Both at high (1 μg) or low (0.2 μg) dose of DNA per well, lipopeptides and DOTAP induced a similar β-galactosidase activity (Table I). Other cell-lines including CV-1, HepG2, C2E12, CHO-K1 and HeLa were also successfully transfected using Dab hydrazide-based lipopeptides with transfection rates similar to those mediated by DOTAP (data not shown).

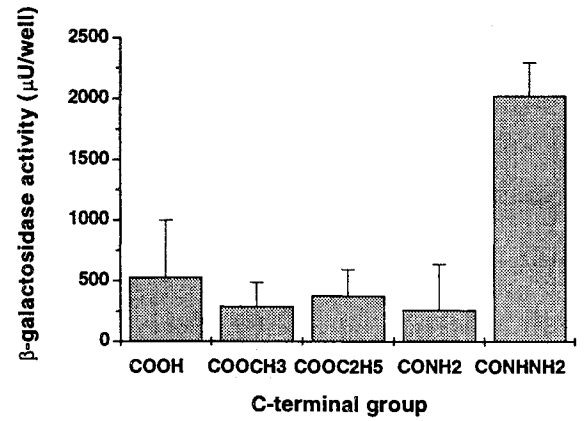
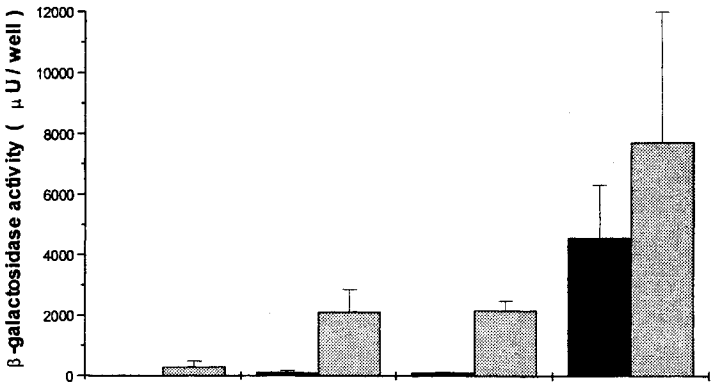



**Fig. 2.** Influence of the C-terminal group on transfection efficiency of lipopeptides. Nα-palmitoyl-(L,Lys-L,Lys) lipopeptides with various C-terminal modifications were complexed with pCH110 plasmid and DOPE was added to the various complexes. Transfecting complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 DOPE/lipopeptide molar ratio. One μg of DNA was applied per well of COS-1 cells. Results are the mean ± SD of 8 transfecting wells in 2 separate experiments.

## Influence of the Helper Lipid

To examine the importance of the DOPE in the transfection process we used other lipids to form the transfecting complex (Figure 6). When DOPE was replaced by DOPC, transfection was almost abolished. Addition of CHEMS or dicapryloyl PC




| DNA dose (μg/well) | 0.2 | 1 | 0.2 | 1 | 0.2 | 1 | 0.2 | 1 |
|---|---|---|---|---|---|---|---|---|
| amino-acid | Lys | Lys | Dab | Dab | Lys | Lys | Dab | Dab |
| C-terminus | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | $NHNH_2$ | $NHNH_2$ | $NHNH_2$ | $NHNH_2$ |

**Fig. 4.** Comparison of the transfection efficiency of various lipopeptides as a function of the dose of DNA, the nature of the amino-acid forming the lipopeptide and the C-terminal modification of the lipopeptide. Nα-palmitoyl-(L,amino-acid-L,amino-acid) lipopeptides were complexed with pCH110 plasmid and DOPE was added to the various complexes. Transfecting complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 DOPE/lipopeptide molar ratio. Results are the mean ± SD of 12 transfecting wells in 3 separate experiments.

**Table I.** Transfection of COS-1 Cells with Dab-Based Lipopeptides or Cationic Lipids[a]

| | 0.2 μg DNA per well | 1 μg DNA per well |
|---|---|---|
| Nα-palmitoyl-(L,Dab-L,Dab) hydrazide | 4584 ± 1742 | 7731 ± 4224 |
| Nα-oleoyl-(L,Dab-L,Dab) hydrazide | 3280 ± 822 | 7294 ± 1969 |
| DOTAP | 4802 ± 1265 | 8050 ± 1911 |

[a] Lipopeptide/DNA/DOPE complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 DOPE/lipopeptide molar ratio. DOTAP was used at a 2/1 +/− charge ratio with DNA. COS-1 cells were transfected in a 96-well plate. Results are expressed as β-galactosidase activity at 48 hr in μUnits per well. Results are the mean ±SD of 12 transfecting wells in 3 separate experiments.

($C_8$PC) to the DOPE dispersion reduced about 3 fold transfection efficiency.

## Physico-Chemical Characterization of Lipopeptides

Determination of the pKa of the amino groups of the Nα-palmitoyl-(L,amino-acid-L,amino-acid) lipopeptides revealed no significant difference between the pKa of the ε amino group of the lysine-based lipopeptide (pKa = 9.30) and the pKa of the γ amino group of the Dab-based compound (pKa = 8.85). The terminal hydrazide group of both compounds had a pKa < 2 and therefore was not charged at physiological pH. Measurement of the critical micelle concentration (CMC) of Nα-palmitoyl-(L,amino-acid-L,amino-acid) lipopeptides in water indicated that neither the nature of the amino-acid nor the C-terminus had an influence on the CMC. CMC values were ranging from 0.8 to 0.9 mM, depending on the compound.

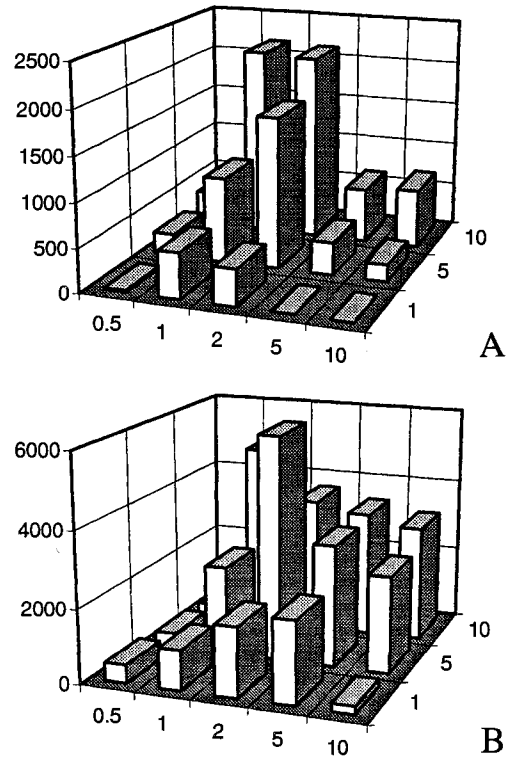



**Fig. 5.** Transfection efficiency of Nα-palmitoyl-(L,Lys-L,Lys) hydrazide (panel A) and Nα-palmitoyl-(L,Dab-L,Dab) hydrazide (panel B) at different lipopeptide/DNA +/− charge ratio (x axis) and DOPE/lipopeptide molar ratio (y axis). Transfecting complexes were applied on COS-1 cells (1 μg of pCH110 plasmid per transfecting well) and β-galactosidase activity (μUnits per well, z axis) was measured 48 h later. Results are the mean of 4 transfecting wells in a single experiment. Please note the different scale of the z axis between both charts.

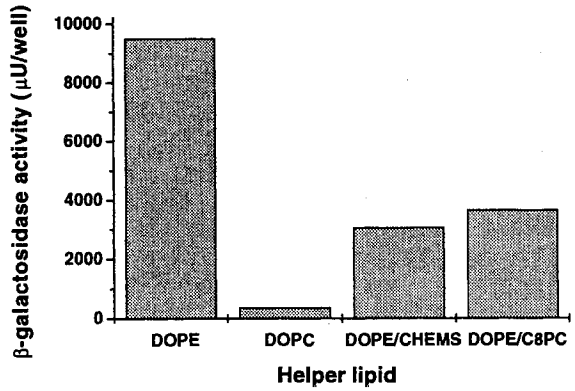



**Fig. 6.** Influence of the helper lipid on transfection efficiency of lipopeptides. Nα-palmitoyl-(L,Dab-L,Dab) hydrazide was complexed with pCH110 plasmid and various helper lipids were added to the lipopeptide/DNA mixture. Transfecting complexes were prepared at a 2/1 +/− lipopeptide/DNA charge ratio and a 5/1 helper lipid/lipopeptide molar ratio. One μg of DNA was applied per well of COS-1 cells. Results are the mean of 4 transfecting wells in a single experiment.

Therefore, at a concentration of about 70 μM used to form a typical complex, lipopeptides should interact with DNA as monomers rather than micelles.

### Lipopeptide/DNA Interactions

EtBr displacement assay indicated that all lipopeptides sharply decreased fluorescence up to a 2/1 lipopeptide/DNA +/− charge ratio (Figure 7). This ratio was found optimal for transfection (Figure 5). Subtle differences in the residual fluorescence levels were observed between the different compounds under investigation. Only Nα-palmitoyl-(L,Dab-L,Dab) hydrazide was able to completely displace EtBr. Addition of DOPE to the Nα-palmitoyl-(L,Dab-L,Dab) hydrazide/DNA mixture yielded no further decrease in EtBr fluorescence (data not shown).

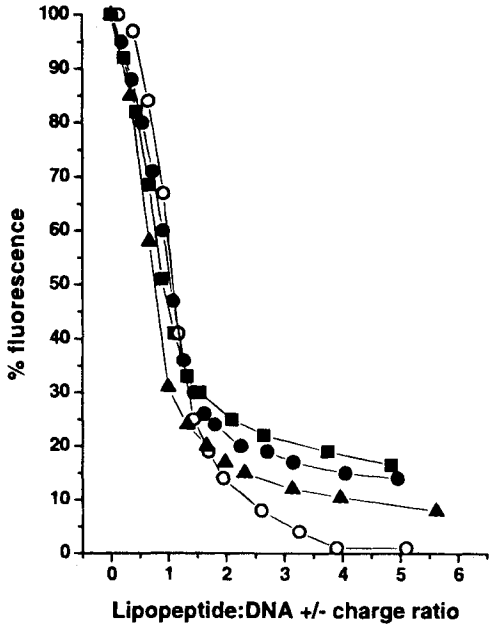



**Fig. 7.** Displacement of ethidium bromide from pCH110 plasmid by the lipopeptides: (■) Nα-palmitoyl-(L,Lys-L,Lys) methyl ester, (●) Nα-palmitoyl-(L,Lys-L,Lys) hydrazide, (▲) Nα-palmitoyl-(L,Dab-L,Dab) methyl ester, (○) Nα-palmitoyl-(L,Dab-L,Dab) hydrazide.

No X-ray structures of small peptide-DNA complexes are known so far. In order to investigate interactions between DNA and lipopeptides, we modelled a number of such DNA-lipopeptides complexes. It is not yet established to what DNA sequences such lipopeptides bind preferrably. Therefore, a number of different DNA sequences and start positions for lipopeptide molecules were chosen to optimize DNA-lipopeptides interactions. Best values were obtained with molecules localized in the minor groove. Indeed, short, basic molecules, such as spermine or short polyamines usually interact with the minor groove of the DNA (16,17). All amide protons of lipopeptides were engaged in H-bonds with DNA in low energy complexes. Hydrazide moieties were always involved in intensive H-bond contacts with DNA whereas no such strong interactions could be formed in the respective methylester compounds. This reflects a preference for hydrazides over methyl esters. Furthermore, the amino groups of amino acid side chains formed salt bridges with phosphate oxygens in all cases. The quality of these salt bridges as well as Van-der-Waals interactions between amino acid side chains and DNA depended on the length of the side chains. Best values were obtained for Dab whereas Dap, ornithine and lysine showed smaller interactions.

## DISCUSSION

Lipopeptides are simple molecules which can be synthesized by rather straightforward chemistry (7). Those compounds are readily biodegradable both chemically and enzymatically and therefore display a very low toxicity but also a low stability in solution (7). By modifying various building blocks of the molecule, we have been able to study the influence of several structural features of the molecule on its gene transfer efficiency. The presence of a basic amino-acid in the lipopeptide was required for gene transfer. Nevertheless, the nature of the basic amino-acid as well as the C-terminal modification had a strong influence on transfection. Dab-based lipopeptides/DNA/DOPE complexes mediated the highest β-galactosidase activity in COS-1 cells. Indeed, at low dose of DNA per transfecting well (0.2 μg), only lipopeptides containing Dab with a hydrazide C-terminal modification transfected the cells.

The reason for the superior transfection activity of Dab hydrazide-based lipopeptides is still not fully understood and could not be explained by the pKas or the CMC of the molecule. However, it may be possible that a high DNA-binding capability of Dab hydrazide-based lipopeptides accounts for part of their high transfection efficiency. Indeed, EtBr displacement assay indicated that Nα-palmitoyl-(L,Dab-L,Dab) hydrazide displayed a slightly higher affinity for DNA than other lipopeptides. Molecular modelling based upon an interaction of the lipopeptides with the DNA minor groove indicated that a hydrazide C-terminal modification was very favorable for an optimal interaction through hydrogen bonding. In addition, the length of the amino side-chain of the Dab was more favorable for phosphate oxygen interactions as well as Van-der-Waals interactions than that of other basic amino-acids. However, beside DNA binding, other factors such as the fate of the

lipopeptide in the cell or the action of the lipopeptide on cellular processes cannot be ruled out and could contribute to the higher efficiency of Dab-based compounds.

Beside the nature of the amino-acid and the C-terminal modification, the fatty acid chain also plays an important role in the transfection process. Indeed, shortening the acyl chain from 16 to 8 carbons completely abolishes transfection. Like other transfection-competent molecules, lipopeptides must therefore possess a fatty moiety of a certain length allowing an optimal interaction with the DOPE and/or the cellular membranes (1,6,19). However, introducing a double-bond in the chain, i. e. replacing the palmitoyl by an oleoyl chain, did not modify transfection efficiency.

Lipopeptide-mediated transfection required the presence of a helper lipid and among the various helper lipids tested, the hexagonal phase-competent DOPE induced the highest transfection. DOPC, which cannot undergo a lamellar-hexagonal phase transition at 37°C, led to a very low transfection. This result suggests that DOPE plays a major role in the lipopeptide-mediated transfection process, perhaps by inducing membrane fusion (20,21). A similar observation has been made for other transfection systems, such as cationic liposomes or cyclic peptide/DNA complexes (22,23). However, in contrast to the gramicidin S-mediated transfection, addition of short-chain phospholipids did not enhance the level of transfection using lipopeptides (12). A pH-sensitive lipid composition, DOPE/CHEMS (4,27) did not improve lipopeptide-mediated gene transfer.

Previous examples of peptide-based gene delivery systems were usually based upon high-molecular-weight polylysine (24). This study demonstrates that L,Dab-L,Dab binds DNA and forms a transfection competent molecule when coupled to a lipophilic chain. Polylysine plays different roles in the transfecting complex. As well as binding and condensing DNA (24), polylysine serves as a scaffold for additional effectors, such as a lipophilic chain (25) or a targeting ligand (26) and interacts with cellular membranes (24). It remains to be seen whether L,Dab-L,Dab shares these various functions. Nevertheless, oligo- or poly-Dab hydrazide could provide a valuable alternative to polylysine in various peptide-based gene delivery systems.

In conclusion, this study allowed us to explore the influence of various features of peptide-based molecules on their transfection efficiency. The findings that Dab and/or an hydrazide C-terminus could greatly enhance gene delivery should allow the design of peptide-based molecules with higher transfection activity than existing systems.

## ACKNOWLEDGMENTS

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