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# Cyclodextrin–Surfactant Complex: A New Route in DNA Decompression

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In the present work, we show a new approach for decompaction of DNA–cationic surfactant complexes, e.g., lipoplexes, by using  $\beta$ -cyclodextrin ( $\beta$ -CD). The DNA decompaction was achieved by dissolving the surfactant aggregates in the complex by making use of the high affinity between the  $\beta$ -CD and the free surfactant in solution. The results from fluorescence microscopy and adiabatic compressibility measurements indicate that coils and globules do not coexist. The reported procedure using  $\beta$ -CD is an efficient way to decompact DNA surfactant complexes because the association constant of surfactants with  $\beta$ -CD is large. The surfactant's interaction with  $\beta$ -CD is specific and the nonspecific interaction between  $\beta$ -CD and biological interfaces is small.

## Introduction

The possibility to treat diseases, in particular hereditary diseases, by the insertion of genes into human cells and tissues, so-called gene therapy, has encouraged many studies in the last few years.<sup>1</sup> This promising new technology is still in its infancy, and therapeutic objective gene delivery requires a precise control of both the uptake into the cell nucleus (transfection) and after the replication into RNA (transcription).

Both DNA compaction and decompaction are needed for successful delivery. First, DNA must be taken up by the natural cell membrane barriers. At this level, DNA compaction or condensation is essential to protect DNA from the nucleases and to allow it to reach the nucleus. In the second step when DNA arrives in the nucleus, it should be decompacted so it is accessible to the enzymatic machinery responsible for transcription into RNA. In living cells, transcription is accompanied by the alteration of chromatin structure in transcribed regions of the genome. Highly compacted structures of DNA and histones are inherently repressive of all DNA dependent processes. In general, regions of the genome that are actively transcribed have a more open and accessible chromatin.<sup>2,3</sup> Consequently, it was recently suggested that agents that bend DNA can be used to control gene expression.<sup>4</sup> It is clear that precise control of DNA decompaction is needed to efficiently control gene expression.

DNA compaction can be achieved by different complexing agents such as multivalent ions, cationic surfactants, lipids, neutral, and cationic polymers or alcohols.<sup>5–12</sup> In general, the choice of the complexing agent will determine the compaction mechanism and the structure of the complex. The most studied complexing agents are polycations, multivalent cations, or surfactants. Compaction with multivalent ions is achieved by ion correlation effects that induce effective attractions between different parts of the DNA molecule.<sup>13</sup> The compaction induced by surfactants is a cooperative process associated with the self-assembly of the surfactant.<sup>14,15</sup> The self-assembly of the surfactant in the presence of DNA starts at the critical association concentration (cac), which is much lower than the critical

concentration at which the surfactants form micelles in the absence of DNA, the so-called critical micelle concentration (cmc). The structure of the surfactant aggregates on the DNA is expected to depend on the type of surfactant. For instance, for CTAB, we have shown by SANS that is likely to be elongated aggregates.<sup>16</sup>

Some pioneering work has been done in order to promote the redissolution or decompaction of DNA, but there is still a need for rational approaches to control the decompaction process. In the case of multivalent cations, decompaction can be achieved by increasing the salt concentration.<sup>17</sup> In the case of cationic surfactants, anionic surfactants can be used to decompact DNA by forming catanionic aggregates that has significantly lower affinity to DNA.<sup>13,18</sup> Chen et al. reported specific formation of bends-on-a-chain on giant DNA by using polyamide derivatives.<sup>19</sup> More recently, Le Ny et al.<sup>20</sup> were able to control the condensation by using light-responsive surfactants. Cyclodextrins have been used in many applications due to their ability to form inclusion complexes with different molecules.<sup>21</sup> In the present work, we show a new approach for decompaction of DNA–cationic surfactant complexes, e.g., lipoplexes, by using  $\beta$ -cyclodextrin ( $\beta$ -CD). The DNA decompaction, studied using fluorescence microscopy, density, and sound velocity measurements, was achieved by dissolving the surfactant aggregates in the complex by making use of the high affinity between the  $\beta$ -CD and the free surfactant in solution.

Despite all the efforts in the synthesis of novel cationic lipids and surfactants, these amphiphilic molecules still pose a problem of toxicity in vivo and therefore their effective use is still not possible. However, we believe that the mechanism for decompaction presented in this study using a model system will be the same independently of the chemical structure of the surfactant that is used.

## Experimental Section

**Materials.** Coliphage T4DNA 166kbp was supplied by Wako Nippon Gene. Hexadecyltrimethylammonium bromide (CTAB) was obtained from Sigma and recrystallized twice in acetone. Fluorescent dyes 4',6-diamidino-2-phenyl-indole (DAPI) and GelStar nucleic acid gel stain were from Sigma and Cambrex, respectively. The latter is supplied as a 10000 $\times$  concentrated stock solution in DMSO. The

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antioxidant ascorbic acid was purchased from Sigma.  $\beta$ -Cyclodextrin ( $\beta$ -CD) was obtained from Sigma

**Sample Preparation.** All stock solutions were prepared in a 10 mM Tris-Cl buffer (pH 7.6). DNA molecules were diluted in the 10 mM Tris-Cl buffer containing 4% of ascorbic acid and fluorescent dye. The final concentration of DNA was  $0.5 \mu\text{M}$  in nucleotide units. DNA was compacted by using a CTAB concentration of  $2.42 \times 10^{-4}$  M. At this concentration, all the DNA molecules were compacted and no coexistence between coils and globules was found.<sup>10</sup> The concentration of  $\beta$ -CD was varied in the decompaction studies and all the other parameters were kept constant. The experiments were made with GelStar and DAPI (the former gave a higher contrast). The density and sound velocity experiments have been carried out without fluorescent dye and antioxidant.

**Fluorescent Microscopy.** The fluorescence microscopy study was conducted by placing a drop of the sample on a thoroughly cleaned microscope slide and then placing a coverslip on top of it. The samples were illuminated with a UV-mercury lamp, the fluorescence images of single DNA molecules were observed using a Zeiss Axioplan microscope, equipped with a 100U oil-immersed objective lens, and then digitized on a personal computer through a highly sensitive SIT Cvideo camera and an image processor, Argus-20 (Hamamatsu Photonics, Japan). The apparent long-axis length of the DNA molecules,  $L$ , was deemed as the longest distance in the outline of the fluorescence image of single DNA. Images of the dynamic motion of single DNA-lipid complexes in solution were recorded by using the C-image software obtained from Hamamatsu. The observations were carried out at room temperature.

**Adiabatic Compressibility Measurements.** The adiabatic compressibility, defined by,

$$\beta_{\text{ad}} = (-1/V)(\partial V/\partial P)_S \quad (1)$$

where  $V$  is the volume of the sample and  $P$  is the pressure and can be calculated directly from the density and sound velocity using the expression,

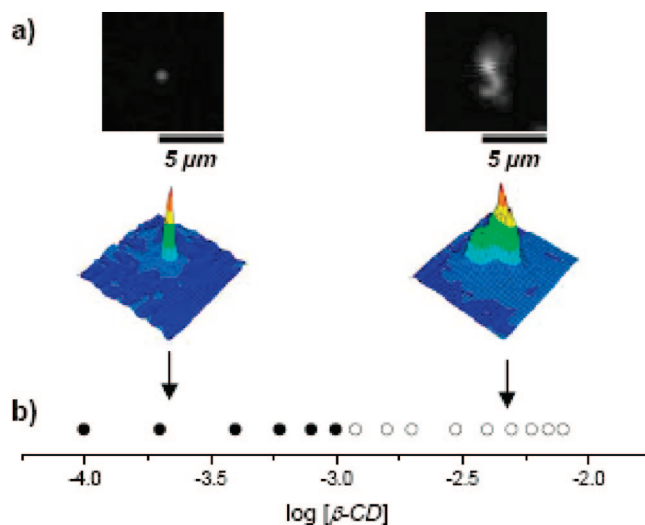
$$\beta_{\text{ad}} = 1/u^2\rho \quad (2)$$

where  $\rho$  is the density and  $u$  is the sound velocity. The changes in density and sound velocities due to the compaction/decompaction process were followed continuously using an Anton-Paar DSA 5000 densitometer and sound velocity analyzer. Because both speed of sound and density are extremely sensitive to temperature, the temperature was kept constant to within  $\pm 10^{-2}^\circ\text{C}$  using the Peltier method. Furthermore, the samples were equilibrated in the instrument for 30 min at  $25^\circ\text{C}$ . The reproducibility of densities and sound measurements was better than  $\pm 5 \times 10^{-6} \text{ g cm}^{-3}$  and  $\pm 10^{-2} \text{ m s}^{-1}$ , respectively.

## Results and Discussion

The conformational state of T4DNA was studied by fluorescence microscopy (FM) following the protocol described in the Experimental Section. By increasing the concentration of  $\beta$ -CD, the DNA was decompacted. Typical fluorescence images and the corresponding intensity profiles are shown in Figure 1a, and the compact and coil states regions as a function of  $\beta$ -CD concentration are indicated in Figure 1b. These images indicate a very sharp transition between the globules and coils with increasing of  $\beta$ -CD concentration.

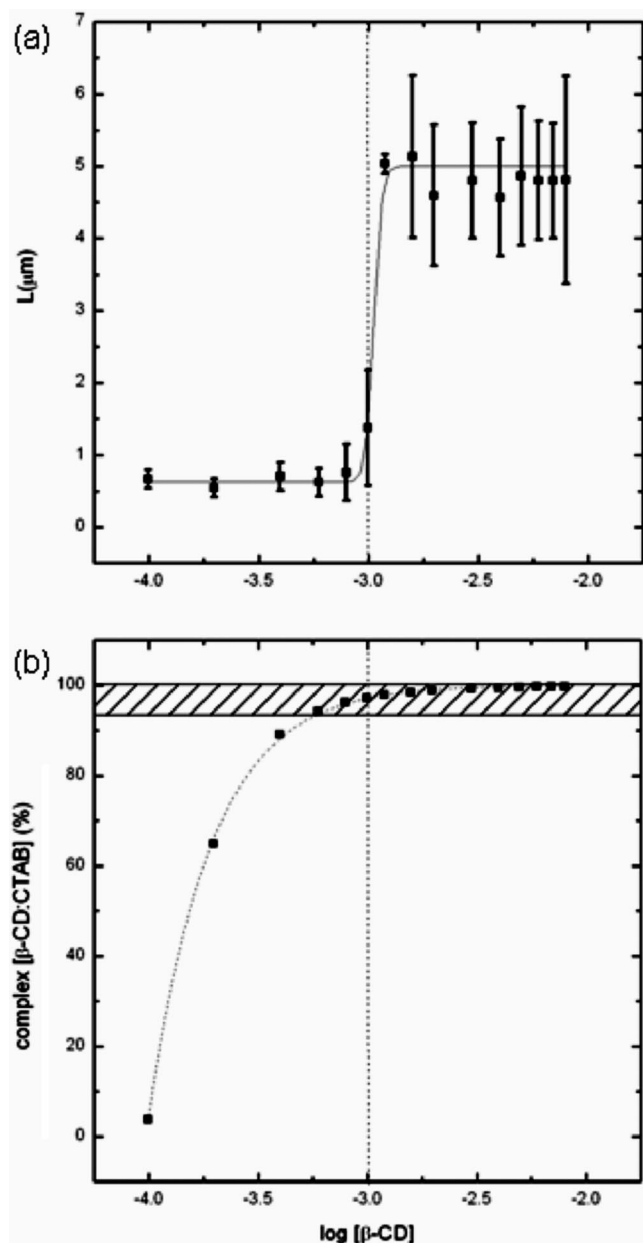
To quantify the compaction/decompaction DNA process, further FM studies were performed and the results in terms of the average length of T4DNA as a function of  $\beta$ -CD concentration are shown in Figure 2a. Every data point is the result of the average of at least 100 images of DNA molecules. At low  $\beta$ -CD concentration, T4DNA exhibits a narrow unimodal size distribution characteristic of the compact state ( $L < 1 \mu\text{m}$ ). At concentrations higher than  $0.01 \text{ M}$ , all the molecules exist in



**Figure 1.** (a) Fluorescent images and the corresponding fluorescent intensity profiles of individual T4 DNA molecules in the globular and coil state. (b) Conformation of single DNA molecules compacted with CTAB as a function of log  $\beta$ -CD concentration at constant CTAB concentration. Filled circles correspond to the globular state and open to the coil state. The experiments were performed using both 4, 6-diamidino-2-phenylindole (DAPI),  $0.5 \mu\text{M}$ , and Gelstar (Cambrex),  $1\times$ , as fluorescent dyes. The results were equivalents using both dyes.

an elongated coil state ( $L = 4\text{--}6 \mu\text{m}$ ). Unlike decompaction obtained by anionic surfactants,<sup>22</sup> these results show no coexistence region between coil and compact states. In Figure 2b, we evaluated the amount of  $\beta$ -CD-CTAB complexes present in solution. The values are expressed in fractions of  $\beta$ -CD-CTAB complexes. This quantity was estimated using the association constants for CTAB and  $\beta$ -CD<sup>23</sup> and assuming that all the surfactant is accessible to the  $\beta$ -CD and that the CTAB has a much higher affinity for  $\beta$ -CD than for DNA. If we compare parts a and b of Figure 2, the results suggest that the DNA decompaction coincides with 98% or more of CTAB bound to  $\beta$ -CD (see hatched region). At lower concentration of  $\beta$ -CD, DNA remains in the compact state DNA, i.e., the surfactant concentration available to DNA is above the cac.

To obtain additional information about the conformational state of DNA as a function of  $\beta$ -CD concentration, we performed adiabatic compressibility measurements at  $25^\circ\text{C}$ . Here we note that the samples were prepared by following the same protocol as used in the fluorescence microscopy experiments, however, these measurements do not require a probe. The adiabatic compressibility has previously been used to investigate the conformational state of proteins in solution,<sup>24</sup> but this is the first time to our knowledge that this approach has been applied to study the compaction/decompaction of DNA in solution. Some of the obtained data are presented in Figure 3, where we show the adiabatic compressibility as a function of the logarithm of the  $\beta$ -CD concentration. The curve shows two well-defined linear regions, and the dramatic change in the slope of the curve suggests changes in the DNA conformation. We also note that the break point coincides with the changes in size reported in Figure 2a. In the globular conformation region, the compressibility is high and almost constant with a low decrease when the  $\beta$ -CD concentration is rising. This is in agreement with the compact state conformation in which we expect to have a higher-order structure formed by T4DNA decorated with some surfactant aggregates. This cluster is likely to be quite hydrated and therefore compressible as well as the surfactant aggregates



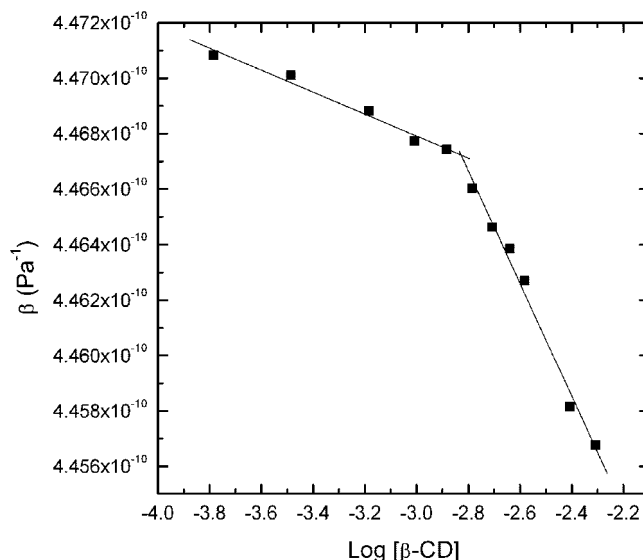
**Figure 2.** (a) Long axis length  $L$  of T4DNA molecules vs  $\beta$ -CD concentration. Error bars indicate the statistical error in size distribution. (b) Fraction of  $\beta$ -CD-CTAB complex as a function of  $\beta$ -CD concentration estimated from the binding constant given in ref 23.

formed within the complex. It is known that the adiabatic compressibility of CTAB micelles is higher than that of the monomers in solution.<sup>25</sup> The slightly decrease in compressibility can be directly related to the increase on  $\beta$ -CD concentration, which has been reported previously when increasing the  $\beta$ -CD concentration at low CTAB concentration.<sup>26</sup>

After decompaction, DNA adopts a coil conformation that is expected to be much less compressible and hence a dramatic drop in compressibility is observed. The reason why compressibility levels out in this region is probably due to the fact that decompaction of DNA occurs before all of the surfactant is removed from the DNA. As discussed above, decompaction occurs when 98% or more of CTAB is bound to  $\beta$ -CD.

### Conclusion

It is well-known that DNA can be compacted with cationic surfactant and that the surfactant aggregates in the surfactant-



**Figure 3.** Adiabatic compressibility, obtained from density and sound velocity measurements at 25 °C for T4DNA compacted using  $2.42 \times 10^{-4}$  M of CTAB at pH = 7.6 as a function of  $\beta$ -CD concentration.

DNA complex is in thermodynamic equilibrium with monomers in solution.<sup>10,27</sup> We have shown that, by using an appropriate cyclodextrin concentration, free surfactant in the bulk will form an inclusion complex. The decrease in the free surfactant concentration will affect the thermodynamic equilibrium in such a way that micelles associated with DNA will be disrupted. This process, which can be regarded as opposite to micelle formation, will eventually lead to a critical concentration where no surface micelles are present and DNA is decompacted. This process can be regarded as a non-first-order transition from globule to coil conformation. The reported procedure using  $\beta$ -CD is a convenient way to decompact DNA when this has been previously compacted using surfactants because the association constant of surfactants with  $\beta$ -CD is large. The surfactant is also removed from the solution by complexation without introducing new ionic species in the bulk that can potentially affect the transcription process in a hypothetical transcription test. The observed decompaction phenomenon was consistently studied by using fluorescence microscopy and adiabatic compressibility measurements. The latter were found to be a promising tool to determine changes of the conformational state of DNA because they do not require probes and can be used for DNA that is too small to be visualized. Furthermore, the adiabatic compressibility can tell us something about the density of the aggregate, i.e., it is a rather convenient way to screen how different compacting agents affect the structure of the DNA-compacting agent complex.

Additionally, other types of cyclodextrins can be used to decompact DNA. Preliminary results in our laboratory show that  $\alpha$ -cyclodextrin is also efficient in DNA decompaction. We expect that this simple approach to DNA decompaction will encourage many studies of ways to decompact DNA by unbinding cationic surfactant compaction agents.

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**Note Added after ASAP Publication.** There was a typographical error in the Conclusion. The sentence “This process can be regarded as a first-order transition from globule to coil conformation.” has been corrected to “This process can be regarded as a non-first-order transition from globule to coil conformation.”. The incorrect version was published ASAP February 8, 2008; the corrected version was published ASAP March 10, 2008.

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