

Study of the Interaction between Progesterone and β -Cyclodextrin by Electrochemical Techniques and Steered Molecular Dynamics

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The interaction of progesterone with β -cyclodextrin (β -CD) was studied by differential pulse polarography. The aim of the present work was to study the effect of β -CD on the electrochemical behavior of progesterone in aqueous solution and also to analyze the molecular interactions involved in formation of the inclusion complex. The complex with stoichiometry of 1:1 was thermodynamically characterized. In addition, steered molecular dynamics (SMD) was used to investigate the energetic properties of formation of the inclusion complex along four different pathways (reaction coordinates), considering two possible orientations. From multiple trajectories along these pathways, the potentials of mean force for formation of the β -CD progesterone inclusion complex were calculated. The energy analysis was in good agreement with the experimental results. In the β -CD progesterone inclusion complex, a large portion of the steroid skeleton is included in the β -CD cavity. The lowest energy was found when the D-ring of the guest molecule is located near the secondary hydroxyls of the β -CD cavity. In the most probable orientation, one intermolecular hydrogen bond is formed between the O of the C-20 keto group of the progesterone and a secondary hydroxyl of the β -CD.

1. Introduction

Cyclodextrins (CDs) are α -1,4-linked cyclic oligomers of D-glucopyranose with their hydrophilic groups on the outside of the molecule and a relatively nonpolar hole down the middle. This hole can form noncovalent inclusion complexes with a variety of guest molecules. Therefore, the inclusion ability of CDs has been used in various fields. Traditionally, they have been used as complexing agents to increase the solubility of poor water-soluble drugs.¹ More recently, they have been applied in other areas: they serve as chiral microenvironments for asymmetric induction² and are used as mobile-phase additives for capillary electrophoresis³ as well as for stationary phases in various types of chromatographies.⁴ Additionally, they have been employed for immobilizing proteins on polymers,⁵ metal surfaces,⁶ and nanoparticles⁷ via supramolecular associations, for enzyme biocatalysis,⁸ as chaperones for enzymes,⁹ etc.

The inclusion of small molecules in the CD cavity induces modification of the physicochemical properties of the "guest" molecules, particularly in terms of water solubility and solution stability.^{10,11} Besides, other properties of an encapsulated molecule are modified by interaction with CDs: photostability,¹² suppression of the bitterness,¹³ reduction of the irritating character,¹⁴ etc. One important aspect is to know optimized CD-containing drug formulations, since a less optimum drug bioavailability can result due too much or too little CD.

The inclusion of steroids on CDs has been reported as an alternative for establishing more efficient therapies.¹⁵ Progesterone has been used in antbirth control and as postmenopausal

therapy.¹⁶ The use of progesterone in therapeutic area is limited since it is slightly soluble in water and is poorly absorbed by oral ingestion unless micronized and in oil. This last formulation may cause allergic reactions in some people. The formation of inclusion complexes with various cyclodextrins showing notable improvement of solubility has been reported.¹⁷⁻¹⁹

The aim of the present work is to investigate the thermodynamics and conformational requirements of the β -CD progesterone complex using a combination of experimental and theoretical approaches. We used differential pulse polarography for obtaining the association constant and thermodynamics parameters involved in formation of the inclusion complex. The orientation and the most probable conformations of the complex were determined using steered molecular dynamics (SMD).

Electrochemical techniques have been used to study host-guest complexation obtaining association constant and thermodynamic parameters.^{20,21} Molecular dynamics (MD) simulations and computational studies on host-guest interactions have been reported for cyclodextrins, but there is no report on SMD.^{22,23} Problems related to molecular modeling of cyclodextrins were extensively discussed in the literature. They include both energy calculation methods²⁴ and sampling problems.²⁵ In this work, SMD is applied with the aim of describing detailed thermodynamics and exploring the conformational space of the β -CD progesterone complex.

2. Materials and Methods

2.1. Reagents and Solutions.

β -CD was obtained from Calbiochem and was used without prior purification. Progesterone was supplied by Sigma. All the other reagents employed were of analytical grade. The supporting electrolyte was Britton-Robinson (BR) buffer prepared in the usual way by adding appropriate amounts of concentrated sodium hydroxide to orthophosphoric acid, acetic acid, and boric acid (0.1 M in

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each). pH measurements were carried out with a WTW microprocessor-controlled standard pH ion meter pMX 3000/pH equipped with a glass pH electrode Sen Tix 81. The standard solutions used for calibration were WTW 4.006, 6.865, and 9.180. Stock solutions of the progesterone were prepared by dissolving an appropriate amount of the steroid in ethanol. All solutions were prepared with ultrapure water ($\rho = 18.2 \text{ M}\Omega \text{ cm}$) from a Millipore Milli-Q system. All the polarographic experiments were obtained after bubbling with N_2 for 10 min in the cell before each run; during measurements, a stream of N_2 was passed over the solution.

2.2. Differential Pulse Polarography and Chronocoulometry. Electrochemical experiments were performed with a totally automated BAS-50 voltammetric analyzer attached to a PC computer with proper BAS 50-W version 2.1 software for total control of the experiments and data acquisition and treatment. A mercury drop electrode (controlling growth mercury electrode, CGME stand of BAS) with a drop area of 0.42 mm^2 as working electrode and platinum as a counter electrode were used. All potentials were measured against Ag/AgCl . For differential pulse polarography (DPP) the CGME stand was used in a CGME mode. Operating parameters were as follows: scan rate 4 mV/s , pulse amplitude 50 mV , sample width 17 ms , pulse width 50 ms , drop time 1000 ms . All experiments were carried out at controlled temperature using a 10 mL thermostatted cell.

Differential pulse polarographic experiments were carried out by keeping constant concentration $1 \times 10^{-4} \text{ M}$ of progesterone in a mixture of 10/90 ethanol/0.1 M BR buffer while varying concentrations of $\beta\text{-CD}$ ($0\text{--}4 \text{ mM}$). The solutions were shaken thoroughly for 10 min and allowed to equilibrate at adequate temperature. Five temperatures were studied: 22, 25.5, 30, 34.5, and $38 \text{ }^\circ\text{C}$. The current titration equation has been described as follows:^{26,27}

$$\frac{1}{[\text{CD}]} = K_i \frac{(1 - A)}{I_p - I_{p,0}} - K_i \quad (1)$$

where K_i is the apparent association constant and $I_{p,0}$ and I_p are the peak currents without and with $\beta\text{-CD}$, respectively. A is a constant.

By using the Gibbs and van't Hoff equations, ΔH , ΔS , and ΔG were calculated as follows:

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

$$\ln K_i = \Delta S/R - \Delta H/RT \quad (3)$$

where K_i represents the complex stability constant $K_{1:1}$. A plot of $\ln K_i$ versus $1/T$ produces the slope $= \Delta H/R$ and the intercept $= \Delta S/R$.

Chronocoulometric measurements were performed for $1 \times 10^{-4} \text{ M}$ of progesterone in a mixture of 10/90 ethanol/0.1 M BR buffer containing various concentrations of βCD ($0\text{--}15 \text{ mM}$). Chronocoulometric curves obey the following equation:²⁸

$$Q = \frac{2nFAC(Dt)^{1/2}}{\pi^{1/2}} + Q_{\text{dl}} + Q_{\text{ads}} \quad (4)$$

wherein Q is the amount of charge in coulombs that has passed at time, t , since the application of the potential step, C and D are the concentration and diffusion coefficients of the reactants, respectively, A is the electrode area, n is numbers of electrons, F is the Faraday constant, Q_{dl} is the double-layer charge (integration of charging current), and Q_{ads} is the faradaic component given by the reduction of adsorbed species. There-

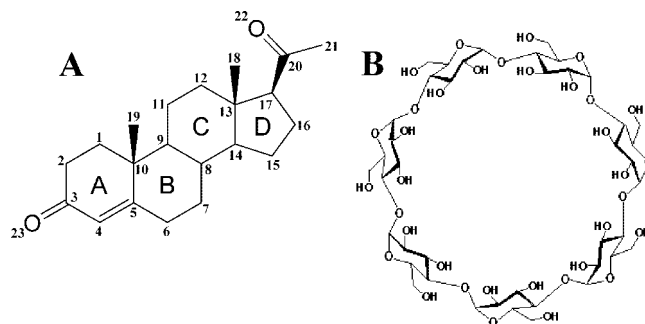


Figure 1. Molecular structures of progesterone and β -cyclodextrin.

fore, from the slope of the linear plots obtained between Q and $t^{1/2}$ (Anson plot) the diffusion coefficient was determined.

2.3. Molecular Structures for Molecular Simulation. The structure of the progesterone was constructed using Sybyl (Tripos, Inc., St. Louis, MO) and then geometrically optimized using the semiempirical quantum-chemical method PM3.²⁹ Mulliken charges on progesterone were calculated at the Hartree–Fock level with the 6-31G* basis set using Gaussian (Gaussian Inc., Pittsburgh, PA). Progesterone was parametrized according to the CHARMM27 parametrization protocol.³⁰ We modified the cholesterol parameters; missing bond, angle, and dihedral parameters were estimated from similar terms within the force field. To set an adequate charge for progesterone, a charge of 0.09 on H bonded to sp^3 carbons was assumed, as it is required by the CHARMM protocol, scaling the Mulliken charges of the bonded atoms to get the desired charges, and consistent with the known parameters. The charges and parameters are available as Supporting Information.

The starting $\beta\text{-CD}$ structure was taken from the “BCD” entry of the Biological Magnetic Resonance Data Bank (<http://www.bmrb.wisc.edu>). This structure was fully minimized (CHARMM27 carbohydrate parameters were employed³¹), and good agreement with the experimental geometry was obtained.³²

2.4. Steered Molecular Dynamics. An MD simulation approximates the motion of atoms in a system of molecules over short intervals of simulated time, typically in the order of picoseconds to nanoseconds. By adding additional restraints, a simulation may be steered to observe the possibility of particular behaviors or to eliminate others over shorter time scales. From this idea, there is increasing interest in SMD, in which external forces or restraints are added to the system in order to study behaviors that might take too long to appear, or to appear often enough, in an unrestrained MD simulation. Such simulations have been widely used to investigate the dynamic behavior of biological systems ranging from identification of ligand binding pathways^{33–36} to the identification of intermediates of unfolding process.^{37–39}

Four models were prepared for SMD studies. In all, $\beta\text{-CD}$ was placed in the origin ($x = 0$; $y = 0$; $z = 0$), with the secondary hydroxyls facing the negative z -axis and the primary hydroxyls facing the positive z -axis (all the glycosidic oxygen atoms near the xy plane). From these models, four pathways were carried out in which the progesterone enters inside the $\beta\text{-CD}$ cavity exploring two orientations O1 and O2 from two initial positions P1 and P2. The reference atoms of the progesterone (atoms on which the steering force was applied: C20 and O23, Figure 1) were placed 2 \AA from the origin in both senses of the z -axis as indicated in Figure 2.

Before SMD simulations, the $\beta\text{-CD}$ progesterone complex was solvated with TIP3^{40,41} water molecules using the SOLVATE command in VMD.⁴² The dimensions of the water box

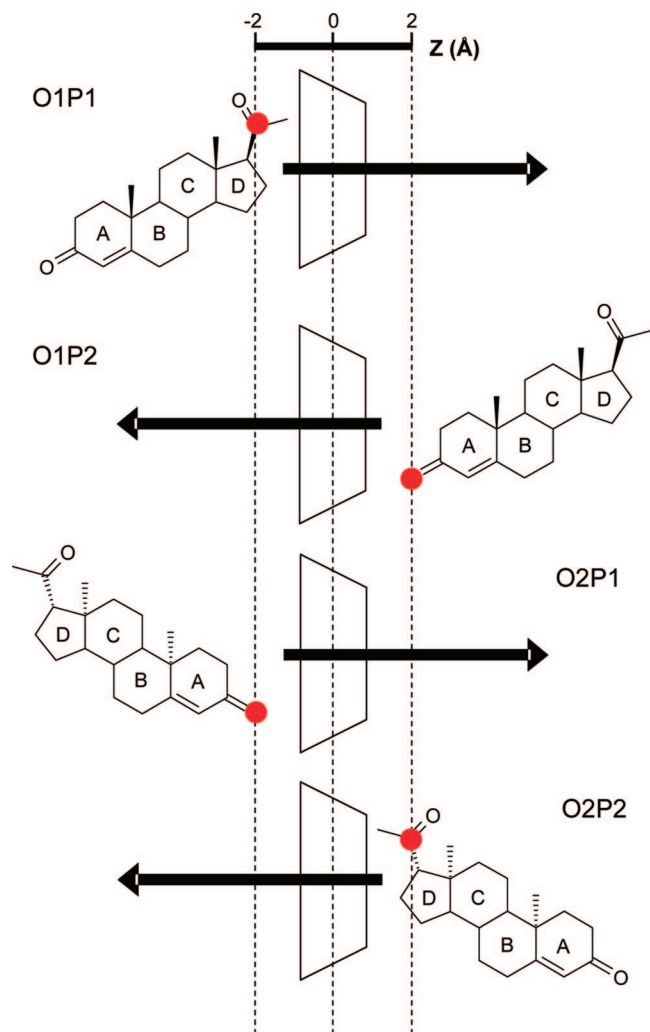


Figure 2. Four pathways along which progesterone was pulled are illustrated by arrows. The pathways were named according to orientation (O) and initial position (P): O1P1, O1P2, O2P1, and O2P2. Red circles indicate the reference atom (atom on which the steering force is applied).

TABLE 1: Thermodynamic Analysis of the β -CD Progesterone Inclusion Complex

K_i ($T = 22$ °C)	126 ± 5
K_i ($T = 25.5$ °C)	220 ± 29
K_i ($T = 30$ °C)	478 ± 18
K_i ($T = 34.5$ °C)	495 ± 35
K_i ($T = 38$ °C)	360 ± 14
slope ^a (K)	-6629
intercept ^a	27.58
ΔH (kcal/mol)	-13.2
ΔS (cal/kmol)	54.8
ΔG° (kcal/mol)	-29.51

^a Equation: $\ln K_i = (\Delta S/R) - (\Delta H/R)T^{-1}$.

were $40 \text{ \AA} \times 40 \text{ \AA} \times 70 \text{ \AA}$, which ensures the whole surface of the complex to be covered along the trajectory through the z -axis. All simulations were carried out using the parallel molecular dynamics program NAMD2⁴³ and the CHARMM27 force field.³⁰ Newly developed parameters for β -CD and progesterone were used. Temperature was controlled by Langevin dynamics. Periodic boundary conditions were applied to obtain consistent behavior. The particle mesh Ewald (PME) method⁴⁴ was employed for computation of electrostatic forces; a cutoff of 12 \AA was used for computing the direct sum of the forces. An integration time step of 1 fs was assumed. The system

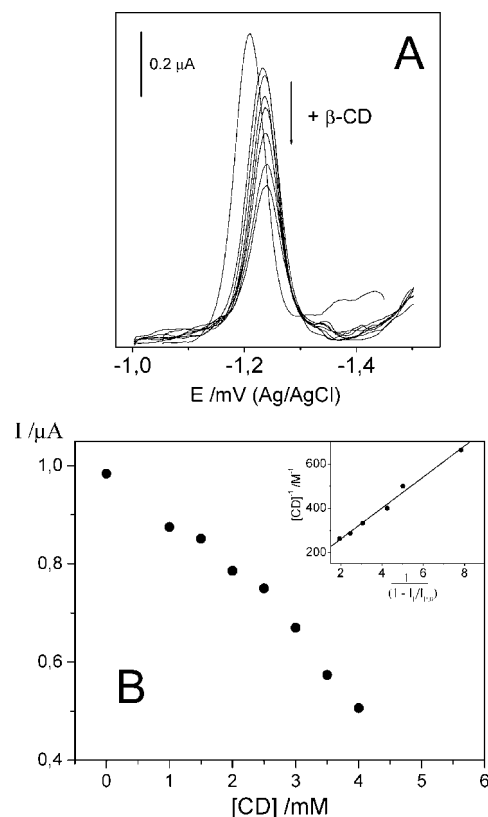


Figure 3. (A) Effect of β -CD concentration on the polarographic behavior of 0.1 mM progesterone solution in a mixture of 10/90 ethanol/0.1 M BR buffer. (B) Dependence of peak current (I_p) on β -CD concentration. Inset: linear plot of eq 1.

was first relaxed for 1 ns . Glycosidic oxygens of the β -CD and O23 and C20 of the progesterone were restrained with a 0.5 and $50 \text{ kcal/mol/\AA}^2$ spring constant, respectively, during relaxation. The final state was saved as a restart point for further SMD study.

Afterward, SMD simulations were performed. In this process, external steering forces were applied on the reference atoms (C20 or O23) to pull the progesterone inside the β -CD cavity, cross along the host cavity, and expose to the solvent again. The pulling velocity was 10 \AA/ns , slower than those used in some previous SMD studies in biological systems.^{34–36} A spring constant of $2.5 \text{ kcal/mol/\AA}^2$ was used to constrain progesterone, and it was large enough to ensure the use of the stiff-spring condition.⁴⁵ During each SMD, the force was only applied along the pulling direction. Progesterone was free from constraint in the plane perpendicular to the pulling direction. The trajectories were saved for every 5 ps , and steering forces were recorded every 0.5 ps . Each trajectory along the four pathways was repeated four times.

The potential of mean force (PMF) is the free energy profile along the reaction coordinate and is determined through the Boltzmann-weighted average over all degrees of freedom other than the reaction coordinate. PMF basically captures the energetics of the process studied. SMD is a nonequilibrium process, since it steers a system by applying a constraint that moves along a prescribed path (a reaction coordinate) in the configuration space. Despite that PMF is an equilibrium property, one must employ nonequilibrium descriptions for the analysis of SMD results. Such a theory has become available through the Jarzynski's equality.^{46,47} This identity connects the ensemble average of an exponential of the total work (W) performed on the system during a nonequilibrium transition from

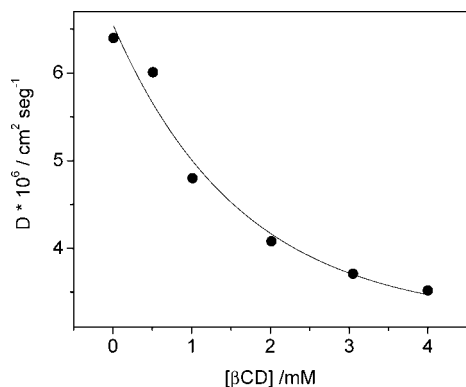


Figure 4. Changes in the observed diffusion coefficients D_{obs} of progesterone in solution of 10/90 ethanol/0.1 M BR buffer vs β -CD concentration.

one state to another to the free energy difference between the two states. According to this, PMF can be derived from SMD trajectories where the error bound on this construction is proportional to the irreversible work done.⁴⁸

Assuming a single reaction coordinate z , and an external potential $U = K(vt - (z - z_0))^2/2$, where K is the stiffness of the restraint and z_0 is the initial position of the restraint point moving with a constant velocity v , the pulling force at time t exerted on the system can be calculated using the following equation:

$$F(t) = K(vt - (z(t) - z_0)) \quad (5)$$

The external work W can be obtained by integration of the force along the pathway from SMD trajectories:

$$W(z) = \int F(z) dz \quad (6)$$

Jarzynski's equality can be used to derive the PMF or free energy difference (ΔG°) from the work W as follows:

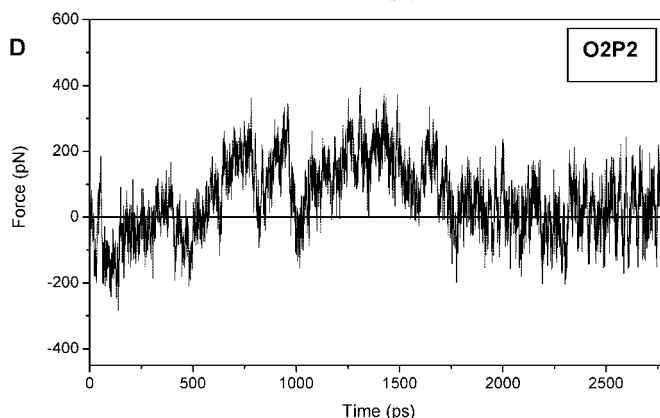
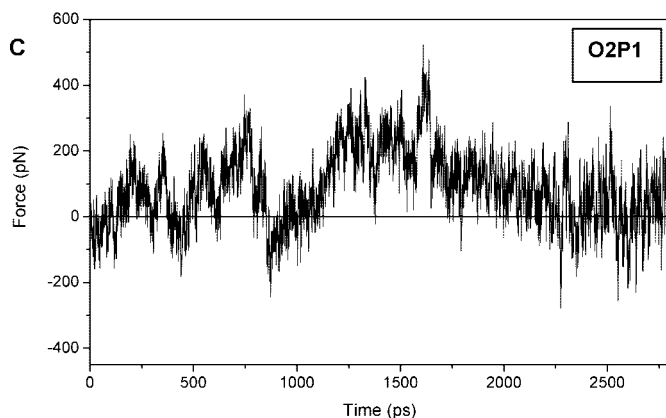
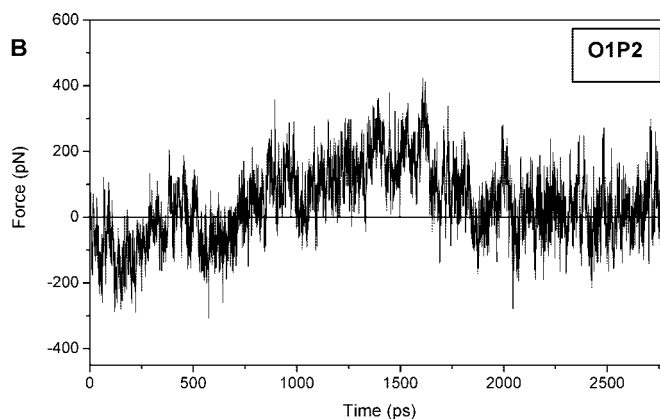
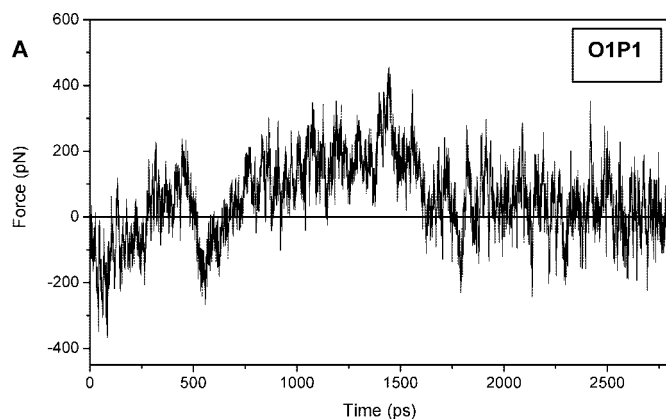


Figure 5. Force profiles in pulling progesterone through four different pathways: O1P1, O1P2, O2P1, and O2P2.

$$\Delta G^\circ = -RT \ln \left\langle \exp \left(-\frac{W}{RT} \right) \right\rangle \quad (7)$$

where R is the universal gas constant and T is the absolute temperature. Equation 7 is valid under the assumption of a converged average, formally needing an infinite number of realizations of the process. Because of the difficulty in estimating the exponential average, a second-order cumulant expansion can be employed, considering that the work distribution from overdamped Langevin dynamics satisfies a Gaussian distribution:⁴⁵

$$\Delta G^\circ = \langle W \rangle - \frac{1}{2k_B T} (\langle W^2 \rangle - \langle W \rangle^2) \quad (8)$$

where $\langle W \rangle$ is the mean work averaged from all trajectories and the second term encloses the standard deviation of the work distribution. Low-order cumulants are relatively easier to estimate from limited sampling; it has been demonstrated that an approximate formula (eq 8) may work better than the exact formula (eq 7) for these conditions.⁴⁵

3. Results and Discussion

Differential pulse polarograms showed a single two-electron irreversible cathodic peak over the whole pH range. This peak has been attributed to the reduction of the 3-keto- δ -4 group in the A-ring of the progesterone molecule.⁴⁹ The effect of β -CD concentration on the polarographic behavior of a 0.1 mM progesterone solution is shown in Figure 3A. As can be seen, the peak current intensity (I_p) decreases progressively with the β -CD concentration. Besides, there is a change on the reduction peak potential (E_p) of 24 mV with the first addition of β -CD, but no significant potential change was noted with additional increase of β -CD concentration. The formation of the inclusion complex causes the decrease of the I_p and the shift of the E_p .

TABLE 2: Description of Force Profiles for the Four Pathways

pathway	t intervals (ps)	slope of the applied F	V_{el} ($\text{\AA}/\text{ns}$) ^a	description
O1P1	0–60	–	31	progesterone partially enters the β -CD cavity
	60–450	+	3	interaction between D-, C-rings of the progesterone and β -CD cavity
	450–540	–	33	above interaction is broken, and the progesterone moves along the β -CD cavity
	540–1450	+	6	interaction between C-, B-, A-rings of the progesterone and β -CD cavity
	1450–1670	–	20	above interaction is broken, and the progesterone gradually stopped making contact with the β -CD
O1P2	1670–2800	~ 0	10	progesterone is pulled through the water
	0–150	–	20	progesterone partially enters the β -CD cavity
	150–450	+	4	interaction between ring A of the progesterone and β -CD cavity
	450–570	–	22	above interaction is broken, and the progesterone moves along the β -CD cavity
	570–890	+	3	interaction between B-, C-rings of the progesterone and β -CD cavity
	890–1020	–	22	above interaction is broken, and the progesterone moves along the β -CD cavity
	1020–1620	+	6	interaction between D-ring and acetyl group of the progesterone and β -CD cavity
1620–1870	–	20	above interaction is broken, and the progesterone gradually stopped making contact with the β -CD	
O2P1	1870–2800	~ 0	10	progesterone is pulled through the water
	0–190	+	8	ring A of the progesterone cannot enter inside the β -CD cavity
	190–440	–	15	ring A of the progesterone enters inside the β -CD cavity
	440–740	+	2	interaction between A-, B-rings of the progesterone and β -CD cavity
	740–870	–	32	above interaction is broken, and the progesterone moves along the β -CD cavity
	870–1600	+	5	interaction between C-, D-rings of the progesterone and β -CD cavity
1600–2270	–	16	above interaction is broken, and the progesterone gradually stopped making contact with the β -CD	
O2P2	2270–2800	~ 0	9	progesterone is pulled through the water
	0–140	–	21	progesterone partially enters the β -CD cavity
	140–390	+	3	interaction between ring D of the progesterone and β -CD cavity
	390–490	–	22	above interaction is broken, and the progesterone moves along the β -CD cavity
	490–780	+	3	interaction between ring C of the progesterone and β -CD cavity
	780–820	–	48	above interaction is broken, and the progesterone moves along the β -CD cavity
	820–960	+	0	interaction between ring B of the progesterone and β -CD cavity
	960–1020	–	54	above interaction is broken, and the progesterone moves along the β -CD cavity
	1020–1310	+	0	interaction between ring A of the progesterone and β -CD cavity
	1310–1780	–	17	above interaction is broken, and the progesterone gradually stopped making contact with the β -CD
1780–2800	~ 0	9	progesterone is pulled through the water	

^a Velocity of pulling considering displacement of the pulled atom in the interval.

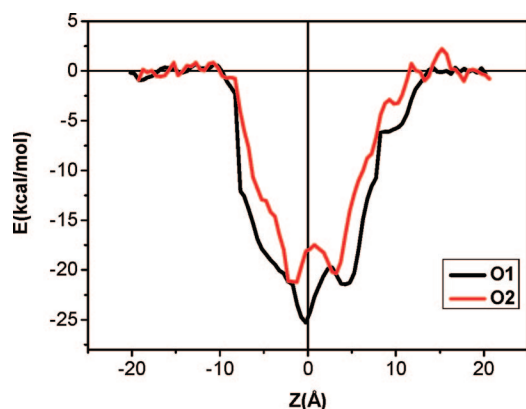


Figure 6. PMF profiles for inclusion of progesterone: black, orientation 1; red, orientation 2.

The decrease of the peak current is due to the decrease of the apparent diffusion coefficient of progesterone, which has formed the inclusion complex with β -CD. The shift of the E_p suggests that the reduction of the inclusion complexes at the mercury electrode needs more activation energy. Nevertheless, this small change is not enough to propose a configuration of the inclusion complex. The analysis with β -CD is only possible with lower concentrations until 4 mM, because an insoluble inclusion complex is obtained with higher concentrations. The decrease of the current is shown in Figure 3B. The association constant (K_i) can be obtained from the linear plot (inset of Figure 3B) according to eq 1. To verify the change in diffusion coefficients, chronocoulometric experiments were performed. Different chro-

nocoulometric curves showed the effect of adding different quantities of β -CD to a solution. A diffusion coefficient value of $6.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for progesterone in the absence of β -CD was determined. This value is similar to values previously reported by other techniques.⁵⁰ As the drug is incorporated into the cavity, its diffusion coefficient is reduced due to the larger complex formed. This decrease is due to the lower diffusion coefficient of the cyclodextrin complex compared with the free guests. The change of the observed diffusion coefficient with increasing of CD concentration has been studied previously.^{51,52} Figure 4 shows the decrease of diffusion coefficients in the presence of different concentrations of β -CD. Therefore, the observed decrease in progesterone diffusion confirms the drug incorporation as the β -CD concentration increases.

The inclusion complex constants obtained at different temperatures are shown in Table 1. Slightly lower values have been obtained using HPLC due to a higher proportion of methanol in the mobile phases.^{53,54} From the corresponding van't Hoff plots (eq 3), the thermodynamic parameters (ΔH and ΔS) were obtained. Thermodynamically, the inclusion complexation of progesterone is driven by both enthalpy ($\Delta H = -13.2 \text{ kcal/mol}$) and entropy ($\Delta S = 54.8 \text{ cal/kmol}$) changes. A negative enthalpy change is attributed to van der Waals interactions between progesterone and the β -CD cavity, whereas positive entropy indicates that the hydrophobic effect (desolvation) is a strong driving force for the progesterone β -CD inclusion complexation.

According to previous reports, steroids which possess A-, B-, C-, and D-rings, are able to penetrate and interact with the

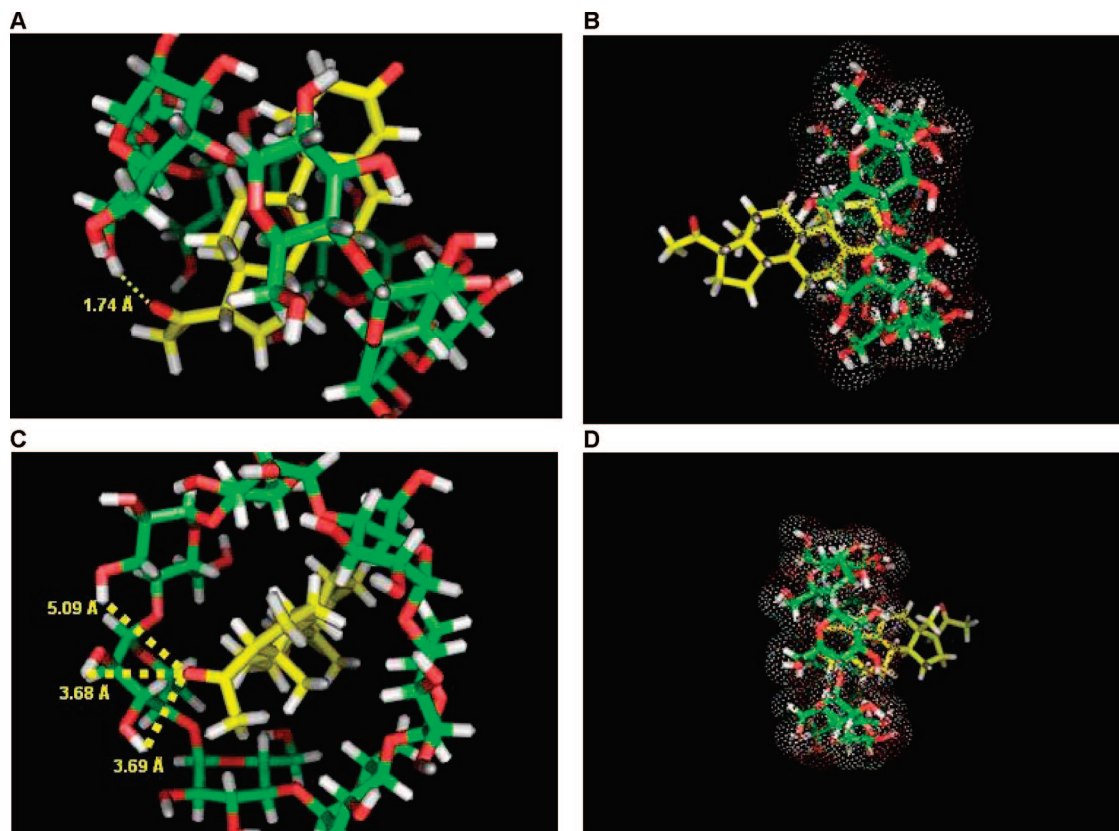


Figure 7. Geometry of inclusion complex at the energy minima for orientations O1 and O2 of the progesterone. O1: (A) $z = -0.24 \text{ \AA}$, (B) $z = 4.74 \text{ \AA}$. O2: (C) $z = -1.26 \text{ \AA}$, (B) $z = 3.22 \text{ \AA}$.

hydrophobic CD cavity by either the A-ring of the steroid molecule or the five-membered cyclopentane ring (D-ring).^{55,56} According to Liu et al.,⁵⁷ the inclusion of the progesterone takes place initially between the D-ring of the steroid molecule and the hydrophobic cavity of β -CD. This 1:1 complex binds with a second β -CD molecule to form the 1:2 complex. In our current experiment, a low concentration of β -CD was used to prevent the formation of 1:2 complex.

In an attempt to explain the progesterone β -CD supramolecular interaction, SMD simulations were carried out. The time scale of the host–guest complexation, of the order of milliseconds or longer, is unreachable for conventional MD simulations which are limited to nanosecond time scales. Energy barriers involved in the inclusion of a guest to a host are too high for the guest to cross the barrier spontaneously on a nanosecond time scale. SMD provides a means of accelerating the process through application of external forces that lower the energy barriers and drive the guest along its inclusion path on nanosecond time scales. By monitoring the forces applied and the response of the guest, it is possible to characterize the pathway and its intermediate states.

Figure 5 shows typical force profiles for pulling progesterone along the four different pathways. The force curves are within typical variations expected in SMD simulations. The force fluctuations around both positive and negative values show that the thermal fluctuation of progesterone is larger than the perturbation from the pulling force, indicating that the process occurs near the equilibrium. The force profiles for the four pathways are described in Table 2. Despite that the pulling velocity was 10 \AA/ns , the advance of the progesterone across the β -CD cavity evolves between slower intervals with a positive slope of the applied force and faster intervals with a negative slope of the applied force. When progesterone leaves the β -CD

cavity, it advances with a velocity around 10 \AA/ns (slope of the applied force ~ 0). Positive and negative slopes of the applied force indicate an increment and decrement, respectively, of the interactions between the guest and the host; slope ~ 0 indicates the absence of interactions when progesterone is pulled through the water media. Specifically, increased force (positive slope) appears when the guest encounters stability due to van der Waals interactions with the β -CD cavity; the system must pass over an energy barrier while breaking this interaction, and the force decreases (negative slope) as the guest leaves this energy barrier. According to this, the inclusion of the progesterone occurs in small steps where the guest is stabilized in each step. During the movement of the guest molecule through the β -CD's interior, van der Waals interactions are established between different parts of the progesterone and the hydrophobic interior of the host. It is recognized that cyclodextrins eventually create a suitable cavity geometry for binding of different classes of hosts.⁵⁸ It became clear that when the progesterone moves through the cavity of the β -CD, the host adopts the conformation that fits best around the guest in each step.

By sampling the external work from repeated trajectories, we calculated the PMF for progesterone inclusion along each pathway using the second cumulant expansion of the Jarzynski's equality (eq 8). Figure 6 shows the results of PMF along the z -coordinate for orientations O1 and O2 of the progesterone. In this figure, the center of mass of β -CD is at $z = 0 \text{ \AA}$, whereas the black and red curves represent the steered movement of progesterone's center of mass through the z -axis for orientations O1 and O2, respectively.

In general, PMFs show low free energy values when progesterone enters the β -CD. Then, the free energy increases when immersion of progesterone in the water media takes place. The qualitative shape of the free energy profiles can be

understood as follows: when the progesterone is inside the β -CD cavity, it is surrounded by a hydrophobic environment which stabilizes the guest by van der Waals interactions. A destabilization takes place when the progesterone is at a particular distance from the β -CD, substituting the hydrophobic environment for the hydration shell.

The PMF for orientation O1 shows two minima. The free energy minimum at pulling distance $z = -0.24 \text{ \AA}$ is achieved when the B- and C-rings of progesterone are inside the hydrophobic β -CD cavity (Figure 7A). In this conformation, a H-bond between O22 of the progesterone and a primary hydroxyl of the β -CD was formed. The other minimum at $z = 4.74 \text{ \AA}$ is produced when ring A of the progesterone is inside the cavity (Figure 7B). The PMF for orientation 2 also shows two minima. In analogy with the PMF profile for orientation O1, the free energy minimum at pulling distance $z = -1.26 \text{ \AA}$ is shown when the B- and C-rings of progesterone are inside the hydrophobic β -CD cavity (Figure 7C). However, in this orientation the H-bond between O22 of the progesterone and secondary hydroxyls of the β -CD cannot be formed since interatomic distances are $>3 \text{ \AA}$. The other minimum at $z = 3.22 \text{ \AA}$ is shown when the ring A of the progesterone interacts with the β -CD cavity (Figure 7D).

As a conclusion, when progesterone is included with orientation O1, the complex is stabilized primarily by van der Waals interactions between the guest and the host cavity and by hydrogen bonds between O of the acetyl group of the progesterone and primary hydroxyls of the β -CD. In contrast, when progesterone is included with orientation O2, the stabilization is due to van der Waals interactions, and the acetyl group of the progesterone cannot interact with secondary hydroxyls of the β -CD. From this analysis, O1 appears to be the preferred orientation between the two possibilities, which is consistent with previous reports.⁵⁷ The energy involved in the inclusion process, according to SMD simulations, was about -26 kcal/mol by taking the average of the difference of the PMF between the lowest minima and ending states along pathways O1P1 and O1P2. This is in good agreement with the experimental ΔG value reported in Table 1.

In a recent report, Yu et al.⁵⁹ examined the host–guest inclusion complexes between cholesterol and three modified CDs using MD simulations and the adaptive biasing force (ABF)⁶⁰ method. As in SMD simulations, the ABF method allows characterizing the molecular association by deriving the PMF along a reaction coordinate. Since a thermodynamic study of the inclusion of a steroid in a CD is the goal of our work, we can establish a comparison between our results and the results of Yu et al. In coincidence with our current report, Yu et al. found free energy profiles with two local minima and a barrier separating them. In the β -CD–cholesterol complex, these authors found a first minimum where the A- and B-rings of cholesterol are buried in the cavity of β -CD and the hydroxyl group of cholesterol is located near the narrow side of the β -CD cavity. Meanwhile, the second minimum is only stabilized by van der Waals interactions and occurs when the cholesterol surpasses an energy barrier of approximately 4 kcal/mol . These characteristics are in agreement to our findings.

4. Conclusions

The inclusion complex between β -CD and progesterone was formed with a stoichiometry of 1:1 and a stability constant $K_i = 220 \text{ M}^{-1}$ at $22.5 \text{ }^\circ\text{C}$, and $\Delta G^\circ = -29.51 \text{ kcal/mol}$. Constant velocity SMD runs revealed that van der Waals contacts established between progesterone and β -CD are of major

importance for the stability of the complex. The movement of the guest molecule through the β -CD's interior occurs in small steps, and at each step the complex is stabilized. A preferred orientation is suggested for complex formation that positions the acetyl group of the progesterone nearer to the primary hydroxyl rim of the cyclodextrin cavity.

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Supporting Information Available: The charges and parameters used in parametrization of progesterone. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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