Temperature Induced Lamellar to Hexagonal Phase Transition in Lipoplexes

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Introduction: Gene therapy provides a new paradigm for the treatment of human diseases. The vectors for the transfection of the cells are subdivided into two classes: the viral vectors (e.g. adenoviruses, retroviruses) and the non-viral vectors (naked DNA, liposomes, polyethylene imines) [1, 2]. Large efforts are actually undertaken for the development of liposomal formulation, which should have the potential to transfect various cell types in vivo [3, 4]. Most of the developed lipids are positively charged, in order to enable a strong condensation of the liposomes with the negatively charged DNA. This condensation leads to the formation of complexes, aggregates called lipoplexes. In various papers, it is described that the structure and the formed lipid phase in such cationic lipid – DNA complexes may have an influence on the transfection efficiency and physical stability of the lipoplexes [1, 2].

The presented study utilises synchrotron X-ray (SAXS: small angle X-ray scattering, WAXS: wide angle X-ray scattering) for the elucidation of the mesomorphic state of the formed aggregates upon mixing DNA with a cationic lipid mixture in buffer. Additionally, the thermotropic phase behaviour is investigated. The used cationic lipid DC-Chol (see below) has already been successfully used for the transfection of an in vivo model (pig) [5].

Materials and Methods: The lipids 3β-[(*N*',*N*'-dimethylaminoethane)-carbamoyl]-cholesterol-HCl (DC-Chol) and dioleoyl-phosphatidylethanolamine (DOPE) were purchased from Avanti Polar Lipids (Birmingham, AL, USA). The binary lipid mixture composed of 40 w% DC-Chol and 60 w% DOPE (denoted as DC-40) was prepared according to standard protocols described in the literature [4].

The ccc-DNA (7 kbp, ccc: covalently closed circular) is obtained from Boehringer Ingelheim Austria. The lipoplex is formed at a weight to weight ratio of DC40:DNA 1:8. The formed hydrated pellet is centrifuged and filled in a capillary. Synchrotron X-ray diffraction investigations were performed on beam line A2 at HASYLAB, DESY in Hamburg [6].

Results and Discussions: Mixing DC-40 (liposome size ~ 250 nm as measured by photon correlation spectroscopy) with cccDNA at a weight to weight ratio of 1:8 induces the formation of a cationic lipid-DNA complex, which is physically unstable in solution. After a few minutes the size of the lipoplexes increases up to 1000 nm and a precipitate is formed with the appearance of aggregates in the range of a 1-1000 μm. These precipitated aggregates were analysed by synchrotron X-ray.
At room temperature two peaks are registered for the SAXS. The ratios of the SAXS peaks indicated the formation of lamellar phase (Fig. 1). The WAXS region showed no signal; a broad halo was detected indicative for melted hydrocarbon chains. This was confirmed by using differential scanning calorimetry (data not shown).
Upon heating the lipoplexes at a heating rate of 1ºC/min, the shape and position of the SAXS reflects changed. At a temperature of about 40-45 ºC the first peak broadens, and with increasing temperature, a second peak (~6.2 nm) appears close to the first peak (~7.1 nm). Additionally, two further peaks are observed at ~3.58 nm and ~3.1 nm. The SAXS ratios of these new peaks corresponds to the sequence 1: √3 : 2, which is consistent with the formation of a 2-dimensional columnar inverted hexagonal phase. Thus, with increasing temperature a bi-phasic system is formed, composed of a lamellar and hexagonal phase. The interplanar repeat as a function of temperature is plotted in Fig. 2. With increasing temperature, the interplanar repeat of the lamellar phase decreases with temperature. Reaching the temperature region, where the conversion of the lamellar (L_α^c) to hexagonal (H_{II}^c) phase is induced, a shaper decrease of the interlamellar repeat is registered.

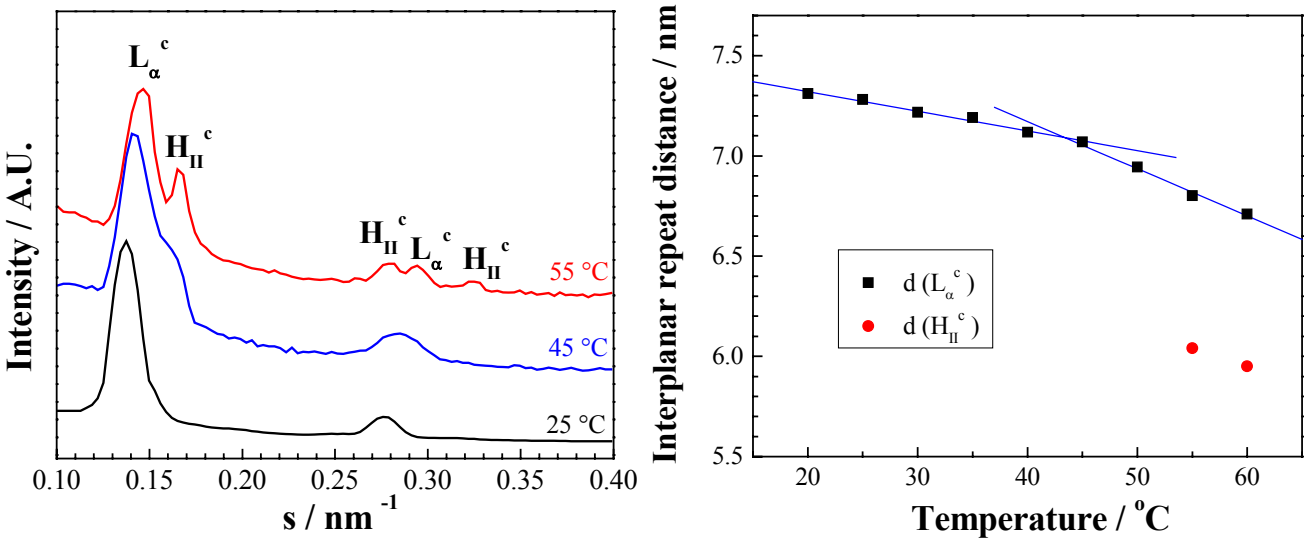

Fig. 1 (left): Small angle X-ray scattering (SAXS) of lipoplexe aggregates composed of DC40 : DNA 1:8 w/w as a function of temperature. L_α^c: multilamellar liquid crystalline phase, H_{II}^c: columnar inverted hexagonal phase. The index "c" referred to the condensed, complexed system [7].
Lamellar phase indexed by: 1 : 2 : 3 : 4 : 5, Hexagonal phase indexed by: 1: √3 : 2 : √7 : 3.

Fig. 2 (right): Interplanar repeat distance of the lamellar (L_α^c) and hexagonal phase (H_{II}^c) as a function of temperature.

Upon cooling to room temperature, the lipoplexe is mainly composed of the L_α^c-phase. However, some traces of the H_{II}^c-phase are still detected, and even after storage for one month at room temperature, the bi-phasic nature of the complex is still obvious.
Recently, Safinya and coworkers have investigated lipoplexes formed of the cationic lipid DOTAP (dioleoyl trimethylammonium propane) with the helper lipid DOPE (dioleoyl phosphatidylethanolamine) or DOPC (dioleoyl phosphatidylcholine) [7-9]. The transformation of a lamellar to hexagonal phase was induced by varying the charge ratio between the cationic lipid and DNA. In contrast to the presented results for DC-Chol lipoplexe, Safinya et al. detected a broad peak in the SAXS which was assigned to the 1-dimensional array of DNA chains.
Such a DC40 lipoplexe as presented in this study could be of relevance for in vivo transfection test, due to the fact, that the transformation from the lamellar phase to the inverted hexagonal phase happens close to 37 ºC. The challenge will be to develop a stable liquid formulation, without precipitating and without the formation of large aggregates.

References

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