Complexes of Nucleosome Core Particles and Chromatin Arrays with Phospholipid Liposomes Investigated by Small Angle X-ray Scattering

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Genetic information is stored in the nucleus as chromatin in all eukaryotic cells. Interphase nucleus consists of nuclear envelope, which surrounds chromatin and made up of outer lipid membrane and inner protein lamina. During cell division, nucleus breaks down to allow chromosome segregation and new nucleus assembly occurs in new cells. Little is known about interactions between chromatin and nuclear membrane during nucleus reorganization. *In vitro* studies reported decondensation of metaphase chromosomes by lipids, and in telophase fusion of membrane vesicles was observed on the chromosomes. Nuclear envelope may influence gene expression by organizing spatially chromatin, where silent heterochromatin is adjacent to envelope, and active euchromatin is in the centre of nucleus. Unit of chromatin is the nucleosome core particle (NCP), consisting of 147 base pairs of DNA wrapped in 1.7 turn of left-hand superhelix around a histone octamer, which consists of two H2A:H2B dimers and one H3:H4 tetramer. We use *in vitro* reconstituted NCP and 12-mer nucleosome array as a chromatin model, to study structure of chromatin-lipid complexes by SAXS. We use liposomes of different charge density from binary mixture of neutral lipid DOPC, with either positive DOTAP, or negative DOPG. We used DNA-liposome mixtures as a control because such systems with both cationic and anionic liposomes have been studied and phase behavior is well known. Also NCP and array aggregation by cations and crowding agents was investigated and multiple phase formation was reported. Complex formation between chromatin models, namely NCP and 12-mer array, and liposomes was not studied before.

We investigated the interaction of NCP and array with cationic DOTAP concentration from 5 to 100 %. Liposomes with $C_{DOTAP} \geq 20\%$ have been found to form lamellar phase with DNA (d_L = 70 – 55 Å), therefore dissociate DNA from histone octamer. NCP and array with liposomes of $C_{DOTAP} \leq 20\%$ (Fig. 1A) show a broad peaks in the range of 500, 200, 70 and 37 Å without DOTAP dependence. Also a scattering in the region of q ≤ 0.05, not characteristic for DNA may be due to NCP forming a smectic, nematic or columnar phases. Array has not produced characteristic chromatin spectra (Fig. 1B), possibly due to overlapping of lipid scattering that is a major contributor. Moreover, demixing for ≤ 15% DOTAP:DOPC mixtures was reported, and NCP and array may coexist in separate phase.

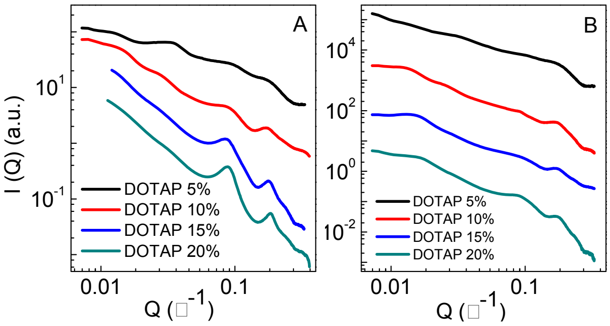

Fig. 1: The SAXS spectra of NCP (A) and array (B) with DOTAP mixed at charge ratio 1.

Mixture of NCP and array with anionic liposomes were prepared for 10 and 25% molar DOPG, and 2, 10 and 50 mM Mg^{2+}. NCP and array were found to remain stable under those conditions. At 2 and 10 mM Mg^{2+} columnar hexagonal, and at 50 mM Mg^{2+} semicrystalline columnar quasihexagonal phases were identified with intercolumnar distance 110 Å and interparticle distance 55 Å. Lipids may have formed lamellar phase, with d_L= 72 Å and 62 Å. Array has demonstrated characteristic scattering for chromatin, with broad peaks at 37, 57, 110 Å regions. No lamellar peaks were observed for lipid-array mixture.

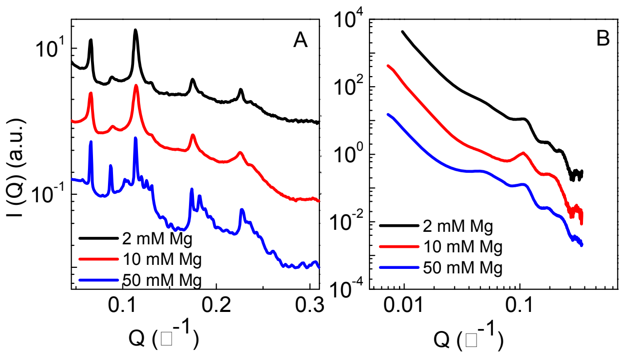

Fig. 2: SAXS spectra for NCP (A) and array (B) with 25% DOPG at charge ratio 1 for C_{Mg}^{2+} 2, 10 and 50 mM.

Fluorescent microscopy of labeled components is going to complement future SAXS studies of the chromatin-lipid system.