

BIOPHYSICAL AND TRANSFECTION PROPERTIES OF DNA-DiC14-AMIDINE COMPLEXES

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DiC14-amidine (N-t-butyl-N'-tetradecylaminopropionamidine) is a cationic lipid used as transfection agent *in vitro* [1] and as a vector for *in vivo* gene therapy applications. DiC14-amidine molecules assemble in a liposomal structure (transition temperature: 23°C - critical micelle concentration: 10⁻⁷M). Compared to other cationic lipids, diC14-amidine does not require a helper lipid. This property significantly simplifies lipoplex preparations and facilitates a biophysical characterization of the lipid-DNA complex.

The interaction of diC14-amidine vesicles with plasmid DNA involves at least 2 steps that were detected by isothermal titration calorimetry. The first step is electrostatic and exothermic in nature and the second, slower step, is endothermic and leads to what is referred as a fused complex [2].

We have used X-ray diffraction to characterize the diC14-amidine-DNA complex. The microstructure of the complex was determined to be a layered type with DNA (in its B structure) sandwiched between lipid bilayers [3]. Within the complex, the lipid chains are tilted with respect to the lamellar plane normal and/or are interdigitated across the bilayer mid-plane. Within the nucleic acid containing layers, DNA polymers run parallel to one another. Some aspects of the transfection activity of diC14-amidine complexes will be discussed more thoroughly.

Preinjection of free diC14-amidine liposomes inhibits the inflammatory response to cationic lipid-DNA complexes injected intravenously in mice and enhance their transfection efficiency (A. Elouahabi et al. "Free cationic liposomes inhibit the inflammatory response to cationic lipid/DNA complex injected intravenously and enhance its transfection efficiency" Human Gene Therapy -submitted). This improvement was correlated with the ability of free diC14-amidine liposomes to inhibit TNF- α but not IFN- γ production resulting from complex injection.

Very few studies have described the immunogenicity of antigens formulated with cationic lipids. Preliminary experiments carried out in our group with recombinant proteins suggest that diC14 amidine can be considered as a Th1/Th2 adjuvant; it shifts Th2 immune response towards Th1.

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