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Bioactive Permethrin/β-Cyclodextrin Inclusion Complex

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Permethrin is popularly used in a variety of therapeutic areas. However, the poor water solubility of permethrin seriously limits its wider clinical applications. The present study demonstrates that solubility of permethrin in aqueous solution can considerably increase in the presence of β-cyclodextrin (β-CD). Extensive experimental data along with computational modeling reveal the formation of stable permethrin/β-CD inclusion complexes, including permethrin(β-CD) and permethrin(β-CD)$_2$, through hydrophobic binding. Both permethrin(β-CD) and permethrin(β-CD)$_2$ complexes coexisted in aqueous solution, and the ratio of the concentration of permethrin(β-CD) complex to that of permethrin(β-CD)$_2$ complex was dependent on the concentration of β-CD. The complexation of permethrin with β-CD significantly improved the bioavailability of permethrin and, therefore, increased the bioactivity. The significant increase of the bioactivity of permethrin in the presence of β-CD provides an effective approach to improve the practical use of permethrin in public health and agriculture.

Introduction

Permethrin, a synthetic pyrethroid, is one of the two currently available "over-the-counter" agents for the treatment of head lice, one of the most common diseases in the U.S. and in most of the developed world.[1] Recently, the increase of opportunistic infections in HIV-infected patients, like scabies, has developed an increasing interest in formulation of this substance for therapy.[2] The use of permethrin has also been extended for the prevention of malaria in tropical areas. In addition, permethrin is also recommended by the World Health Organization to be used as the active ingredient of public hygiene insecticidal products for treatment of mosquitos, fleas, flies, mites, and cockroaches.[3] However, the poor water solubility of permethrin seriously limits its practical clinical applications. In efforts to overcome the limitation, some approaches, including the use of cosolvents, solubilizers, and surfactants, have been considered to improve the water solubility of permethrin, although all of these existing approaches have their own limitations. A promising approach for the solubilization of synthetic pyrethroids could be the use a cyclodextrin (CD), which imparts its beneficial physicochemical properties through formation of an inclusion complex, as CDs have been used for delivery of other pharmaceutical agents.[4,5] CDs are cyclic oligosaccharides composed of six (α-), seven (β-), or eight (γ-) D-glucopyranose residues linked by α-(1,4) bonds.[6] As important host compounds, CDs have high molecular recognition ability toward guest molecules with a suitable polarity and dimension because of their hydrophobic internal cavity and hydrophilic external surface. This ability has been widely used in pharmaceutical applications with the aim to enhance water solubility, chemical stability, and bioavailability of insoluble or poorly soluble drugs, to reduce toxicity, and to control the rate of release.[7]

Here we report the preparation and characterization of a new type of inclusion complexes formed from permethrin and β-cyclodextrin, with an aim to improve the water solubility and bioavailability of permethrin. The obtained inclusion complexes were found to significantly improve the water solubility of permethrin and demonstrated a 4-fold increase of the insecticidal activity. The work described in this report is based on an integrated experimental−computational approach. Compared to the previously used pure experimental approaches, the integrated experimental−computational approach not only demonstrates some macroscopic information about the solubility and possible intermolecular interactions through wet experimental measurements but also provides valuable information about the microscopic binding through the computational modeling.

Materials and Methods

Materials. All materials were obtained from commercial sources and were used as received unless stated otherwise. Specially, β-cyclodextrin (≥99%) was obtained from Shanghai Boao Life Science and Technology Corp. (China). *trans*-(1*R*,3*R*)-Permethrin was recrystallized from ethanol and dried in low temperature.

Preparation and Experimental Characterization of the Permethrin/β-Cyclodextrin Inclusion Complexes. An ethanol solution of permethrin (0.l mM, 0.0391 g) was added to aqueous solution of β-CD (0.2 mM, 0.227 g) in 80 mL of water. The resulting mixture was stirred at 30 °C for 3 days. After removal of 20 mL of a mixture of ethanol−water under vacuum, the reaction mixture was cooled at 2 °C overnight. The precipitate was filtered and washed by a little hot water in order to remove unreacted β-CD. The obtained residue was dried overnight at 30 °C under high vacuum.

Phase solubility studies were carried out according to Higuchi and Connors' method.[8] An excess amount of permethrin was added to 10 mL aqueous solutions containing different concentrations of β-CDs (0−14 mM). Flasks were sealed to avoid

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changes due to evaporation, and the solutions were magnetically stirred for a week in a thermostated bath at 25 °C. After equilibrium was reached, a small volume of the supernatant was withdrawn, filtered through a 0.45 μm hydrophilic membrane filter.

The concentration of permethrin in the filtrate was determined by UV spectroscopy. UV spectra were recorded on a UV-2550 (SHIMADZU) UV spectrophotometer. A circular dichroism spectrum (CDS) was recorded in a quartz cell (10 mm in length) on a JASCO J-810 spectropolarimeter. Differential scanning calorimetry (DSC) analyses were performed on a NETZSCH DSC 200PC. ESI-MS experiments were performed on a LCQ ion trap mass spectrometer (Finnigan) equipped with an Electrospray ion source.

Insecticidal Activity Assay. Samples were dissolved with dimethylforamide (DMF) and diluted to the required test concentrations with water. Treated with DMF and water leaves were considered as controls. Mites were released on the leaves. These leaves were dipped into the respective test solutions for 15 s. After air-drying, all assays were kept under standard laboratory conditions. Mortality was investigated 24 h later, and IC_{50} values were obtained by the Probit method.

Molecular Modeling. The Tripos molecular modeling software package Sybyl 7.0 [9] was used to construct the starting structures of permethrin, β-CD, and possible permethrin/β-CD complexes. The Sander module of the Amber7 program package[10] was used to perform molecular dynamics (MD) simulations on the structures of the free molecules and complexes. The partial atomic charges of permethrin and β-CD required for the MD simulations were calculated by using the RESP protocol implemented in the Antechamber module of the Amber7 package following electrostatic potential (ESP) calculations at ab initio HF/6-31G* level with the Gaussian 03 program.[11]

The general procedure for carrying out the MD simulations in water is essentially the same as that used in our previously reported other computational studies.[12−14] Each aforementioned starting structure was solvated in a rectangular box of TIP3P water molecules[15] with a minimum solute-wall distance of 10 Å. The solvated systems were carefully equilibrated and fully energy-minimized. These systems were gradually heated from $T = 10$ K to $T = 298.15$ K in 35 ps before a production MD simulation run for 500 ns or longer, making sure that we obtained a stable MD trajectory for each of the simulated systems. The time step used for the MD simulations was 2 fs. Periodic boundary conditions in the NPT ensemble at $T = 298.15$ K with Berendsen temperature coupling[16] and $P = 1$ atm with isotropic molecule-based scaling[16] were applied. The SHAKE algorithm[17] was used to fix all covalent bonds containing hydrogen atoms. The nonbonded pair list was updated every 10 steps. The particle mesh Ewald (PME) method[18] was used to treat long-range electrostatic interactions. A residue-based cutoff of 10 Å was utilized to the noncovalent interactions. The coordinates of the simulated systems were collected every 1 ps during the production MD stages.

The computations were performed on a supercomputer (Superdome, with 256 shared-memory processors for parallel computing) at University of Kentucky Center for Computational Sciences and on SGI Fuel workstations and a 34-processors IBM x335 Linux cluster in our own laboratory.

Results and Discussion

Water-Solubility and Bioactivity of Permethrin in the Presence of β-CD. One of the most important applications of cyclodextrins in pharmaceutical sciences is to enhance the water solubility of insoluble organic compounds through complexation. In the absence of β-CD, permethrin is insoluble in water, with a solubility of $\sim 2 \times 10^{-4}$ g/L or ~500 nM.[19] Depicted in Figure 1 is the phase-solubility diagram, i.e., plot of the solubility of permethrin as a function of the β-CD concentration ([β-CD]), of the complex formation between permethrin and β-CD. As seen in Figure 1, the solubility of permethrin in water considerably increases by increasing the concentration of β-CD. The considerable increase of the water solubility of permethrin is likely due to the formation of some soluble inclusion complexes between permethrin and β-CD. A β-CD-involved phase-solubility diagram usually shows some features to reflect the composition of the formed inclusion complexes.[20−22] Figure 1 indeed shows some interesting features. The water solubility of permethrin increases almost linearly with increasing [β-CD] when [β-CD] < ~4 mM. The water solubility of permethrin also increases linearly by increasing [β-CD] when [β-CD] > ~8 mM. However, the solubility increase is negligible from [β-CD] = ~4 mM to [β-CD] = ~8 mM. These features imply that the composition of the formed permethrin/β-CD inclusion complex changes at the high β-CD concentration. In general, if the inclusion complex has a composition as $(\text{permethrin})_n(\beta\text{-CD})_m$ (i.e. the host:guest ratio is defined as m:n) when [β-CD] < ~4 mM, then it is likely that the inclusion complex becomes $(\text{permethrin})_n(\beta\text{-CD})_{2m}$ (i.e. the host:guest ratio becomes $2m$:n) when [β-CD] > ~8 mM. Specific host:guest ratios cannot be determined by using Figure 1 alone but can be determined otherwise (see below).

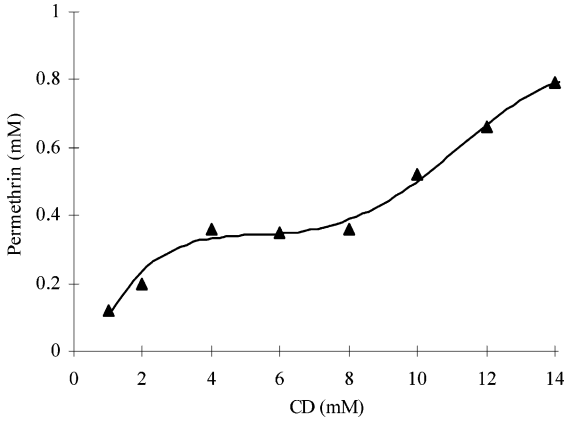

Figure 1. Phase solubility diagram for permethrin in the presence of β-CD in water at 25 °C.

Bioassay revealed that the IC_{50} value of the inclusion complex against mites is 97.5 nM, whereas the IC_{50} value of permethrin against mites is 385 nM. This means that the complexation of permethrin with β-CD significantly improved the bioavailability of permethrin due to the improvement of the water solubility.

Spectroscopic Properties of Permethrin/β-CD Complex. The UV spectrum depicted in Figure 2 indicates that the complexation of permethrin with β-CD resulted in a slight red shift (7 nm) of the UV absorption band compared to that of permethrin, which may be explained by the high electron density of the guest (permethrin) located inside the β-CD cavity.[23] DSC analysis further supports the formation of a permethrin/β-CD inclusion complex. As seen in Figure 3, permethrin showed a thermogram endothermic peak at 43.3 °C and β-CD demonstrated thermogram endothermic peaks at 117.5 and 261.3 °C, whereas the thermogram endothermic peaks of the permethrin/β-CD inclusion complex appeared at 104.6 and 277.2 °C.

Mass spectrometry (MS) has become an increasingly interesting tool for examination of noncovalent complexes because it

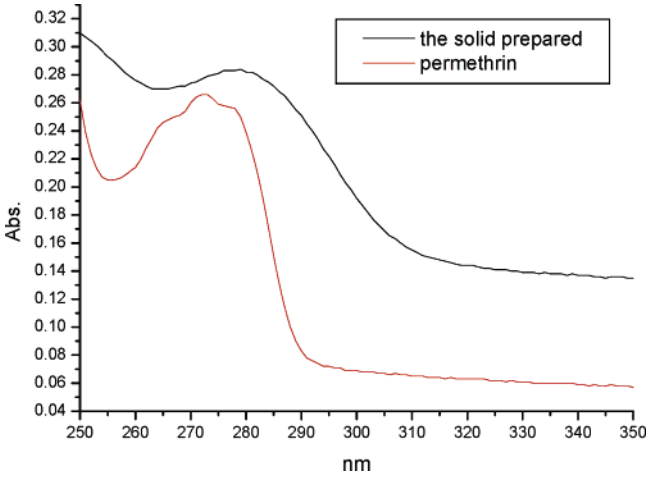

Figure 2. UV absorption spectra of permethrin in ethanol and the permethrin/β-CD inclusion complex in water.

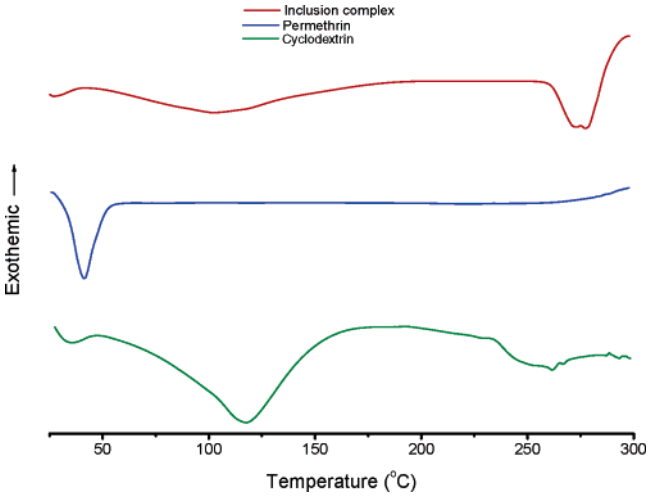

Figure 3. DSC thermograms of permethrin, β-CD, and permethrin/β-CD inclusion complex.

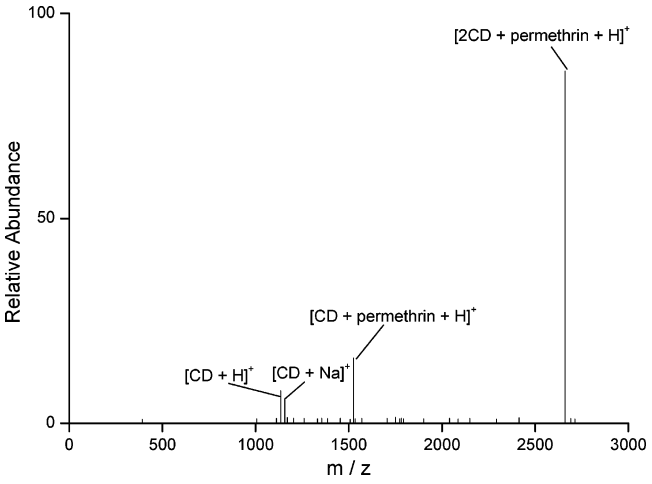

Figure 4. ESI-MS of the permethrin/β-CD complexes.

is more sensitive compared to other analytical methods, it is relatively simple, and, most importantly, it provides molecular weight data that allow one to determine stoichiometry.[24] Figure 4 illustrates the mass spectra of the inclusion complexes of permethrin with β-CD. From Figure 4, the peaks observed at *m*/*z* 1135, 1157, and 1527, correspond to $[\beta\text{-CD} + H]^+$, $[\beta\text{-CD} + Na]^+$, and $[\beta\text{-CD} + \text{permethrin} + H]^+$, respectively. The most abundant peak at *m*/*z* 2661 corresponds to $[2\beta\text{-CD} + \text{permethrin} + H]^+$. These experimental data reveal a dominant host:guest ratio of 2:1 for the permethrin/β-CD inclusion complex in which each permethrin molecule binds with two β-CD molecules. At the same time, the mass spectrum also reveals a smaller amount of the permethrin/β-CD inclusion complex with the host:guest ratio of 1:1.

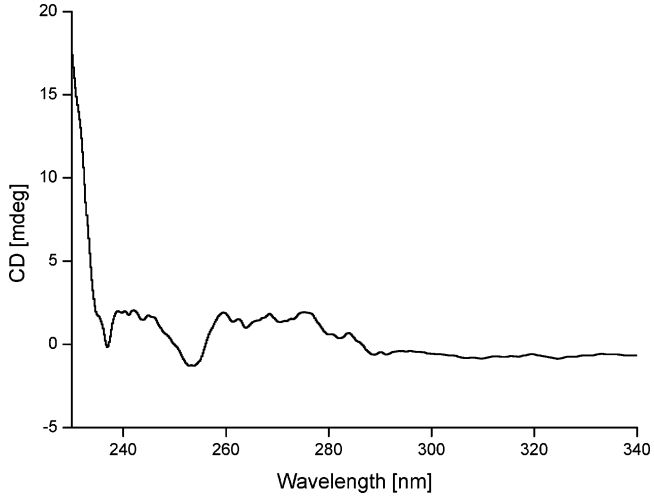

Figure 5. Circular dichroism spectrum of the inclusion complex in water.

In addition, the circular dichroism spectrum of the inclusion complex in water was performed to deduce the conformation of permethrin in the cavity of β-CD. The circular dichroism spectrum of the inclusion complex showed two strong positive Cotton shifts from 260 to 275 nm and from 240 to 250 nm, while a weak negative Cotton shift around 255 nm was also observed (Figure 5). According to the experimental rules on the circular dichroism spectrum of CD complexes,[25] the observed spectrum indicates that permethrin was embedded in the hydrophobic cavity almost parallel to the axis of β-CD.

Structures and Dynamics of the Permethrin/β-CD Inclusion Complexes. To understand the detailed structures of the permethrin/β-CD inclusion complexes, we carried out molecular modeling and molecular dynamics (MD) simulations on various possible microscopic permethrin/β-CD binding modes. Molecular modeling and MD simulations were first performed to find the most stable structure of the hydrophobic binding between one permethrin molecule and one β-CD molecule, i.e.,

$$\text{permethrin} + \beta\text{-CD} \rightarrow \text{permethrin}(\beta\text{-CD})$$

On the basis of the modeling, β-CD was able to selectively recognize the *trans*-1*R*,3*R* permethrin isomer to form a stable permethrin(β-CD) complex. Depicted in Figure 6 are the obtained most stable structures of permethrin, β-CD, and permethrin(β-CD). The orientation of the permethrin inside the cavity of β-CD is consistent with that deduced from the observed circular dichroism spectrum in Figure 5. As seen in Figure 6, a benzene ring of the permethrin molecule stays inside the hydrophobic cavity of β-CD, with another benzene ring of the permethrin molecule staying outside of the cavity on the tail side of β-CD. The other side of the permethrin molecule, including the two chlorine atoms, stays outside of the cavity on the head side of β-CD.

Further, we considered the possible binding of permethrin-(β-CD) complex with a second β-CD molecule to form a permethrin(β-CD)$_2$ complex:

$$\text{permethrin}(\beta\text{-CD}) + \beta\text{-CD} \rightarrow \text{permethrin}(\beta\text{-CD})_2$$

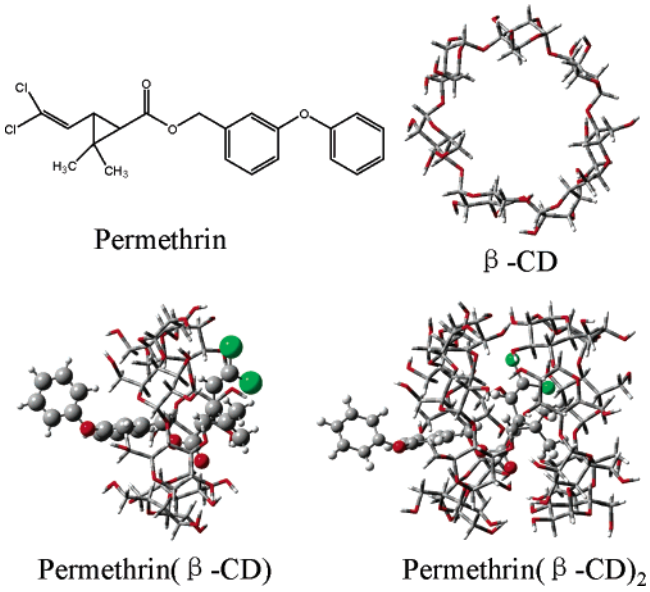

Figure 6. Energy-minimized geometries of *trans*-(1*R*,3*R*)-permethrin, β-CD, permethrin(β-CD), and permethrin(β-CD)$_2$.

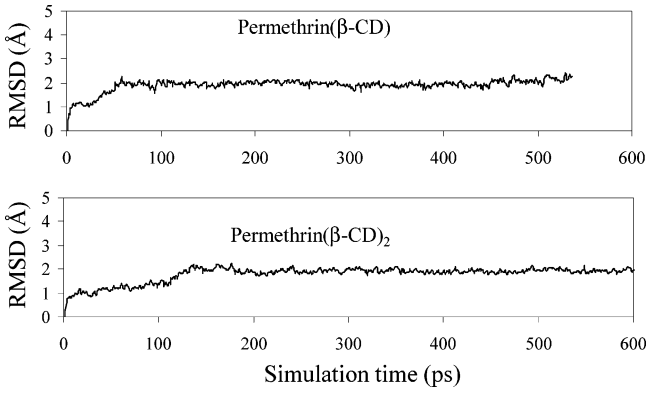

Figure 7. Time dependence of the root-mean-square deviations (RMSD) of atomic positions in the MD-simulated complexes from those in the corresponding energy-minimized structures.

Obviously, the second β-CD molecule could use its head or tail side to approach the head or tail side of the β-CD molecule in the permethrin(β-CD) complex. Hence, we examined four possible binding modes of the permethrin(β-CD)$_2$ complex, i.e. the head−head, head−tail, tail−head, and tail−tail binding modes, and found that the head−head is the most stable binding mode (see Supporting Information). The energy-minimized structure of the permethrin(β-CD)$_2$ complex in the head−head binding mode is also depicted in Figure 6.

Figure 7 shows the time dependence of the root-mean-square deviations (RMSD) of the MD-simulated atomic positions of the permethrin/β-CD complexes from the corresponding atomic positions of the energy-minimized structures. These plots are based on the MD simulations in water performed at $T = 298.15$ K and $P = 1$ atm. For each MD simulation, the RMSD values were calculated for the positions of all atoms in the simulated complex. As seen in Figure 7, the trajectories of the MD simulations on both permethrin(β-CD) and permethrin(β-CD)$_2$ complexes in water were quickly stabilized during the MD simulations. The MD trajectory was stabilized after ∼50 ps for the permethrin(β-CD) complex and after ∼130 ps for the permethrin(β-CD)$_2$ complex. After the MD trajectories were stabilized, the average RMSD values were 1.98 Å for the simulated permethrin(β-CD) complex and 1.92 Å for the permethrin(β-CD)$_2$ complex. These results indicate that both permethrin(β-CD) and permethrin(β-CD)$_2$ complexes are very stable in water, although the permethrin(β-CD)$_2$ complex with a slightly smaller RMSD value is slightly more stable than the permethrin(β-CD) complex.

These computational results can be used to better understand the aforementioned experimental observations. The combined use of the computational results with the data in Figure 1 reveals the following important binding information: (1) when [β-CD] < ∼4 mM, the concentration of the permethrin(β-CD) complex was dominant; (2) when [β-CD] > ∼8 mM, the concentration of the permethrin(β-CD)$_2$ complex was dominant; (3) when [β-CD] changed from ∼4 to ∼8 mM, the concentration of the permethrin(β-CD) complex gradually increased, while the concentration of the permethrin(β-CD)$_2$ complex gradually decreased, without significant change of the total concentration of the permethrin(β-CD) and permethrin(β-CD)$_2$ complexes; (4) when [β-CD] = ∼6 mM, the concentration of the permethrin-(β-CD) complex should be roughly equal to that of the permethrin(β-CD)$_2$ complex.

Conclusion

The solubility of permethrin in aqueous solution was observed to considerably increase in the presence of β-cyclodextrin (β-CD). Extensive experimental data along with computational modeling reveal the formation of stable permethrin/β-CD inclusion complexes, including permethrin(β-CD) and permethrin(β-CD)$_2$, through hydrophobic binding. Both the permethrin(β-CD) and permethrin(β-CD)$_2$ complexes coexisted in aqueous solution, and the concentration ratio, i.e., [permethrin-(β-CD)]/[permethrin(β-CD)$_2$], was dependent on the concentration of β-CD, i.e., [β-CD]. When [β-CD] < ∼4 mM, the concentration of the permethrin(β-CD) complex was dominant. When [β-CD] > ∼8 mM, the concentration of the permethrin-(β-CD)$_2$ complex was dominant. When [β-CD] changed from ∼4 to ∼8 mM, the concentration of the permethrin(β-CD) complex gradually decreased, while the concentration of the permethrin(β-CD)$_2$ complex gradually increased, without significant change of the total concentration of the permethrin(β-CD) and permethrin(β-CD)$_2$ complexes. When [β-CD] = ∼6 mM, the concentration of the permethrin(β-CD) complex should be roughly equal to that of the permethrin(β-CD)$_2$ complex.

The complexation of permethrin with β-CD significantly improved the bioavailability of permethrin and, therefore, increased the insecticidal activity. The significant increase of the bioactivity of permethrin in the presence of β-CD provides an effective approach to improve the practical use of permethrin in public health and agriculture.

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Supporting Information Available: Additional figures and a table about the structures and interaction energies calculated for the permethrin/β-cyclodextrin inclusion complexes (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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