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Quercetin Affects Nucleosome Structure

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Quercetin (Fig. 1 B) is a natural flavonoid with multiple biological activities: radical-suppressing, antioxidant, antibacterial, anti-inflammatory, immuno-modulating, antiviral and anticancer. It is currently used for treatment of obesity and cardiovascular diseases [1]. Quercetin is supposed to possess anticancer effect due to oncogene suppression and tumor suppressor gene re-activation via epigenetic alterations [2]. Quercetin interacts with DNA. Modes of its interactions include: binding to DNA grooves, intercalation, electrostatic and hydrogen bond binding [3-5]. However, the impact of quercetin on a nucleosome structure is not known; it is addressed here.

Interactions of quercetin with nucleosomes were studied using single particle fluorescence microscopy based on the Förster resonance energy transfer (spFRET microscopy) [6] and mononucleosomes (Fig. 1 C), which were assembled as described elsewhere [7] on the 187 bp DNA template using chicken core histones. DNA template contained the 603 nucleosome positioning sequence (147 bp) flanked by two 20 bp linker fragments as well as Cy3 and Cy5 labels placed at 13 and 91 bp from the boundary of the 603 sequence. These labels are positioned in close proximity in neighboring gyres of DNA in assembled nucleosomes and efficiently participate in FRET. Quercetin-induced structural changes in nucleosomes were evaluated by measuring the proximity ratio EPR that reflects FRET efficiency from single freely diffusing nucleosomes followed by analysis of the frequency distributions of nucleosomes by EPR (Fig. 1 A). The EPR profiles of nucleosomes were fitted by three Gaussians (Fig. 1 D), and fractions of nucleosomes possessing low EPR (LEPR, EPR< 0.3) were calculated (Fig. 1 E). Quercetin was dissolved in 100% DMSO and added to nucleosomes for 20 min. DMSO concentration in the studied samples did not exceed 2% and did not affect the nucleosome structure (Fig. 1 D, E).

spFRET microscopy revealed that quercetin induces concentration-dependent alterations in the structure of nucleosomal DNA. An increase in the LEPR fraction of nucleosomes was observed at the quercetin concentration higher than 6 μM (Fig. 1 D, E). LEPR fraction increases from 1-5% in the absence of quercetin to ~40% at 24 μM quercetin (Fig. 1 E). These alterations can be interpreted as unwrapping of DNA from the histone octamer, which involves 20 bp of linker DNA and at least 13 bp of the 603 DNA sequence. Since the dissociation constant of the quercetin complex with DNA was reported to be ca. 14 μM [3], structural changes in nucleosomes are most probably directly related to interactions of quercetin with nucleosomal DNA. Polyacrylamide gel electrophoresis revealed that nucleosome-quercetin interactions are not accompanied by nucleosome dissociation with a release of DNA in the studied quercetin concentration range (data not shown).

Quercetin-induced unwrapping of nucleosomal DNA is likely a part of epigenetic activity of quercetin that increases access to nucleosomal DNA for proteins involved in DNA repair, transcription and replication and can modulate these processes.

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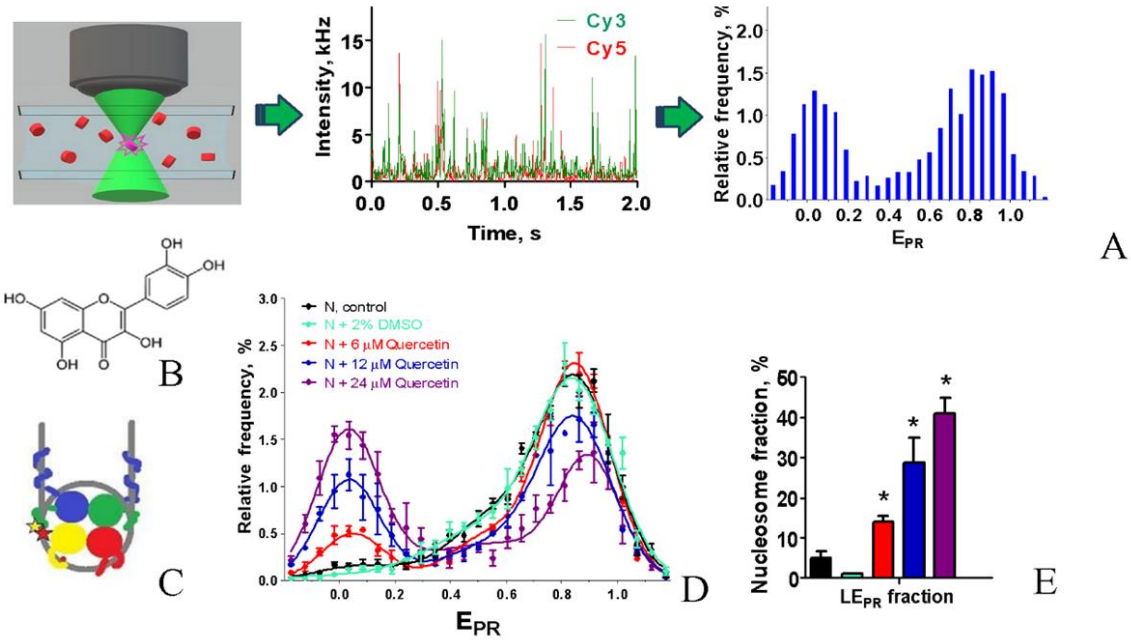

Figure 1. spFRET microscopy study of quercetin interactions with nucleosomes. (A) A scheme of spFRET experiment and data analysis. Fluorescence intensities of Cy3 and Cy5 labels were measured from single freely diffusing nucleosomes. EPR value was calculated for each measured nucleosome. The frequency distributions of nucleosomes by EPR were calculated and analyzed. (B) Structure of quercetin. (C) Structure of mononucleosomes and position of labels (asterisks). Octamer of histones is shown by different colors. (D) Relative frequency of nucleosome (N) distribution by EPR value in the absence and presence of different concentrations of quercetin (mean±SEM, n=3). Buffer - 150 mM KCl, 5 mM MgCl2, 1 mM β-mercaptoethanol, 20 mM Tris HCl, pH=8.0. (E) Content of low-EPR (LEPR) fraction of nucleosomes in different samples (*relative to control, $p<0.05$). Color designation of samples is the same as in panel D.

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