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# Control of stability and structure of nucleic acids using cosolutes

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ABSTRACT

The stabilities, structures, and functions of nucleic acids are responsive to surrounding conditions. Living cells contain biomolecules, including nucleic acids, proteins, polysaccharides, and other soluble and insoluble low-molecular weight components, that occupy a significant fraction of the cellular volume (up to 40%), resulting in a highly crowded intracellular environment. We have proven that conditions that mimic features of this intra-cellular environment alter the physical properties affect the stability, structure, and function of nucleic acids. The ability to control structure of nucleic acids by mimicking intra-cellular conditions will be useful in nanotechnology applications of nucleic acids. This paper describes methods that can be used to analyze quantitatively the intra-cellular environment effects caused by cosolutes on nucleic acid structures and to regulate properties of nucleic acids using cosolutes.


## 1. Introduction

Controlled formation of structures by nucleic acids has enormous potential in the fields of nanobiotechnology and biomedical technology. Nucleic acids can undergo structural transitions from the canonical duplex structure to non-canonical structures such as a triplex, a quadruplex, i-motif, or cruciform in response to environmental stimuli. Such stimuli include pH, [1–3] small molecules (e.g., cosolutes and ions), [4–7] macromolecules (e.g., oligonucleotides and proteins), [8] and electrical signals. [9] Researchers have taken advantage of the switching properties of nucleic acid structures to develop nucleic acid-based sensors, logic devices, circuits, and drugs. We and other researchers also have developed logic devices that respond to input molecules (e.g., pH and cations) that monitor the change in DNA structures from G-quadruplexes and i-motifs to duplexes, and these are of considerable interest in DNA computing. Thus, control of the structures formed by nucleic acids is of interest in diverse research fields including medical, pharmaceutical, and material sciences.

The following five factors play major roles in determining the stabilities and structures of nucleic acids: hydrogen bonding, base stacking, conformational entropy, water binding, and cation binding. Hydrogen bonding, base stacking, and conformational entropy are determined by sequence of nucleic acids [10–13]. In contrast, extents of water and cation binding are determined by conditions surrounding the nucleic acid such as cosolute and cosolvent conditions [14–19]. Thus, a convenient method for control of the structures formed by nucleic acids and the stabilities of those structures is to change the surrounding conditions.

In living cells, organelles such as nuclei, the endoplasmic reticulum, and the mitochondria are present (Fig. 1a). [20,21] Moreover, biomolecules such as nucleic acids, proteins, polysaccharides, and other soluble and insoluble low-molecular weight components occupy a significant fraction of the cellular volume (up to 40%) (Fig. 1b) [21–23]. The effect of high concentrations of biomolecules is referred to as molecular crowding. Under the crowded conditions, the chemical potentials and activity coefficients of biomolecules are changed due to the excluded volume effect. Moreover, the physical properties of intra-cellular conditions such as osmotic pressure, viscosity, and the dielectric constant are different from those of dilute solutions [23]. We have investigated the effect of intra-cellular conditions induced by cosolutes on the structures and stabilities of nucleic acids: Cosolutes, especially cosolutes with low-molecular weight (below about 200), change the physical properties of solution and perturb the water and cation binding to nucleic acids [23–27]. This paper describes the methods for quantitative analysis for water and cation binding to DNA in the presence of cosolutes and describes how one can take advantage of the properties of cosolutes to regulate stabilities and structures of nucleic acids.

## 2. Sample preparation

### 2.1. Preparation of nucleic acids

Oligodeoxyribonucleotides are suitable for quantitative studies because association and dissociation of the structures usually

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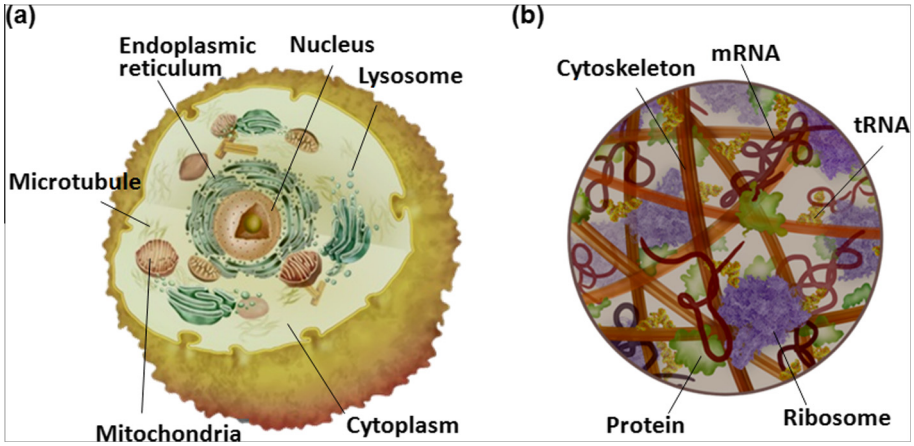


**Fig. 1.** Schematic structures of a highly crowded environment in cell resulting from the presence of (a) organelles and (b) macromolecules.

occur through two-state transitions; this property makes it possible to evaluate the thermodynamic parameters of the conformational transition [24,25,28]. In addition, many short oligonucleotides adopt the same structures in both dilute and in molecular crowding conditions [24,25,27,29]. In contrast, long nucleic acid strands tend to fold into compact and aggregated structures under crowding conditions that differ from those adopted in dilute solution [24,30]. Oligonucleotides are synthesized using a solid-phase phosphoroamidate procedure on an automated nucleic acid synthesizer. The synthesized oligonucleotides are purified by reversed-phase HPLC (typically using a C18 column with a methanol gradient). The purity of the purified oligonucleotides may be analyzed by HPLC or mass spectroscopy. Oligonucleotides of less than 95% purity should not be used for quantitative data analysis. HPCL-purified oligonucleotides can be purchased from a number of companies (e.g., Wako Pure Chemical, Tokyo Chemical Industry, and Sigma–Aldrich Japan). Single-strand concentrations of DNA and RNA oligonucleotides are determined from the absorbance measured at 260 nm at 90 °C using the single-strand extinction coefficients calculated from the mononucleotide and dinucleotide data based on the nearest-neighbor approximation model [31].

### 2.2. Preparation of crowding solution

Various compounds have been used as cosolute molecules to mimic the conditions that occurs naturally in cells. The cosolute concentrations are often represented as weight percent per volume and the cosolutes are generally used at 5–40 wt%. Cosolutes used to mimic cellular crowding should meet the following criteria: *(1)* Molecules should be basically inert (i.e., the cosolutes should not interact directly with nucleic acids), *(2)* cosolutes should be soluble in water (to at least several weight percent), and *(3)* in the case of large cosolutes, a range of polymer sizes should be available [20,22,23,32].

The commonly used large cosolutes are poly(ethylene glycol) (PEG), dextran, and Ficoll. PEG is often used because different molecular weight PEGs are available [23]. Proteins such as albumin, hemoglobin, and lysozyme are also utilized as crowding cosolutes [32]. Commonly used small cosolutes are ethylene glycol (EG), PEG 200 (PEG with average molecular weight of 200), alcohols, glycols, amino acids, and betaine [17,24,33]. These cosolutes have been used in experimental studies to study the equilibria and kinetics of many biochemical reactions. These cosolutes can be purchased from many commercial sources (e.g., Wako Pure Chemical, Tokyo Chemical Industry, and Sigma–Aldrich Japan).

### 2.3. Thermodynamic analysis for formation of nucleic acid structures

Methods to obtain the thermodynamic parameters of enthalpy ($\Delta H^\circ$), entropy ($\Delta S^\circ$), and free-energy changes at 25 °C ($\Delta G^\circ_{25}$) for the formation of a nucleic acid structures are described below; DNA duplex formation is taken as an example. Data are typically analyzed with a two-state model, which assumes that each strand is either completely paired or unpaired. The equilibrium for the duplex formation is represented as either a self-complementary or non-self-complementary association as follows [34,35]:

$$2A \rightleftarrows A_2 \tag{1}$$

$$B + C \rightleftarrows B \cdot C \tag{2}$$

where A, B, and C indicate the single strands of DNA and $A_2$ and $B \cdot C$ indicate the double-stranded DNA.

The equilibrium for a unimolecular transition, for example, of a hairpin, is represented as:

$$D_{ss} \rightleftarrows D_f \tag{3}$$

where $D_{ss}$ and $D_f$ indicate the single-stranded (unfolded) and folded DNA structures, respectively.

For self-complementary (Eq. (1)) or non-self-complementary equilibria (Eq. (2)) with equal concentrations of B and C, the observed equilibrium constant is given by:

$$K_{obs} = (\alpha/2)/(C_t/s)(1-\alpha)^2 \tag{4}$$

$C_t$ is the total strand concentration:

$$C_t = [A] + 2[A]\ (\text{self} - \text{complementary duplex}) \tag{5}$$

$$C_t = [B] + [C] + [B \cdot C]\ (\text{non-self} - \text{complementary duplex}) \tag{6}$$

$s$ has a value of 1 for self-complementary duplexes and 4 for non-self-complementary duplexes. $\alpha$ is the fraction of strands in a duplex. For a unimolecular transition,

$$K_{obs} = \alpha/(1-\alpha) = [D_f]/[D_{ss}] \tag{7}$$

where

$$\alpha = [D_f]/([D_{ss}] + [D_f]) \tag{8}$$

Fig. 2 shows a UV melting curve for the self-complementary duplex (5′-ATGCGCAT-3′). At low temperatures, the strands are in duplex form and the absorbance is low. As the temperature is increased, the duplex dissociates into single strands. The difference in absorbance between duplex and single strands is referred to as hyperchromicity. For self-complementary or