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Energy Transfer Evidence for Cross-Linking of DNA by 1,4-Bis((N-methylquinolinium-4-yl)vinyl) Benzene

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There are few examples of ligands that were reported to exhibit interstrand cross-linking abilities. Luzopeptin, an antitumor antibiotic containing two substituted quinoline rings linked by a cyclic peptide spacer, is the first example for which interstrand bisintercalation has been evidenced [1]. Lowe *et al*. reported on the cross-linking of DNA by ligands containing intercalating units (*e.g*., phenanthridinium or acridinium) linked by a rigid connector [2]. The detection of intermolecular bisintercalation is a special problem. The proposed techniques, including analysis of mobility of DNA-bisintercalator products [1] or ligation assay [2], have been proved difficult and present some limitations (*e.g.,* inhibition of ligase action at higher ligand concentration). Therefore, we decided to examine an alternative approach that exploits fluorescence resonance energy transfer (FRET) process, the method that has been successfully applied to study structural and dynamic properties of nucleic acids [3,4]. The idea behind our assay is to titrate a mixture of two single-labeled DNA duplexes (one labeled with a donor and the other with an acceptor molecule) with the bisintercalator. In the absence of cross-linking no FRET signal will occur since both duplexes are randomly distributed in solution, with the separation distance between donor and acceptor far exceeding Förster critical radius (R_0). On the contrary, the interstrand cross-linking of duplexes will result in their spatial organization and in an appearance of FRET signal.

Here, we report the preliminary FRET results on DNA interstrand cross-linking by 1,4-bis((N-methylquinolinium-4-yl)vinyl) benzene (abbreviated as pMQVB hereafter). Due to the photoisomerization, pMQVB can adopt *E,E* and *E,Z* conformations [5]. Planar *E,E* isomer is expected to exhibit bisintercalating abilities, contrary to its non planar partner, *E,Z* isomer (one arm in *cis* configuration). The *E,E* pMQVB was prepared by known procedure [6]. Photoisomerization product, *E,Z* isomer, was separated using HPLC. Deoxyribonucleotides used in FRET studies were synthesized by TaKaRa Inc., (Tokyo, Japan). The 8-mers (5′-CGTACGGC-3′) labeled at 5′ end with fluorescein (ssONF) or x-rhodamine (ssONXR) were used as a donor and an acceptor, respectively. Duplexes dsONF and dsONXR were prepared by incubation

of preliminary heated (65°C) 1:1 mixture of labeled and unlabeled complementary strands at 10°C for 30 min.

The Förster critical radius, (R_0 = 50 Å) for dsONF/dsONXR energy transfer system has been calculated assuming a value of 2/3 for the orientation factor κ^2, n = 1.36, and ϕ^{ONF} = 0.5 [3,4,7]. In a typical experiment, sample solution containing dsONF and dsONXR (0.5 μM each), TE buffer pH 7.8 (10 mM Tris–HCl, 1 mM EDTA) and 50 mM NaCl was equilibrated in a quartz cell at 10°C for 10 min. After successive additions of concentrated solution of pMQVB, fluorescence emission spectra were recorded using a set of polarizers aligned at the "magic angle". The emission spectra showed dramatic quenching of donor emission (at *ca*. 515 nm) accompanied with minor changes around 600 nm where sensitized emission of acceptor was expected (the cross-linking efficiency was reported to be usually very low [1,2]). Observed large decrease in donor fluorescence was mainly caused by pMQVB quenching of label emission as it was evidenced by titration of single-component systems (dsONF and dsONXR alone) with pMQVB. Values of quenching constants of *E,E* pMQVB were 1.4×10^6 and 3.6×10^6 for dsONXR and dsONF, respectively. The corresponding values of K_{SV} for *E,Z* pMQVB were $6.7\times10^5$and 2.4×10^6. Very high values of K_{SV} can be explained by local accumulation of the quencher in the vicinity of DNA that is consistent with the high value of binding constant of pMQVB with DNA ($K_b \sim 1\times10^6$ [7]). Since high quenching shields energy transfer signal, the total emission from the system was analyzed in order to isolate x-rhodamine spectrum alone. PhotochemCAD software that allows quantitative multicomponent analysis of a spectrum composed of several components was used for this purpose [8]. Extracted fluorescence spectra of x-rhodamine were next processed to plot quenching dependencies. The plot for *E,E* isomer showed significantly lower quenching for dsONF/dsONXR system comparing to that for dsONXR alone. This is an evidence of energy transfer contribution to the x-rhodamine fluorescence because energy transfer adds sensitized emission to the directly excited spectrum of x-rhodamine and resulting quenching is apparently less efficient. On the contrary, in case of *E,Z* isomer both plots (for dsONXR alone and dsONF/dsONXR mixture) showed similar quenching efficiency. Thus, *E,Z* isomer appeared to be a poor cross-linking agent in accordance with its non-planar structure.

The presented results show that FRET can be successfully applied for the investigation of cross-linking of DNA. The pMQVB appeared to be an interesting example of cross-linking ligand, for which energy transfer takes place only when it is present as a planar *E,E* isomer that suggests an importance of bisintercalation process in the interstrand cross-linking of DNA. Further studies that will prove independently cross-linking properties of *E,E* pMQVB are in progress.

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