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Stimuli responsive polymorphism of C_{12}NO/DOPE/DNA complexes: Effect of pH, temperature and composition

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ABSTRACT

N,*N*-dimethyldodecylamine-*N*-oxide (C_{12}NO) is a surfactant that may exist either in a neutral or cationic protonated form depending on the pH of aqueous solutions. Using small angle X-ray diffraction (SAXD) we observe the rich structural polymorphism of pH responsive complexes prepared due to DNA interaction with C_{12}NO/dioleoylphosphatidylethanolamine (DOPE) vesicles and discuss it in view of utilizing the surfactant for the gene delivery vector of a pH sensitive system. In neutral solutions, the DNA uptake is low, and a lamellar L_α phase formed by C_{12}NO/DOPE is prevailing in the complexes at $0.2 \leq C_{12}NO/DOPE < 0.6$ mol/mol. A maximum of ~30% of the total DNA volume in the sample is bound in a condensed lamellar phase L_α^C at C_{12}NO/DOPE = 1 mol/mol and pH 7.2. In acidic conditions, a condensed inverted hexagonal phase H_{II}^C was observed at C_{12}NO/DOPE = 0.2 mol/mol. Commensurate lattice parameters, $a_{HC} \approx d_{LC}$, were detected at $0.3 \leq C_{12}NO/DOPE \leq 0.4$ mol/mol and pH = 4.9–6.4 suggesting that L_α^C and H_{II}^C phases were epitaxially related. While at the same composition but pH ~ 7, the mixture forms a cubic phase (*Pn3m*) when the complexes were heated to 80 °C and cooled down to 20 °C. Finally, a large portion of the surfactant ($C_{12}NO/DOPE > 0.5$) stabilizes the L_α^C phase in C_{12}NO/DOPE/DNA complexes and the distance between DNA strands (d_{DNA}) is modulated by the pH value. Both the composition and pH affect the DNA binding in the complexes reaching up to ~95% of the DNA total amount at acidic conditions.

1. Introduction

Complexes of DNA with cationic liposomes (CL) are intensively studied as potential non-viral vectors for gene therapy [1–4]. Cationic lipids and surfactants strongly interact with the polyanion of DNA due to electrostatic attraction and, also, due to the stabilization of DNA surfactant complexes through hydrophobic interactions between lipophilic moieties of surfactant molecules. The compensation of the negative charge of DNA by cationic species, allows the complex to approach the cytoplasmic membrane. In addition, cationic surfactants have been reported to collapse individual DNA molecules and to form small particles, which allow an efficient internalization of these complexes into the cells [5,6]. The transport mechanism of DNA into the cells includes several key steps. The complex internalized inside the cell by endocytosis has to escape from the endosome through the activated fusion with the endosomal membrane. After the escape from the endosome, DNA must be released by the dissociation of these complexes into the cytoplasm. Whereas for a successful escape from the endosome a high positive surface charge density of complexes is needed as it facilitates the fusion with the negatively charged endosomal membrane, for the dissociation of complexes into the cytoplasm a high surface charge density is undesirable as it increases their stability and prevents complex dissociation [7,8]. One of the ways to overcome this problem is the use of pH-sensitive surfactants with pK_a within the range of 4.5 to 8. These pH-sensitive surfactants are at acidic pH inside the endosomes in their cationic form, which enables the membrane fusion of the complexes with the endosome. At neutral pH, in the cytoplasm, they are mainly in their non-ionic form enabling an easy dissociation of the complex [9–11].

Relationships between the structure of gene delivery vectors and their transfection efficiency have been studied widely for many years, and the conclusions are not all consistent [12–14]. A condensed lamellar phase (L_α^C) with DNA strands packed regularly between cationic phospholipid bilayers [15] and an inverted hexagonal phase where DNA is arranged inside of tubules formed by inverted micelles and packed in hexagonal symmetry (H_{II}^C) [16] are the most discussed structures,

Abbreviations: SAXD, small angle X-ray diffraction; C_{12}NO, *N*,*N*-dimethyldodecylamine-*N*-oxide; DOPE, dioleoylphosphatidylethanolamine; HT-DNA, DNA from herring testes; L_α^C, condensed lamellar phase; H_{II}^C, condensed hexagonal phase; CL, cationic liposome

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although bicontinuous cubic phases [17,18] were also revealed in morphologies of gene delivery vectors. For the L_{α}^{C} phase, the optimal surface charge density of a cationic membrane ($\sigma_M \sim e/100$ Å^2) was found as a key parameter for the fusion of cationic liposome–DNA complexes with the endosomal membrane [7]. X-ray structural studies show that the rate of DNA release from lipoplexes as well as transfection activity correlate well with non-lamellar phase progressions observed in cationic–neutral lipid mixtures [12,14,19]. The transfection behavior of the inverted hexagonal H_{II}^{C} phase of cationic liposome–DNA complexes is independent of the membrane charge density.

N,*N*-dimethyldodecylamine-*N*-oxide ($C_{12}NO$) (Fig. 1) is a non-ionic surfactant in solutions with neutral pH. However a strong polar N – O bond with a high electron density on oxygen yields in the protonation of molecules ($C_{12}N^{+}OH$) at acidic pH (pK ~ 5) [20–23].

$C_{12}NO$ as an amphiphile incorporates into biological membranes and can induce changes of fluidity [24,25] and thickness [26–29] of lipid bilayers. At high concentration, $C_{12}NO$ destabilizes lipid bilayers, and forms non-bilayer phases and mixed micelles [30,31]. Generally, C_nNO (in CnNO, *n* is the number of carbons in the alkyl substituent) displays antimicrobial [25,32], immunomodulatory [33] or antiphotosynthetic activity [34]. Also functions of membrane proteins such as sarcoplasmic reticulum Ca^{2+}-ATPase are modulated [35]. However, all available information on amine oxides demonstrates their low-to-moderate level of toxicity [36–38].

The toxicity of *N*,*N*-dimethylalkylamine oxides expressed as their lowest, still effective concentration at which the growth of the microorganisms is inhibited, or the so called minimum inhibitory concentration (MIC) supports the statement above. For example, the MIC of $C_{12}NO$, the most effective of the C_nNOs, n = 6–18 homologous series, detected at *Escherichia coli* and *Staphylococcus aureus* was found 3–340 times higher [32,39] in comparison to gemini surfactant pentane-1,5-diyl-bis(dodecyldimethylammonium bromide) used in the liposomal DNA delivery vector in transfection experiments [40,41]. In absolute unit scale, Warisnoicharoen et al. [38] found 0.08 mg/ml (~350 μM) as the $C_{12}NO$ concentration that caused 50% cell death (i.e. IC_{50}) tested on human bronchial epithelium cells. Recently, 0.023 mg/ml of $C_{12}NO$ (100 μM) was recognized as the concentration causing the increase in mammalian cells' lethality [37].

Among the C_nNO homological series, $C_{12}NO$ is widely used in pharmaceutical and cosmetic formulations, as detergent in household dishwashing liquids and surface cleaners and in various areas of industry [36,37,42]. Due to its large use, the concentration of C_nNO in some rivers in Japan was monitored, and was found at 0.01–0.07 μg/l (<0.3 nM if related to $C_{12}NO$ only) as declared in the report of the Japan Soap and Detergent Association (JSDA, Tokyo, Japan), cited in Fukunaga et al. ([37] and references therein). For comparison, common concentrations of cationic surfactants in transfection experiments are in the range of 2–50 μM [43,44].

DNA interaction with $C_{12}NO$ or $C_{12}NO$/phospholipid is poorly reported in literature. The first experiments, focused on DNA coil–globule phase transition in the presence of $C_{12}NO$ and modulated by pH, came from the group of Lindman [45]. Using fluorescence microscopy for visualizing the DNA compaction process by surfactant micelles, they observed that the minimum surfactant concentration necessary to compact DNA increases with pH. The phase map of DNA/$C_{12}NO$ compaction shows a strong DNA interaction with protonated $C_{12}N^{+}OH$ at pH < 6.4, while DNA coil did not collapse due to the surfactant interaction when pH increases above 7.4. Using SAXS, the authors identified the hexagonal structure in DNA/$C_{12}NO$ complexes at acidic conditions. The experiments thus revealed the strong influence of pH on DNA/$C_{12}NO$ interaction. Turbidimetric and light scattering experiments of Wang et al. [46] analyzed DNA interaction with $C_{12}NO$ micelles focusing on the degree of $C_{12}NO$ protonation and micelle shape as a function of pH. Additional experimental methods, dielectric spectroscopy and circular dichroism [47], fluorescence with pH sensitive probe [48], dynamic light scattering and viscosity measurements [49] were employed with the aim of shedding light into the interaction and establishing the pH range of $C_{12}NO$ micelle protonation sufficient for an effective DNA/$C_{12}NO$ complexation. DNA interaction with the surfactant/lipid mixed system was discussed only by Mel'nikova and Lindman [45]. DNA compaction by unilamellar vesicles prepared from an equimolar $C_{12}NO$/DOPE mixture was followed by fluorescence microscopy. The authors found the mixture more efficient in DNA compaction as compared to the surfactant alone in the same pH range. SAXD experiments have shown a lamellar structure with the repeat distance of 6.28 nm for the DNA/$C_{12}NO$/DOPE complex at $C_{12}NO$/DOPE = 1 mol/mol and pH = 5.5. Under alkaline conditions, pH = 8.5, DNA interaction with vesicles resulted in their aggregation and flocculation as the authors derived from images of the cryo-TEM technique.

Despite the large use of C_nNO in different fields of industry and its non-zero concentration in the environment, very little is known about interactions between the surfactant and biologically important molecules. For many years our workplace studies the biological activity and physico-chemical properties of C_nNO on real systems or models of biological membranes [24,26,28–30,32–35]. Our preliminary static light experiments on the $C_{14}NO$/DNA system confirmed the high pH sensitivity of the surfactant/DNA interaction in good agreement with results described above. SAXD on DNA/$C_{14}NO$/dimyristoylphosphatidylcholine (DMPC) complexes revealed that the distance between DNA strands (d_{DNA}) packed in a condensed lamellar phase can be modulated in the range 6.0–3.8 nm changing pH from 8 to 4, respectively [50].

The present study extends the investigation of the polymorphic behavior of C_nNO/lipid/DNA complexes. We focused our experiments on DNA condensation with $C_{12}NO$/DOPE mixtures as a function of composition, pH and temperature. SAXD experiments revealed a large variety of liquid-crystalline mesophases, differences in their stability as well as in their capability to accommodate DNA when the pH of the solutions changes from neutral to acidic. The binding efficiency of the $C_{12}NO$/DOPE mixture for DNA was examined by UV–vis spectrophotometry. We routed our experiments towards the possibility of utilizing the surfactant for the gene delivery vector of a pH sensitive system. However, we believe that the obtained knowledge about the surfactant–lipid–DNA polymorphism modulated through pH can attract a larger audience from the areas of pharmacy, chemistry or biophysics.

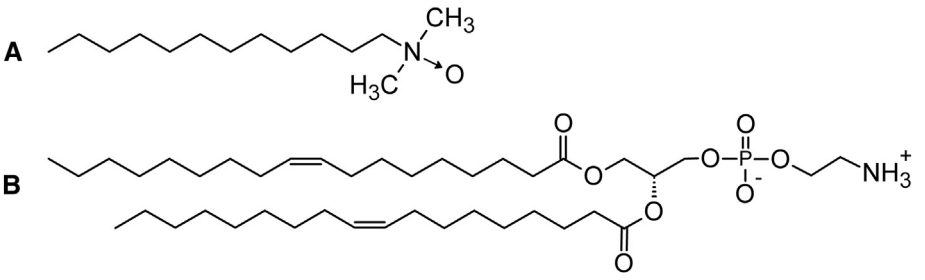

Fig. 1. The structure of *N*,*N*-dimethyldodecylamine-*N*-oxide ($C_{12}NO$) (A) and dioleoylphosphatidylethanolamine (DOPE) (B).