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Structural studies of the formation of lipoplexes between siRNA and selected bis-imidazolium gemini surfactants

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ABSTRACT

Dicationic (gemini) surfactants are agents that can be used for the preparation of stable complexes of nucleic acids, particularly siRNA for therapeutic purposes. In this study, we demonstrated that bis-imidazolium gemini surfactants with variable lengths of dioxyalkyl linker groups (from dioxyethyl to dioxydodecyl) and dodecyl side chains are excellent for the complexation of siRNA. All of these compounds effectively complexed siRNA in a charge ratio range (*p/n*) of 1.5–10. The low resolution structure of siRNA oligomers was characterised by small angle scattering of synchrotron radiation (SR-SAXS) and *ab initio* modelling. The structures of the formed complexes were also analysed using SR-SAXS, circular dichroism studies and electrophoretic mobility tests. The most promising agents for complexation with siRNA were the surfactants that contained dioxyethyl and dioxyhexyl spacer groups.

1. Introduction

The sequence-specific process of post-transcriptional gene silencing (PTGS) through RNA interference (RNAi) utilises short fragments of double-stranded RNA called siRNA (approximately 19–28 nucleotides in length) [1–3]. Because controlling the gene silencing mechanism has tremendous therapeutic potential, siRNA molecules are an attractive tool for gene therapy aimed at treating a broad range of diseases [4–6]. A number of attempts to apply innovative siRNA therapeutics in the treatment of various human diseases have been reported, particularly cancers, genetic diseases and viral infections [5,7–10]. Some of these proposed siRNA-based therapeutics have reached the second phase of clinical trials [11,12].

A crucial point in the broad range of medical applications of siRNA-therapeutics is the selection of effective and safe systems for introducing siRNA oligomers into the cell. These siRNA therapeutics are able to bind and protect siRNA molecules and support their efficient transfer into cells through the cell membrane (for details, see review articles: [13,14]). However, one major problem associated with the therapeutic use of siRNA is its low stability in solution (e.g. in serum) and during the transfection process, which is caused by its susceptibility to digestion by endonucleases prior to cellular internalisation [15–17]. These problems can be reduced by the use of appropriate delivery systems (also known as transfection vectors or delivery vehicles) that support the transfection process while simultaneously protecting the siRNA molecules from degradation [18,19]. However, the delivery systems dedicated to enhancing the transfection process should not exhibit any toxicity to the transfected cell lines or target organisms. In recent years, a number of synthetic vectors supporting transfection have been developed and implemented (for review see: [20,21]). A wide range of synthetic gene delivery tools, based on cationic lipids, that are useful for transfection have been generated [22,23]. These carriers exhibit good transfection efficiency against a wide variety of cell lines. Unfortunately, significant toxicity towards some mammalian cell lines (especially primary mammalian cells, such as primary osteoblasts, neurons or dendritic cells) has also been reported when these compounds have been used as components of delivery systems [24,25]. Therefore, new compounds or systems suitable for the effective formation of lipoplexes with nucleic acids, especially siRNA, are a point of interest for therapeutic and research purposes.

Numerous structural and transfection studies have demonstrated the effectiveness of dicationic (also known as gemini, siamese or dimeric) surfactants to complex with DNA for effective delivery to a broad range of cell types [26,27]. Recently,

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several groups of dicationic surfactants with imidazolium-based hydrophilic heads and polar spacer groups have been reported to efficiently compact the plasmid DNA, showing promise as components of gene transfection systems [28–30]. In our previous studies, we have shown that a series of surfactants with variable alkyl or dioxyalkyl linker groups is suitable for the preparation of complexes with long, double-stranded, linear DNA or with plasmid DNA [31,32]. Systems based on gemini surfactants with cycloalkyl side chains can also effectively complex both linear and plasmid DNA [32]. The structures of different types of DNA lipoplexes have also been intensively studied using small angle scattering of synchrotron radiation.

Despite these previous advances, there are few reports concerning structural studies of siRNA lipoplexes using synchrotron radiation. This is partly due to the significant susceptibility of siRNAs to digestion by nucleases, which has been previously described, as well as the potential damage caused by long-term exposure to X-rays of high intensity. Only the structure of lipoplexes based on bis(quaternary ammonium) bromides with spacer groups of different lengths (12-3-12, 12-6-12, 12-12-12) and a weak electrolyte surfactant (SH14) with a triazine head has been studied by neutron scattering [33]. The kinetics of lipoplex formation with a series of gemini surfactants has also been described on the basis of time-resolved SAXS measurements using synchrotron radiation. Falsini and co-workers [34] have shown that complexation between siRNA and micelles formed by dicationic surfactants is a rapid process (time scale <50 ms).

The aim of our study was to investigate the ability of short, potentially therapeutic oligomers of double-stranded siRNA to complex with a series of gemini bis-imidazolium surfactants consisting of dioxyalkyl spacer groups of variable-length (from dioxyethyl to dioxydodecyl group) and dodecyl side chains. The microstructures of these complexes were characterised by small angle scattering of synchrotron radiation (SR-SAXS), circular dichroism studies and electrophoretic mobility tests.

2. Experimental

2.1. Sample preparation

We used a double-stranded siRNA oligomer with the following nucleotide sequence:

sense: 5′-pGUUACGACAAUCCUGUUUCdTdT-3′

antisense: 5′-pGAAACAGGAUUGUCGUAACdTdT-3′

The siRNA molecule (also known as siCUG-BP1) [35] selected for our research possesses a specific sequence that blocks CUGBP1 protein biosynthesis. This protein is a key regulator in myotonic dystrophy type 1 (DM1), or Steinert disease [36], and other genetic diseases.

All siRNA samples were synthesised by FutureSynthesis Sp. z o.o. (Poznań, Poland) and were used without additional purification. Lyophilised oligonucleotides were directly dissolved in 10 mM sodium phosphate buffer pH 7.0 to a working concentration of 1.2 mg/ml (88 μM). Reference ds-siRNA without the addition of gemini surfactants was dissolved in 20 mM sodium phosphate buffer pH 7.0 for use in SAXS studies.

The dicationic (gemini) surfactants were synthesised on the basis of our previously described procedure [31] and were a generous gift from Dr Andrzej Skrzypczak (Poznań University of Technology, Poland). The chemical structures of the dicationic surfactants studied are presented in Fig. 1. We selected six dicationic compounds with different spacer group lengths (increasing from dioxyethyl to dioxydodecyl) and dodecyl side chains.

Complexes (lipoplexes) between siRNA and the studied gemini surfactants were prepared by directly mixing the surfactant

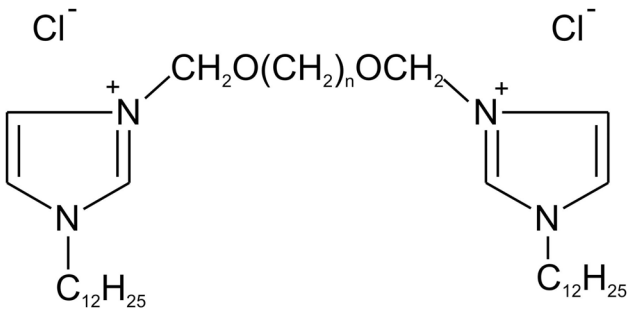

Fig. 1. The chemical structure of the studied dicationic surfactants C12JCn, where n = 2, 4, 6, 8, 10 and 12.

solutions with an siRNA solution. All samples were incubated for approximately 15–20 min at room temperature prior to taking measurements. A series of different lipoplexes characterised by charge ratio (*p*/*n*) of 0.5 – 10 was prepared. The *p*/*n* ratio is defined as the positive-to-negative electric charge ratios of lipoplexes and can be described by equation 1 [37]:

$$p/n = \frac{2C_{gem}}{nC_{RNA}} \quad (1)$$

where C_{RNA} is the molar concentration of siRNA, C_{gem} is the molar concentration of gemini surfactant and n represents the quantity of negative charges attributable to the siRNA molecules. The siRNA molecule used in this study consisted of 21 bp and n = 42, and the final concentration of siRNA in lipoplexes was 0.6 mg/ml (44 μM).

2.2. Small angle scattering of synchrotron radiation (SR-SAXS)

The SAXS data for the siRNA/gemini surfactant lipoplexes were collected on the BM29 BioSAXS Beamline [38] at ESRF (Grenoble, France) using synchrotron radiation (wavelength λ = 0.9919 nm). The distance between the sample and the detector was 2.867 m, which corresponds to an *s*-axis range of 0.06 – 4.2 nm^{-1} ($s = 4\pi\sin\theta/\lambda$). The scattering experiments were performed at 15 °C using a continuous flow cell with automated filling (typical sample volume = 30 μl). The SAXS measurements of the siRNA/gemini surfactant lipoplexes were conducted for the following set of *p*/*n* values: 0.5, 1, 1.5, 2, 3, 4, 6 and 8. Scattering patterns were recorded on a Pilatus 1M 2D detector (active area: 169 × 179 mm^2), and for each sample, 10 frames (10 s exposure/frame) were collected. The scattering vector *s* was calibrated on the positions of the diffraction peaks of silver behenate [39]. The possible radiation damage was monitored by comparing the subsequent frames. Finally, the solution scattering data were processed (correction for detector response, normalisation to the incident beam intensity and subtraction of the buffer scattering were applied) using the program PRIMUS [40] from the ATSAS package [41]. The basic structural parameters (the radius of gyration R_G, pair distance distribution function *p*(r) and the maximum intramolecular distance D_{max}) were calculated using indirect Fourier transformations implemented in the GNOM module from the PRIMUS package.

2.3. Modelling of the low-resolution structures of siRNA

The low-resolution structures of the siRNA duplexes in solution were determined by using two independent programs: DAMMIN [42] and DAMMIF [43]. The SAXS data collected for the siRNA sample without surfactants were used. For the first step of data analysis, at least 20 independent bead models were generated using DAMMIF. These models were then averaged to determine the most common shapes of the siRNA oligomers using the programs DAMAVER and SUPCOMB [44]. Then, the next step of modelling the