A novel class of cationic *gemini* surfactants showing efficient *in vitro* gene transfection properties

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Five novel peptide-based cationic *gemini* surfactants have been synthesised and their ability to transfect plasmid DNA containing the luciferase gene has been examined. Three of these detergents, differing in the number of positive charges per molecule at neutral pH, mediated transfection on their own. However, their efficiency increased markedly on the addition of a neutral colipid and a basic polypeptide.

Over the last ten years various cationic lipids have been synthesised for complexation with DNA and the *in vitro* delivery of genes to mammalian cells, leading to successful expression of the corresponding proteins.[1–3] The hydrophobic 'tails' of the majority of these cationic detergents consist of one or two saturated or mono-unsaturated hydrocarbon chains containing 16 to 18 carbon atoms; examples are cetyltrimethylammonium bromide (CTAB) and 2,3-dioleoyloxy-*N*-{2-[1,4-bis(3-aminopropymino)-2-butylcarboxamido]ethyl}-*N*,*N*-dimethyl-1-propylammonium bromide (DOSPA).[4]

The polar 'head-groups' of cationic lipid vectors have generally consisted of monovalent quaternary ammonium salts (as in CTAB and 1,2-dioleoyloxypropyl-*N*,*N*,*N*-trimethylammonium chloride, DOTAP). However, lipids such as DOSPA with a multivalent 'head-group' have been found to transfer genes *in vitro* more efficiently than the monovalent analogues.[5]

We report a novel class of non-viral gene-transfer vectors based on *gemini* surfactants (Fig. 1). Unlike several cationic lipids used in gene transfection studies, GS1 to GS5 *gemini* surfactants are solids, easy to handle and readily soluble in aqueous media over a wide range of pH. We use a thioether rather than the disulfide linkage for reasons of chemical stability. However, the amide linkages in the 'head-groups' and between the 'head-groups' and the hydrophobic alkyl chains confer a degree of biodegradability, reducing the potential cytotoxicity of these detergents.

Complexation of cationic lipids with DNA is a key design factor for these vehicles. The binding interaction between the cationic *gemini* molecules and DNA was confirmed by agarose gel electrophoresis studies (Fig. 2). Various concentrations of *gemini* surfactants were incubated with the pCMV-luciferase

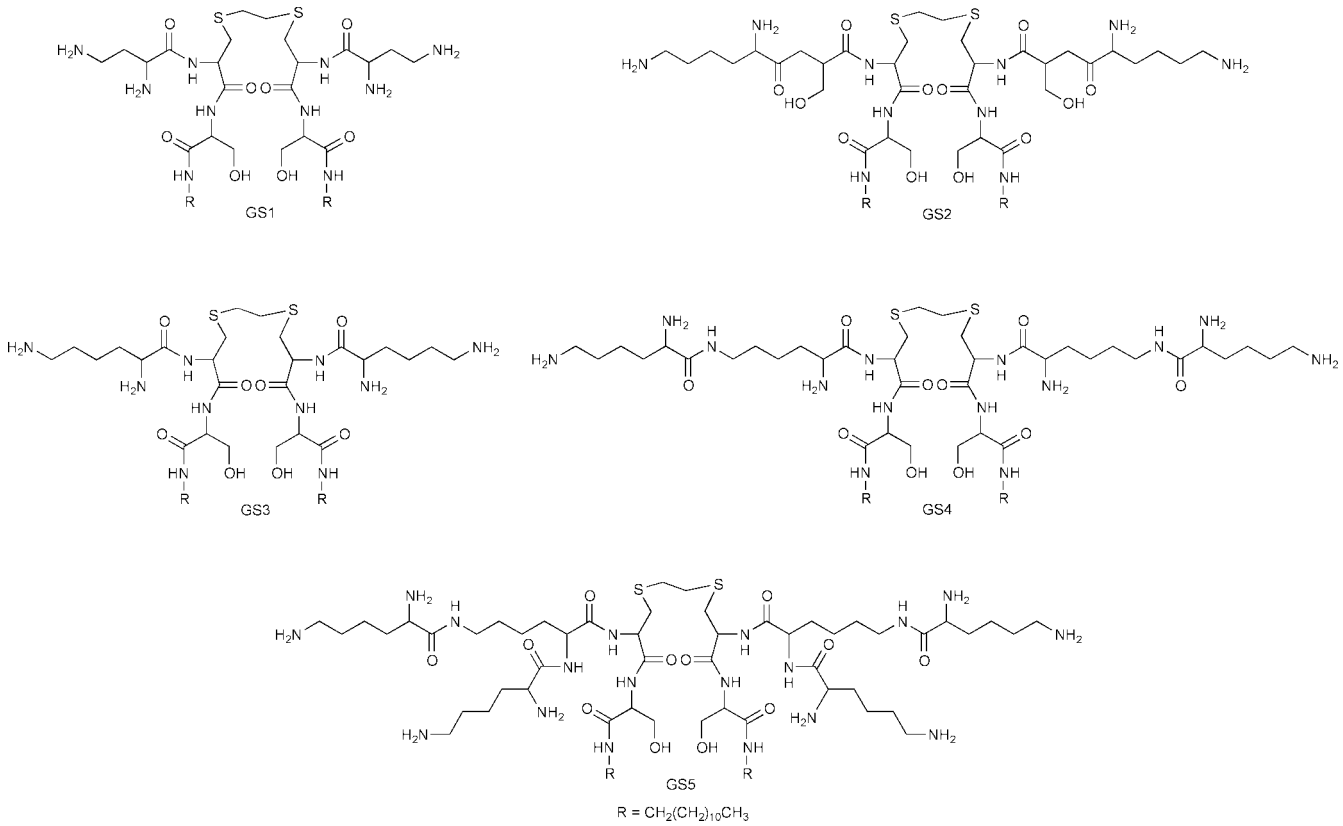

Fig. 1 Chemical structures of *gemini* surfactants GS1 to GS5. All details on the synthesis of these surfactants are provided in patent application, WO 99-29712 (SmithKline Beecham plc).

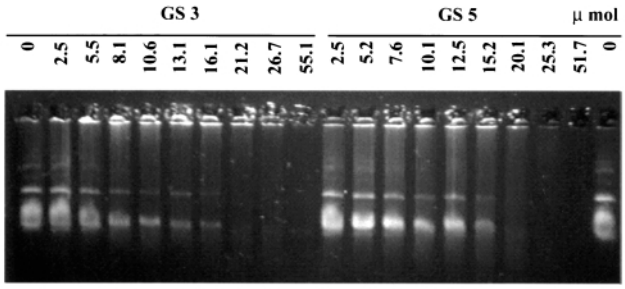

Fig. 2 Interaction of *gemini* surfactants with DNA in the presence of ethidium bromide. Agarose gel electrophoresis assay with GS3 and GS5.

plasmid DNA complexed with ethidium bromide.† These mixtures were then electrophoresed in agarose gel, and subsequently visualised under ultraviolet light. These experiments indicate that all five molecules were effective in releasing the nucleic acid from the DNA–ethidium bromide complex, consistent with binding of the cationic *gemini* to the DNA.

Relative transfection efficiencies for surfactants GS2, GS4 and GS5 are shown in Fig. 3. GS2 and GS5 are most efficient at about 10 μM, whereas for *gemini* GS4 the optimal concentration is slightly higher, at 13 μM. Concentrations used in the transfection experiments are well below the cmc values (0.3 ± 0.1 mM) so that the formation of micelles or liposomes (multilamellar forms) does not appear to be necessary for transfection to occur. Although the electrostatic nature of complex formation of these *gemini* molecules with DNA is likely to be important for complexation with DNA, it is not the only factor that influences gene transfection. Thus surfactants GS1 and GS3 complex with DNA, but do not mediate transfection. It may be that the complexes formed by these two surfactants are too strong to release DNA at a critical point, or that interactions other than electrostatic contribute to transfection efficiency.

It is well known that the DNA transfection efficiency of cationic lipids improves in the presence of a neutral colipid ('helper') and/or a basic polypeptide.[6,7] Thus LIPOFECTAMINE PLUS™ (Life Technologies), one of the leading commercial products for *in vitro* gene transfection, consists of a mixture of DOSPA, dioleoylphosphatidylethanolamine

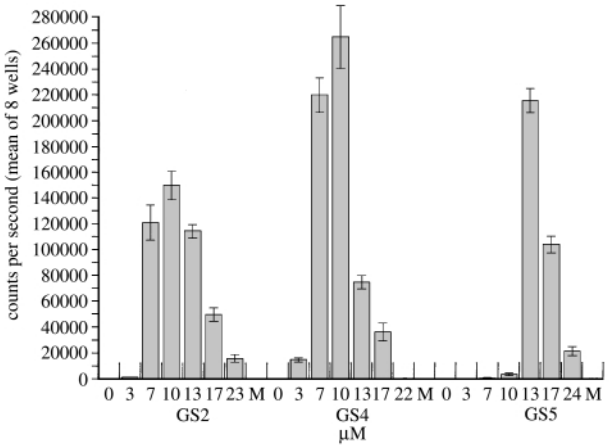

Fig. 3 Transfection experiment in CHO-K1 cells. The cells were incubated overnight at 37 °C with DNA and different concentrations of GS2, GS4 and GS5 or GS without DNA (M). Luciferase activity was measured using the stable light signal reporter gene assay and the counts per second from eight wells were averaged.

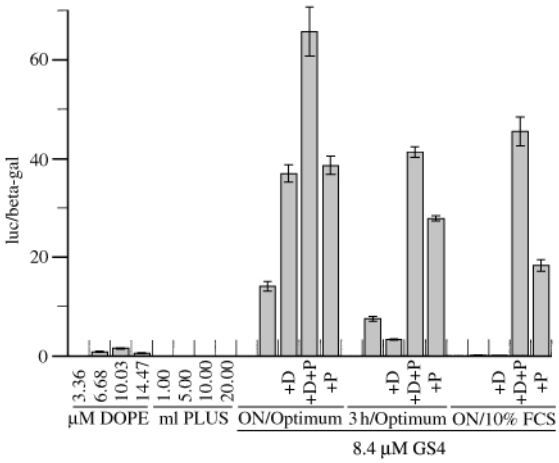

Fig. 4 Transfection experiment in CHO-K1 β-gal cells. The cells were incubated in Opti-MEM® or complete medium with the transfection mixtures overnight (ON) or 3 h at 37 °C. GS4 was used alone or in combination with DOPE (+D), with 'PLUS'-reagent (+P) or in a combination of both (+D+P). The luciferase activity of eight wells was normalized against the corresponding β-galactosidase activity. As controls, various amounts of DOPE and 'PLUS'-reagent alone were incubated with DNA and used for transfection (over night/Opti-MEM®).

(DOPE) and a basic peptide. The transfection efficiencies of GS4 alone and in the presence of (i) DOPE, (ii) the 'Plus' reagent (a basic peptide) and (iii) DOPE and the 'Plus' reagent is shown in Fig. 4. Addition of either the neutral lipid or the basic peptide increases transfection efficiency more than twofold. However, the biggest increase in efficiency occurs when both neutral lipid and basic peptide are added to GS4. It is remarkable that overnight incubation of the DNA with serum in the presence of GS4, DOPE and the 'Plus' reagent gave the maximum protection from degradation by serum nucleases. A similar result was reported recently for the transfection vehicle 3β-[*N*-(*N*′,*N*′-dimethylaminoethyl)carbamyl]cholesterol in the presence of polylysine.[7] In the majority of experiments, the observed expression level with GS4 was of the same order of magnitude as that with LIPOFECTAMINE PLUS™ (data not shown).

Notes and references

† The IUPAC name for ethidium bromide is 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide.

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