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Studying the effect of crowding and dehydration on DNA G-quadruplexes

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ABSTRACT

Intracellular environment is crowded with biomolecules that occupy a significant fraction (up to 40%) of the cellular volume, with a total concentration in the range 300–400 mg/ml. Recently, the effect of crowding/dehydrating agents on the DNA G-quadruplexes has become a subject of an increasing interest. Crowding and/or dehydrating agents have been used to simulate how G-quadruplexes behave under cell-mimicking conditions characterized by a large excluded volume and a lower water activity. Indeed, the presence of both steric crowding and a lower water activity can affect G-quadruplex stability, their folding/unfolding kinetics, as well as their binding processes with proteins or small ligands. Many of these effects can be explored experimentally by measuring the dependence of the conformational stability, isomerisation kinetics and equilibria on the concentration of cosolutes which do not interact with the molecules (G-quadruplexes) under investigation.

Spectroscopic methodologies, like circular dichroism, UV and fluorescence, have been widely employed to study G-quadruplexes in dilute solution. Here we focus on some aspects that need to be taken into account when employing such techniques in the presence of large amount of a cosolute. Additionally, we discuss possible problems/artifacts that arise in setting experiments in presence of these commonly employed cosolutes and in interpreting the results.

1. Introduction

G-quadruplexes are distinctive DNA secondary structures comprising a stack of two or more G-tetrad subunits linked by the phosphodiester backbone [1]. The G-tetrads are based on the formation of a planar array of four guanine bases associated through Hoogsteen-like hydrogen bonds. G-quadruplexes are stabilized by the specific binding of metal ions, preferentially Na^+ and K^+, that position themselves in the central cavity produced by stacking of G-tetrads [2,3]. In the case of a unimolecular structure, the organization of the phosphodiester backbone gives rise to a range of possible topologies, thus exhibiting a broad structural diversity and polymorphism compared to duplex DNA [1]. For example, the G-rich single-stranded human telomeric sequences have been shown to adopt different G-quadruplex structures in solution, depending on sequence type, length and presence of different cations in solution [4]. K^+ and Na^+, certainly the most relevant cations for their abundance in the aqueous environment of living cells, may frequently induce different G-quadruplex conformations on telomeric as well as on non-telomeric sequences [5,6]. Such conformations are often in equilibrium with each other in solution.

With the aim of mimicking the typical condition in cell nuclei, it is of special significance to investigate the effect of cosolutes acting as crowding and/or dehydrating agents (that do not interact directly with DNA) on the structure, stability and interactions of G-quadruplexes. Indeed, intracellular environment is crowded with biomolecules that occupy a significant fraction (up to 40%) of the cellular volume, with a total concentration in the range 300–400 mg/ml [7], thus generating a large excluded volume effect and, likely, decreasing the water activity.

It is known that molecular crowding may alter the rates and equilibria of biomolecular reactions [8,9], as well as a change in water activity can affect conformational transitions involving uptake or release of water molecules.

Recently, the effect of cosolutes on the DNA G-quadruplexes has become a subject of an increasing interest. Large amount of cosolutes have been used to simulate molecular crowding and/or dehydrating conditions in the attempt to clarify how G-quadruplexes behave under cell-mimicking conditions [10–14].

Crowding/dehydrating conditions can affect G-quadruplex stability, their folding/unfolding kinetics as well as their binding processes with proteins or small ligands. Many of these effects can be explored experimentally by measuring the dependence of the conformational stability, isomerisation kinetics and equilibria upon the concentration of cosolutes (crowding/dehydrating agents) that

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do not directly interact with the molecules (G-quadruplexes) under investigation.

Spectroscopic methodologies, like circular dichroism (CD), UV and fluorescence, have been widely employed to study quadruplex in molecular crowding. Many details on the use of these techniques to study quadruplex in conventional buffer solutions (dilute conditions) have been already reported [15,16], here we focus on some aspects to be taken into account when employing such techniques in the presence of a large amount of cosolutes. Additionally, we discuss possible problems/artifacts that arise in setting experiments in presence of these cosolutes and in interpreting the results.

2. Methods

2.1. Choice of the cosolute as crowding and/or dehydrating agent

To simulate physiological conditions a cosolute is commonly added to the DNA at a total concentration in the range 300–400 mg/ml. The chosen cosolute should do not interact directly with DNA but should only affect DNA indirectly through excluded volume effect or by changing the water activity. So far, the most widely used cosolute employed in the quadruplex field has been the highly water-soluble synthetic polymer polyethylene glycol (PEG), usually with low molecular weight (PEG 200 and PEG 400). It has been used in the attempt to mimic steric crowding. However, doubts have been recently raised on the use of PEG to simulate crowding conditions as it could interact with macromolecules rather than being an inert partner and its effect could not be described in terms of excluded volume alone [8,14]. Further, it has been shown that presence of high concentration of PEG lowers significantly the water activity and its effect on DNA is mainly due to dehydration rather than steric crowding [13].

A variety of other water-soluble proteins (BSA, hemoglobin, and lysozyme), large molecules (ficoll, dextrans) or small molecules (ethanol, acetonitrile, DMSO, betaine) can be employed as cosolute to generate crowding and/or to decrease the water activity (Fig. 1). Among these, small cosolutes (like alcohols and glycerols) being employed at high molar fractions, reduce greatly the water activity and act mainly as dehydrating agents, whereas larger molecules such as proteins or polysaccharides generate a significant excluded volume (crowding) also at lower molar concentration and have a small effect on water activity.

Although, it is still unclear which of these cosolutes is better to simulate cellular environment, surely, proteins and polysaccharides have the advantage of mimic more closely the types of biomolecules in the cellular environment. Particularly, it has been shown that ficoll 70 affects the G-quadruplex conformation in similar way with respect to the more natural biological environment represented by Xenopus laevis egg extract [14].

2.2. Preparation of the samples

Quadruplex samples can be prepared by using a concentrate stock solution of DNA in the buffer without the crowding/dehydrating agent (“dilute buffer”) and a solution of the same buffer containing the crowding/dehydrating agent at the desired concentration. The concentration of the crowding/dehydrating agent in the buffer is usually fixed at 40% (w/v), mimicking the total concentration of biomacromolecules in the living cells. The sample for the measurement can be prepared by diluting the DNA stock solution, prepared in “dilute buffer”, with the buffer containing the crowding/dehydrating agent until the desired concentrations of the DNA is reached. This procedure is suitable when the DNA concentration in the final sample is very low if compared with his concentration in the stock solution, thus the preparation of the final sample from the DNA stock solution involves a negligible dilution of the cosolute previously added to the buffer. This is the case for a wide range of experiments requiring low G-quadruplex concentration (e.g. CD, UV and fluorescence spectroscopy). If higher DNA concentrations are required (e.g. for NMR experiments) may be convenient to dilute the quadruplex stock solution with a concentrate solution of crowding/dehydrating agent until reaching the desired quadruplex and crowding/dehydrating agent concentrations.

2.3. Relevance of the annealing procedure

A standard procedure for the preparation of G-quadruplex samples for biophysical studies is to subject the DNA sample to an annealing procedure, consisting in heating the sample at high temperature, and then slowly cooling it at room temperature. This procedure helps in dissolving completely the sample and allows to reach the thermodynamically more stable conformation, thus avoiding artifacts due to the initial presence of possible kinetically trapped conformations. In many cases, the DNA conformation

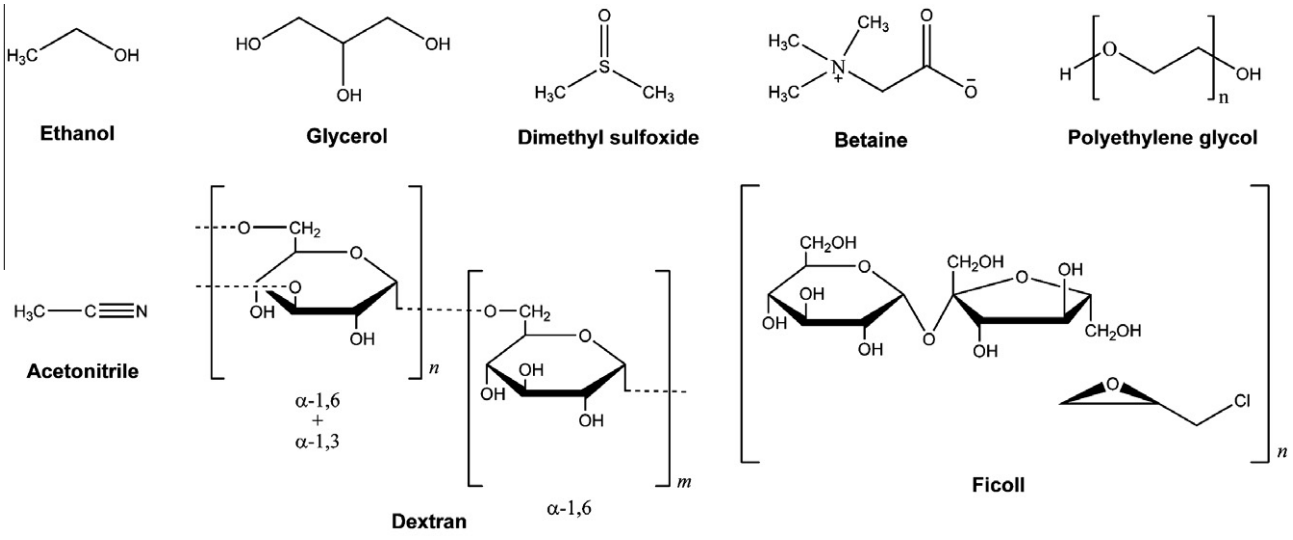

Fig. 1. Chemical structures of some large or small molecules commonly employed as cosolutes.