Inclusion Complexation and Solubilization of Paclitaxel by Bridged Bis(*β*-cyclodextrin)s Containing a Tetraethylenepentaamino Spacer

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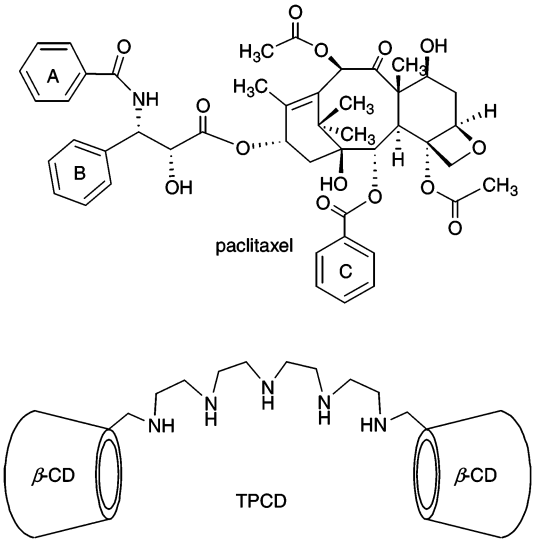
Received July 16, 2003

Abstract: A novel water-soluble paclitaxel complex has been prepared by inclusion complexation with bridged bis(*β*-cyclodextrin)s and characterized by means of ^{1}H NMR, SEM, powder X-ray diffraction patterns, TG-DTA, DSC, FT-IR, and 2D NOESY. The cyclodextrins were able to solubilize paclitaxel to levels as high as 2 mg/mL. Furthermore, the cytotoxicity of the novel complexes was assessed using a K562 leukemia cell line which indicated that drug concentrations of 10 pg/mL elicited an inhibitory effect.

Since paclitaxel was first isolated by Wani et al. from the Pacific Yew in 1971,[1] tumor inhibitory properties[2] have been extensively investigated. It is well-known that the poor water solubility[3] of paclitaxel seriously reduces its wider clinical application. In an effort to overcome these limitations, techniques designed to improve the water solubility of paclitaxel have been considered including prodrugs[4−6] and the use of cosolvents and solubilizers. These approaches each have their own set of limitations. Various cosolvents such as DMSO, PEG's, and others do not provide for adequate solubility upon administration due to subsequent dilution.[7] One promising approach for the solubilization of paclitaxel is the use of cyclodextrin which imparts its beneficial physicochemical properties through the formation of inclusion complexes.[8]

Cyclodextrins, which generally contain six to eight D-glucose units linked by α-1,4-glucose bonds, can act as host molecules interacting with appropriately sized guests via their lipophilic center[9] and consequently have been applied extensively to form inclusion complexes with all sorts of drugs in formulation and delivery systems.[10] While simple cyclodextrins have been applied to the solubilization of paclitaxel,[11] poor solubility continues to be an issue for this anticancer agent, meaning that additional attempts to further increase the solubility of paclitaxel in a stable, biocompatible solvent systems are highly sought after. Recently, Moser and co-workers[12] investigated the inclusion complexation behavior of cyclodextrin dimer with paclitaxel in the H_2O−DMSO mixed solution and determined the biological action of the complex with human tumor cells. In the present communication, we wish to report the preparation of a novel water-soluble paclitaxel inclusion complex by bridged bis(*β*-cyclodextrin)s and its solubilization effect for paclitaxel.

Chart 1. Structures of Paclitaxel and Tetraethylenepentaamino-Bridged Bis(*β*-cyclodextrin)



Tetraethylenepentaamino-bridged bis(*β*-cyclodextrin)s (TPCD as abbreviated, Chart 1) were synthesized according to our reported procedures.[13] To generate drug−cyclodextrin complexes, paclitaxel (0.03 mM) and TPCD (0.01 mM) were completely dissolved in a mixed solution of ethanol and water (v:v = 1:5) and stirred for 3 days at room temperature. After evaporating the ethanol from the mixed solution, the uncomplexed paclitaxel was removed by filtration. The filtrate was again evaporated to remove water and dried in a vacuum to give the paclitaxel−TPCD inclusion complex 38.5 mg (93% yield).

^{1}H NMR spectrum of the paclitaxel−TPCD complex (in D_2O)[14] (Figure 1) provided direct evidence for the formation of the inclusion complex as well as information on its stoichiometry. Owing to its exquisitely poor water solubility, paclitaxel is transparent to ^{1}H NMR under most conditions when D_2O is used as solvent.[3] Assessment of the paclitaxel−TPCD by ^{1}H NMR demonstrated the presence of the structural protons of the paclitaxel molecule consistent with significant solubilization. In addition, integration of the areas associated with the phenyl protons of the paclitaxel (δ 7−8 ppm) and the H-1 protons of the TPCD, respectively, suggested a ratio of two paclitaxel molecules to one TPCD molecules. This is consistent with a 2:1 complex in which the bidentate TPCD surrounds and complexes the paclitaxel entity. The complex formation is corroborated by IR spectrophotometry since the C═O stretching vibration is shifted from 1725 cm^{-1} in paclitaxel to 1720 cm^{-1} in the paclitaxel−TPCD complex.

To further confirm the binding mode, a 2D NOESY experiment of the complex was completed with the spectrum illustrated in Figure 2. It is apparent that the H-3 and H-5 protons of the cyclodextrin cavities are strongly correlated with the ortho and meta protons of the A and B phenyl rings and relatively weak interactions with the ortho protons in ring C of paclitaxel,

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10.1021/jm034148f CCC: $25.00
Published on Web 09/23/2003

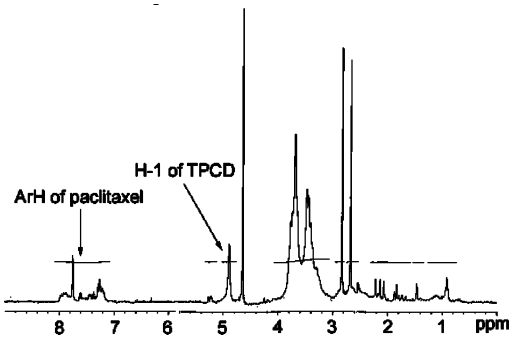

Figure 1. ^{1}H NMR spectrum of paclitaxel−TPCD complex.

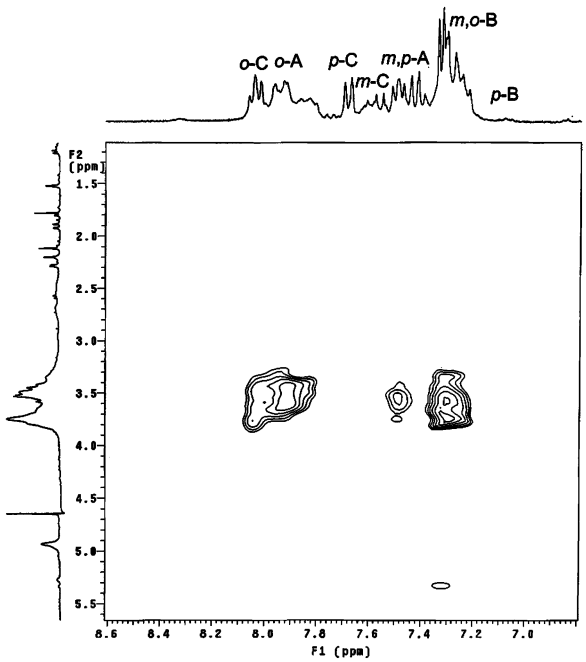

Figure 2. 2D NOESY spectrum of paclitaxel−TPCD complex in D_2O at 298.1 K with a mixing time of 400 ms.

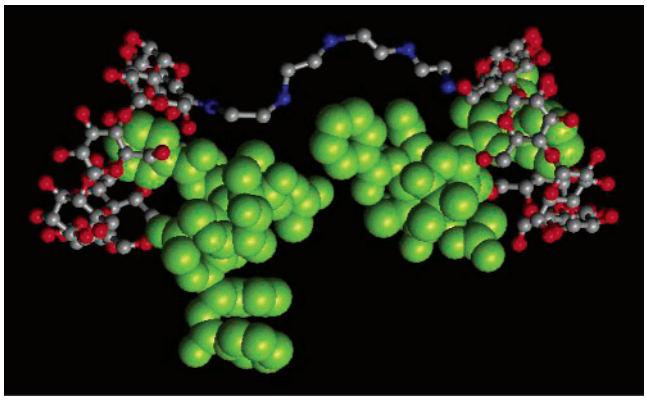

Figure 3. A possible binding mode of paclitaxel−TPCD complex.

suggesting that the cyclodextrin cavities include the A and B rings in a bridging structure. A structural representation of this complex based on the generated 2D NOESY data is given in Figure 3. To assess the thermodynamics of complexation, isothermal titration mircrocalorimetry (ITC) was performed using an ethanol−water (1:1) solution at 25 °C. A sequential two-step binding model was applied to generate an apparent binding constant ($K_1 \times K_2$) for the 2:1 inclusion complex

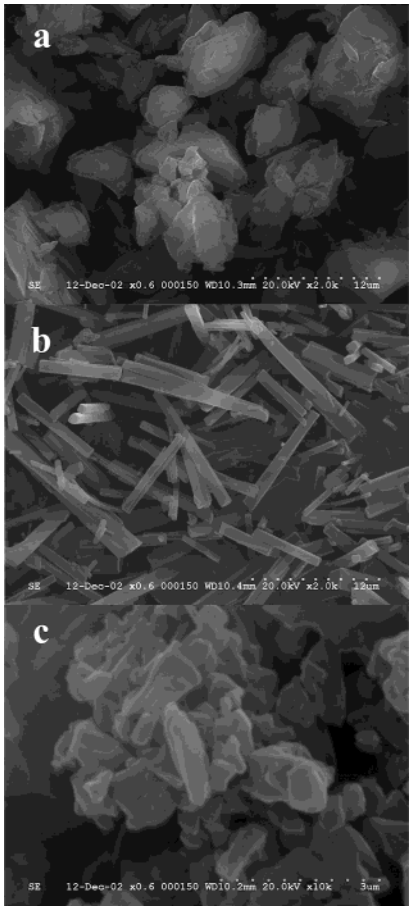

Figure 4. SEM images of (a) tetraethylenepentaamino-bridged bis(β-cyclodextrin), (b) paclitaxel, and (c) paclitaxel−TPCD complex.

of 2.04×10^8 M^{-2}. The derived thermodynamic parameters indicated that the inclusion complexation is an entropically driven process ($\Delta H°_1 + \Delta H°_2 = -10.2$ kJ·mol^{-1}, $T\Delta S°_1 + T\Delta S°_2 = 37.3$ kJ·mol^{-1}), indicating that the desolvation effect greatly contributes to the overall inclusion process. This is contrast to the thermodynamics of binding for simple cyclodextrin hosts and their molecular guests in that these interactions tend to be enthalpically driven.[15]

The surface morphology of powders derived from paclitaxel, TPCD, and their inclusion complexes, as assessed by scanning electron microscopy (SEM), is provided in Figure 4. A comparison of the images revealed that the complex was structurally distinct from the isolated components, those being the unmanipulated drug and TPCD. Specifically the complex could be characterized as a regular platelike morphology, the TPCD as a cubelike morphology, while the parent drug manifests a more sluglike morphology.

The powder X-ray diffraction patterns of TPCD, paclitaxel, physical mixture, and the inclusion complex were obtained using a Rigaku D/max-2500 diffractometer with Cu Kα radiation. Paclitaxel has a diffractogram consistent with its crystalline nature (Figure 5b) while the TPCD is amorphous (Figure 5a). The physical mixture of paclitaxel and TPCD (Figure 5c) gives a diffractogram that is a superimposition of crystalline paclitaxel and the amorphous cyclodextrin. By contrast, the inclusion complex (Figure 5d) gives a different pattern especially in the 15−25° (2θ) area where peaks

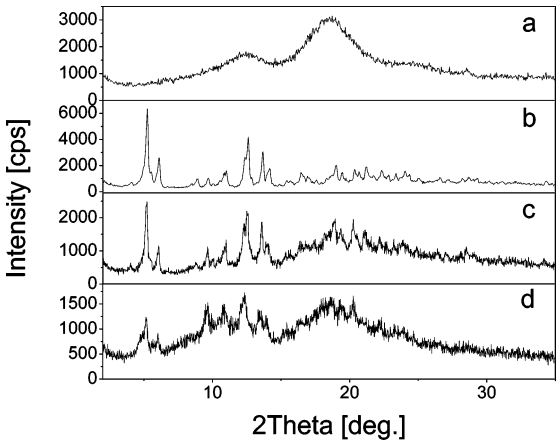

Figure 5. The X-ray powder diffraction patterns of (a) tetraethylenepentaamino-bridged bis(β-cyclodextrin), (b) paclitaxel, (c) physical mixture of TPCD and paclitaxel, and (d) paclitaxel−TPCD complex.

associated with crystalline paclitaxel have almost disappeared. In addition, an assessment of the 3−8° (2θ) region suggests differences from the spectra associated with the physical mixture both in terms of peak shape and relative intensities, meaning possible conformation changes for paclitaxel in the inclusion complex.

To investigate the thermal stabilities of the inclusion complex, thermogravimetric (TG) and differential thermal analysis (DTA) were recorded with a RIGAKU Standard type. The TG and DTA results cooperatively show that the TPCD decomposes at 315 °C and the paclitaxel decomposes at 244 °C. Therefore, the peak at about 228 °C for the complex represents the temperature of the complex dissociating to TPCD and paclitaxel, indicating that the complex is very stable in thermal viewpoint. Simultaneously, differential scanning calorimetry (DSC) performed with a NETZSCH DSC 204 instrument gives further evidences. The curve for paclitaxel shows an endothermic peak at 222 °C, due to the melting of the drug. However, along with the inclusion of TPCD and paclitaxel, the endothermic peak at 222 °C disappeared and a new exothermic peak at 234 °C emerged in the DSC spectrum of the TPCD−paclitaxel inclusion complex, suggesting the dissociation of the two components.

The solubility of resulting complex of paclitaxel and bridged bis(β-cyclodextrin) is assessed by the preparation of saturated complex solution.[10c] Excess complex was put into 5 mL water (pH 6) and stirred for 1 h. After removing the undissolved substance, the solution was dried in a vacuum and dosed by weighing method. The water solubility of the complex, comparing with that of paclitaxel (30 μg/mL;[3a] 0.7 μg/mL;[3b] 6 μg/mL[3c]), is dramatically increased to approximately 2.0 mg/mL (calculated as paclitaxel residue).

Antiproliferative activity of this paclitaxel−TPCD complex was tested using the MTT cytotoxicity assay. The complex at concentration of 1×10^{-6} mg/mL shows an inhibitive ability of 57.7% for K562 erythroleukemia after 72 h.[16,17]

This solid-state complex obtained in a simple way is composed of only paclitaxel and cyclodextrin dimer, which could have a wide expectation in clinic application. Further investigation about the percent cell survival at different concentrations, the maximum tolerated dose (MTD) and the antitumor mechanism of this TPCD−paclitaxel complex is in progress.

Acknowledgment. This work was supported by NNSFC (No. 29992590-8 and 20272028), the Tianjin Natural Science Fund (No. 013613511), and Special Fund for Doctoral Program from the Ministry of Education of China (No. 20010055001), which are gratefully acknowledged.

Supporting Information Available: FT-IR, TG-DTA, and DSC of TPCD, paclitaxel, and the inclusion complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Paclitaxel−TPCD complex: ^{1}H NMR (300 MHz, D_2O, tetramethylsilane (TMS)): *δ* 7.08−8.10 (m, 30 H, ArH of paclitaxel), *δ* 4.78−5.04 (s, 14 H, H-1 of TPCD), *δ* 3.04−4.11 (m, 84H, H-2−6 of TPCD) *δ* 1.88−3.04 (m, 35 H, $NHCH_2$ of TPCD, paclitaxel protons), *δ* 0.78−1.88 (m, 26H, paclitaxel protons).
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(16) Cell and treatments: Cells were cultured at 5×10^5/mL in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were seeded at 5×10^4/mL and treated with the indicated amounts of the complex.
(17) Measurement of cytotoxicity: the effect of the complex was evaluated as cell survival after treatment. Cell viability was evaluated by a microculture tetrazolium reduction assay using MTT (3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma). Briefly, 50 mL of MTT stock solution (2 mg/mL in PBS) was added to 150 mL cell cultures in 96-microwell flat-bottom plates for 4 h incubation at 37 °C. Plates were then centrifuged, and MTT-containing culture medium was removed. Precipitated formazan was dissolved in 150 mL of DMSO. Results were read with 15 min in a spectrometer at 577 nm, and the means of triplicates were calculated. Cell inhibition rate is expressed as percentage of control samples.

JM034148F