

Complexation Kinetics of Cyclodextrin with Hydrophobic Molecules Confined in an Isolated Droplet in Water

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Received January 18, 2000. In Final Form: May 3, 2000

Inclusion complexation kinetics of methyl- β -cyclodextrin (m- β -CD) to hydrophobic molecules confined in an isolated droplet was investigated with optical tweezers. The radius of the droplet shrank over time as m- β -CD was introduced into the sample chamber due to complexation between the hydrophobic molecules and the m- β -CD molecules. The short time behavior of the droplet shrinkage can be described by a model based on Brownian kinetics. The probability of complexation was greater for a branched 2-hexadecanol than for hexadecane. The probability decreased with increasing chain length for short alkanes. The long time behavior of the droplet shrinkage is surprising because the droplets stopped shrinking even when the uncomplexed m- β -CD concentration was still high. We speculate that surface activities of m- β -CD might have slowed the reaction.

1. Introduction

β -Cyclodextrins (CD) are cyclic oligosaccharides made up of seven glucopyranose units. Their hydrophilic outer surface and lipophilic inner cavity allow them to form water-soluble inclusion complexes with a wide variety of molecules.^{1,2} The ability to form inclusion complexes gives these molecules important applications in areas where selective solubilization, selective molecular transport, and targeted drug delivery are not easily achievable otherwise.^{3–5}

In many applications where CD molecules are used for complexation, the hydrophobic molecules exist in a confined geometry. One example is the use of methyl- β -cyclodextrin (m- β -CD) for removing cholesterol from a plasma membrane.⁶ Another example is the use of CD in emulsion polymerization as transport agents for hydrophobic monomers to synthesize highly hydrophobic polymers.⁷

For insoluble molecules in a confined geometry, their complexation with CD molecules can occur primarily when CD molecules make contact with the confining surfaces. This Letter describes our investigation of the inclusion complexation kinetics in such a configuration. Our experimental approach involved holding a single oil droplet in aqueous solution by an optical tweezer^{8,9} (see Figure 1) and using video microscopy to determine the droplet size as a function of time after the m- β -CD molecules were introduced into the solution. Here, we focus on the scenario

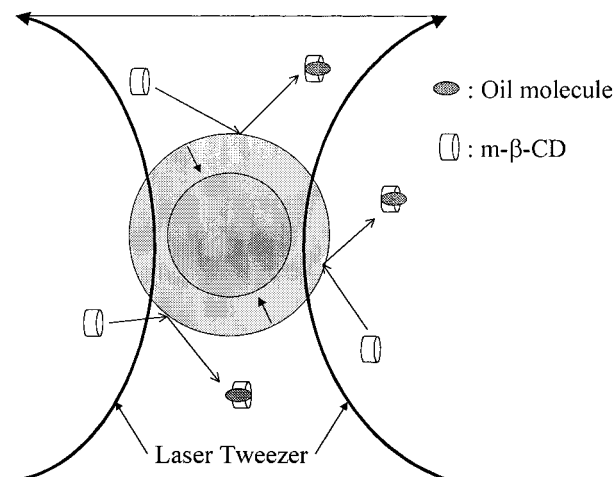


Figure 1. Schematic representation of the inclusion complexation of m- β -CD molecules with oil molecules confined in an optically trapped oil droplet. The oil droplet, trapped by the optical tweezer, shrank as oil molecules were taken away by the β -CD molecules.

where the oil molecules have negligible intrinsic solubility, so that the shrinking of the droplet is due only to the complexation reaction at the droplet surface.

The Theoretical Model

From mass balance, the rate of removal of the hydrophobic molecules from the droplet surface, or surface reaction rate dN_{HP}/dt , can be related to the rate of change in the radius of the droplet (dR/dt) by the expression

$$\frac{d}{dt} N_{\text{HP}} = \frac{4\pi\rho R^2}{M_w} \frac{dR}{dt} \quad (1)$$

where N_{HP} is the number of mole of molecules in the droplet, ρ is the density of the hydrophobic molecules, R is the radius of the droplet at time t , and M_w is the molecular weight of the hydrophobic molecule. Assuming that the rate-determining complexation kinetics is first order, i.e., one m- β -CD molecule and one oil molecule per complex, we can rewrite eq 1 as

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$$\frac{d}{dt} N_{\text{HP}} = -\alpha(\varphi C_{\beta\text{CD}}(t) - \varphi' C_{\beta\text{CD-HP}}(t)) \quad (2)$$

where α is the Brownian collision rate at unit concentration, φ is the probability of forming a complex when m - β -CD molecules collide with the droplet, $C_{\beta\text{CD}}(t)$ is the concentration of free β -CD, φ' is the dissociation probability of the complex upon collision, and $C_{\beta\text{CD-HP}}(t)$ is the concentration of the complexes. The Brownian collision rate at unit concentration, α , according to von Smoluchowski,¹⁰ is

$$\alpha = \frac{2kT(R+r)^2}{3\eta Rr} \approx \frac{2kTR}{3\eta r}$$

for $R \gg r$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of water, and r is the hydrodynamic radius of the m - β -CD molecules.

Since the volume of a single oil droplet (≈ 10 fL) was negligible compared with the volume of the m - β -CD solution in the sample chamber (≈ 6 μL), the depletion of free m - β -CD molecules due to complexation was extremely small. Thus, we can assume $C_{\beta\text{CD}}(t) \approx \text{constant}$ and $C_{\beta\text{CD-HP}}(t) \approx 0$. Equation 2 can then be solved to obtain an expression for the time evolution of the droplet radius

$$R^2(t) = R_0^2 - \frac{\varphi k T M_w C_{\beta\text{CD}} t}{3\pi\eta r} \quad (3)$$

With an experimentally measured $R(t)$, the slope of a plot of $R^2(t)$ vs t would yield the probability of complexation, φ .

2. Experimental Section

The m - β -CD samples (Sigma Chemical Co., St. Louis, MO) were used as received. They were 2,6-methyl- β -cyclodextrin with an average substitution of 1.6 methyl groups per saccharide ring. This substitution gives m - β -CD over 90% solubility in water. The m - β -CD concentrations used were 3.5×10^{-2} to 1.75×10^{-1} M prepared by diluting a stock solution (0.5656 M) with distilled and deionized (DDI) water.

The oil molecules, hexadecane, tetradecane, and decane (Aldrich, Milwaukee, WI) and 2-hexyldecanol (ISOVOL-16, Condea Vista, Houston, TX), were used without further purification. The alkanes were all liquids at room temperature. ISOVOL-16 is a mixture of branched 2-hexyldecanol isomers, which has a melting temperature range of -15 to -21 °C. (We found that the solid droplets formed by low melting temperature alcohols did not shrink with added m - β -CD.)

The flow chamber was made of commercial microscope slides and cover slips. A layer of vacuum grease 12–40 μm thick was used as a spacer between the slide and the cover slip for liquid flow. The total volume of the flow cell was approximately 5–7 μL . The optical tweezer was created at the focal plane of an inverted microscope (Olympus IX-70) by tightly focusing (NA = 1.35 oil objective) a laser beam (Spectra-Physics Millennia Nd:YVO₄ laser at 532 nm wavelength). A schematic diagram of the optical tweezer setup used in this experiment is shown in Figure 2. Our setup is designed to create two tweezers simultaneously, but only one optical tweezer was used in this study.

Oil droplets with radii (R_0) between 1 and 2.5 μm were prepared by sonicating a mixture of oil and water. The oil droplets in the present experiment were prepared without the use of surfactant. We isolated a single droplet by selectively trapping the droplet by the optical tweezer and gently flushing the rest of the droplets out of the sample chamber. The single droplet approach avoids the potential of droplets coalescing during experiments.

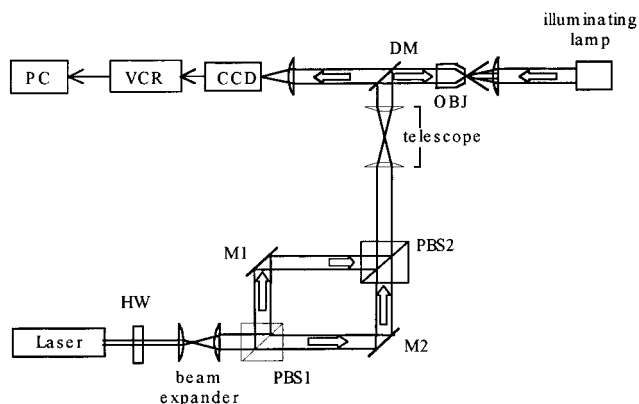


Figure 2. Schematic of the experimental setup of optical tweezers. HW is a half-wave plate, PBS1 and PBS2 are polarizing beam splitters, M1 and M2 are mirrors, and DM a dichroic mirror. The sample chamber is located directly to the right of a high NA microscope objective OBJ. Two optical traps can be set up in the scheme, but only one trap was used in the current study.

Solutions of m - β -CD of known concentrations were flowed into the sample cell, and the radius of the droplet, R , was measured as a function of time using video image analysis. We used a CCD camera (MTI CCD72) and a S-VHS video recorder (Mitsubishi HS-U770) to record the time evolution of the droplet images during the reaction. The recorded image frames were analyzed to give droplet radii. Since there was an error of 15–20% in using video image analysis to measure the droplet radius, each measurement was repeated multiple times. To minimize heating of oil droplets by optical tweezers, less than 2 mW laser power at the focal point of the optical tweezer was applied. It is estimated to have caused < 1 K raise in local temperature near the droplet.

3. Results and Discussions

To ensure that the reduction of droplet radius was not caused by the intrinsic solubility of these hydrophobic molecules at the droplet surface, we measured the radius of an optically trapped droplet over time in the absence of m - β -CD. For all the oil samples examined, we did not find any reduction in the radius of the droplet held by the optical tweezer over a duration of 10 min, data not shown. Figure 3 shows the square of the radius (R^2) vs time for both the 2-hexadecanol and the tetradecane droplet for $C_{\beta\text{CD}} = 0.035$ M at $T = 293$ K. In both main figures, the $t = 0$ mark corresponds to the time of injection of m - β -CD into the flow cell. The insets show the behavior of the droplet radius for the entire observation times, including a period of time before the m - β -CD was injected into the sample cell.

Figure 4 shows the short time behavior of each of the oil molecules studied. The linear regressions shown in the figure were used to obtain the value for φ using $r = 0.75$ nm and $\eta = 0.01$ P for the radius of the m - β -CD and viscosity of water, respectively. The results of the reaction probabilities from the short time behavior are summarized in Table 1.

The magnitudes of the complexation probability φ for the alkanes were found to be in the range of 2×10^{-4} to 4×10^{-3} . The 2-hexadecanol (ISOVOL-16) had a larger complexation probability than those of alkanes. If the complexation involved more than one m - β -CD per 2-hexadecanol, we expect to see a clear trend in the calculated φ . For comparison, the φ values relative to that of hexadecane are shown in the table. The complexation probability, φ , demonstrated a weak dependence on $C_{\beta\text{CD}}$, but no clear trend was apparent. We suspect that the variation reflects the uncertainties in our experiments; the errors in estimating the droplet radius by digital image

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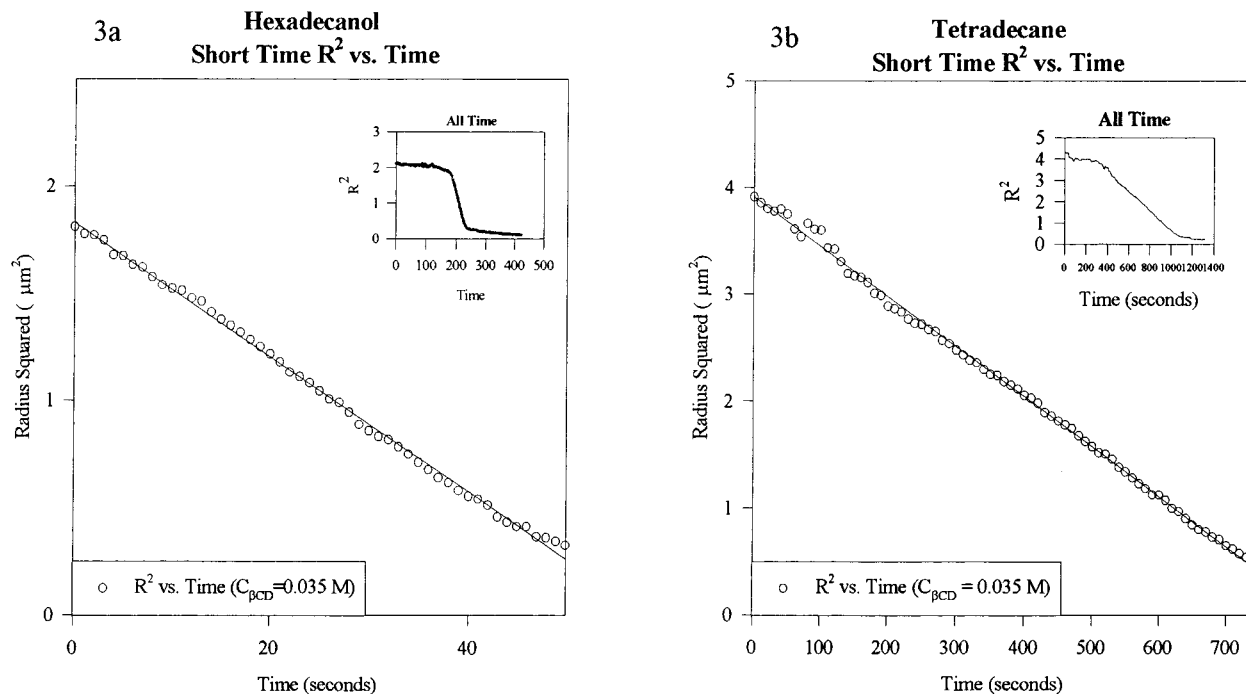


Figure 3. (a, b) Linear behavior of $R^2(t)$ at short times for (a) 2-hexadecanol, and (b) tetradecane is representative of the behavior of the other oils. The inset in each figure shows the time-dependent behavior of the droplets over the whole observation times.

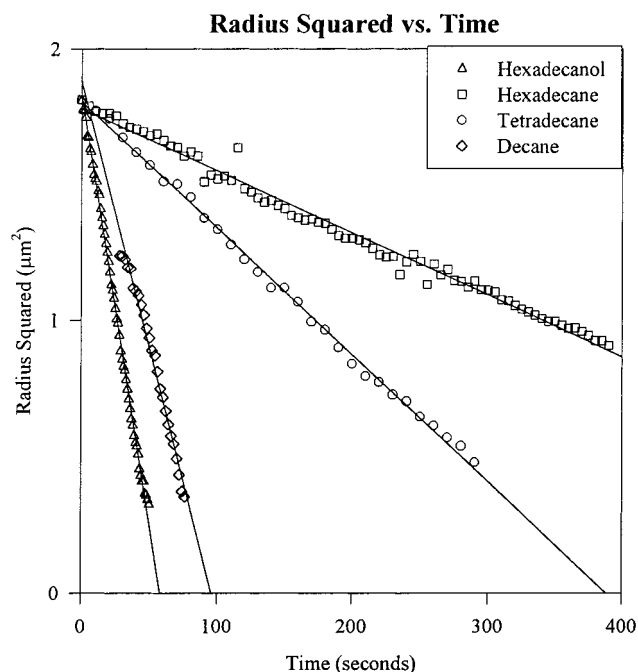


Figure 4. General behavior of R^2 vs time for each of the samples. The concentration of $m\text{-}\beta\text{-CD}$ was 0.035 M for all cases here. Linear regressions were used to calculate φ .

Table 1. Complexation Probabilities

oil/alcohol	$C_{\beta\text{CD}}$ (M)	φ value	rel φ value
decane	0.035	$(4.72 \pm 1.37) \times 10^{-3}$	13.2
tetradecane	0.035	$(1.46 \pm 0.75) \times 10^{-3}$	4.1
hexadecane	0.035	$(3.57 \pm 1.08) \times 10^{-4}$	1
2-hexadecanol	0.035	$(6.08 \pm 0.92) \times 10^{-3}$	17.0
2-hexadecanol	0.175	$(9.15 \pm 0.13) \times 10^{-3}$	25.6
2-hexadecanol	0.070	$(15.6 \pm 1.7) \times 10^{-3}$	43.7

analysis were about 15–20%, which could have caused the variations in the φ value.

The value for complexation reaction probability determined for hexadecanol $(6.08 \pm 0.92) \times 10^{-3}$ was an order

of magnitude higher than that for hexadecane $((3.57 \pm 1.08) \times 10^{-4})$. For linear alkanes, the shorter the chain length, the larger the complexation probability. Several studies have found that association constants increase as a function of hydrophobic chain length for hydrophobic molecules in solution with $m\text{-}\beta\text{-CD}$.^{3,11} However, the φ measured here should not be confused with the association constant K_a . The complexation probability reflects a competition between the $m\text{-}\beta\text{-CD}$ molecule–oil molecule interaction and the oil molecule–oil molecule interaction, while the association constant K_a is the ratio of the complexation rate to the dissociation rate under homogeneous conditions.

The behavior shown in the insets of Figure 3 was typical to all the other samples. For all of the observations, the droplets stopped shrinking at a diameter of about $0.5 \mu\text{m}$. This was not an artifact due to optical or video resolution. It is easy to estimate, from the concentration and volume of the sample cell, the variation of $m\text{-}\beta\text{-CD}$ concentration over the duration of the experiments. The concentrations of the free, uncomplexed $m\text{-}\beta\text{-CD}$ when the droplets stopped shrinking were essentially the same as those at time $t=0$, and the concentrations of complexed molecules were negligible ($\approx 10^{-9}$ M). As a matter of fact, according to our model, the oil droplets should completely “dissolve”. This indicates that we may have made an error in the assumptions of our model. One that may be questionable is the assumption that the surface activity of $m\text{-}\beta\text{-CD}$ was negligible. If, for whatever molecular mechanism, a layer of $m\text{-}\beta\text{-CD}$ or complexes were absorbed at the oil–water interface, complexation kinetics could indeed become slower.

4. Conclusions

Inclusion complexation kinetics of methyl- β -cyclodextrin with hydrophobic molecules confined in a single oil droplet has been investigated with the optical tweezer technique

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coupled with digital video microscopy. Experimental results showed that the probabilities for complexation increased with decreasing alkane chain length. Furthermore, the complexation probability for 2-hexadecanol was much greater than that of hexadecane. These results demonstrate that a higher association constant for hydrophobic molecules in solution does not necessarily correspond to a more efficient complexation process when considering complexation at a hydrophobe–aqueous interface. Results also demonstrated a weak dependence of the probability for complexation on the concentration of m- β -CD used, although this dependence cannot be clearly defined from current data.

The short time behavior of the droplet shrinkage supports a simple kinetic model presented here. The long time behavior, where droplet shrinkage stopped prematurely, was not expected. However, a m- β -CD rich layer at the oil–water interface could explain the slowing down

of droplet shrinkage. A more thorough investigation of the surface activity of m- β -CD at the oil–water interfaces is in progress. This new method is found effective for studying molecular reaction in a confined geometry. Because the sample required for each experiment is less than a few nanograms, the method can be attractive for studying nanoscale biological samples.

Acknowledgment. The work was supported in part by a grant from NSF (CTS-9805887). E.E.M. was also supported by the NSF-REU program at Lehigh University. H.D.O.-Y. appreciates the discussion with Carlos Marques concerning the possibility of cyclodextrin surface activities. Comments from Andy Bunn, Kristin Weidemaier, and Jack C. Thibeault of Rohm and Haas Co. are greatly appreciated.

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