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Preparation, spectroscopy and molecular modelling studies of the inclusion complex of cordycepin with cyclodextrins

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ABSTRACT

The inclusion complexes of cordycepin with cyclodextrins (CDs) were prepared, the resultant complexes were characterised by UV–vis, FTIR, DSC, SEM, XRD, ESI-MS and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). The stoichiometry was established using a Job plot and the inclusion mechanism was clarified using molecular dynamic simulations. Molecular modelling calculations have been carried out to rationalise the experimental findings and predict the stable molecular structure of the inclusion complex. The stability of the inclusion complexes were confirmed by energetic and thermodynamic properties (ΔE, ΔH, ΔG and ΔS) and HOMO, LUMO orbital. The 1:1 binding model of complexes were visually proved by ESI-MS experiment. Our results showed that the purine group of cordycepin molecule was deeply inserted into the cavity of CDs.

1. Introduction

Cordyceps militaris, a species of the fungal genus cordyceps, is an ingredient of traditional Chinese medicine that has been prescribed for inflammatory diseases and cancer.[1,2] Cordycepin[3] (Fig. 1A), the adenosine analogue 3′-deoxyadenosine, which was first isolated from the ascomycete fungus *C. militaris*, had been reported to exert anti-inflammatory and anti-oxidant actions. It is an entomopathogenic fungus that grows parasitically on lepidopteron larvae and insect pupae. The genus cordyceps is well-known in traditional Chinese medicine as it exhibits a variety of clinical health effects, including immunomodulatory, anticancer, antioxidant, anti-inflammatory and anti-microbial activities.[4–6] It's medicinal values and diversity of biological activity have gradually been recognised in recent years, making the study of cordycepin a hot topic worldwide.

However, the use of cordycepin as a natural herbal medicine is greatly limited by its low water solubility and bioavailability.[7,8] Although much effort has been made to improve the water solubility by introducing some nanodispersion or enzymatic condensation techniques, it is still not possible to sufficiently dissolve cordycepin in water, which prevents its usage for therapeutic applications. Therefore, the search for an efficient and nontoxic carrier of cordycepin has become important in order to further promote its clinical applications.

Cyclodextrins (CDs) are non-toxic cyclic oligosaccharides, consisting of six to eight glucose units linked by α-1,4-glucosidic bonds. Because of their hydrophobic internal cavity and hydrophilic external surface, their unique molecular structure can form supramolecular host–guest complexes with various hydrophobic molecules.[9–11] Inclusion complexes of CDs are being formulated and studied for varied purposes, such as dissolution rate enhancement, solubility of poorly water soluble drugs, stability of the system and even as drug carriers.[12,13] Based on their distinct physical and chemical properties,[14] CDs are used in various fields, including biological medicine,[15–17] food technology[18] and the pharmaceutical industry.[19,20]

More recently, our group has reported that the inclusion complexation of CDs with natural products[19–21] significantly enhanced the water solubility and bioavailability of the products. Their behaviour, characterisation and binding ability were investigated in both solution and the solid state by means of UV–vis,

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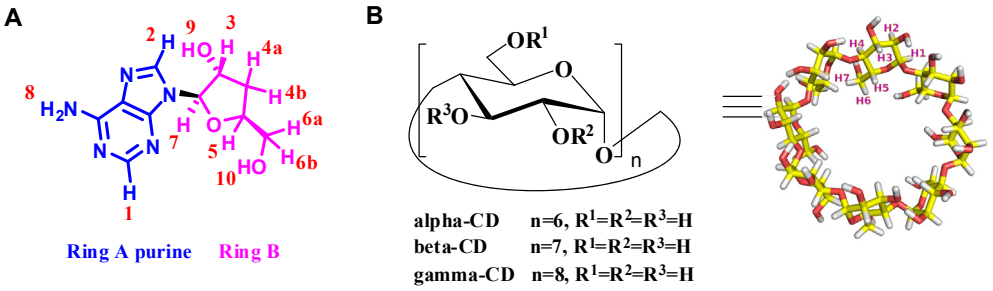

Fig. 1. Chemical structure of Cordycepin (A) and cyclodextrins (B).

NMR, XRD, DSC and SEM. In this paper, we investigated the inclusion complexes of natural cordycepin with cyclodextrins. Different from previous works, we have now obtained new direct evidence in ESI-MS[22–24] experiment for the 1:1 binding model of CDs and cordycepin. In addition, we have carried out solubility measurements and molecular modelling of these complexes to fully characterise the binding behaviour between cyclodextrin and cordycepin. Meanwhile, the cordycepin/CDs solid inclusion complex gave a stronger binding energy value in the following order: α-CD>β-CD>γ-CD. This work indicates the formation of 1:1 binding models between α-, β- or γ-cyclodextrin, and it provides evidence for further research into cyclodextrin.

2. Materials and methods

2.1. Materials

Cordycepin (FW=251.24, purity >99%) was obtained from Sigma–Aldrich (Dorset, UK). α-cyclodextrin (FW=972, purity >99%), β-CD (FW=1135, purity >99%) and γ-CD (FW=1297, purity >99%) were purchased from Acros (Belgium) and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

2.2. Preparation

The 1:1 molar ratio cordycepin-CDs inclusion complexes were prepared using the co-evaporated method. Accurately weighed amounts of α-, β- and γ-CD were dissolved in double-distilled water. Subsequently, the solution of cordycepin in ethanol was added slowly to the aqueous CDs solution. The resulting suspension was stirred at room temperature for 48 h. It was then filtered through a 0.22-μm membrane filter and dried under reduced pressure (<10 Pa) for 12 h in a vacuum desiccator to obtain the solid complexes.

The physical mixtures used to test for possible inclusion were prepared by mixing the powders in a 1:1 molar ratio of cordycepin and CDs in an agate mortar.

2.3. Stoichiometry determination: Job's method and phase-solubility studies

The UV–vis absorption spectra of cordycepin, CDs and the inclusion complexes were recorded using a UV-2401 spectrophotometer (Shimadzu, Tokyo, Japan) in the range of 200–600 nm.

The stoichiometry of inclusion complex was determined by the continuous variation Job's method.[25] To implement Job's method experimentally, equimolar 2.15×10^{-5} M solutions of cordycepin and CDs were mixed to a standard volume containing a fixed total concentration of the species. In the solutions, the R (R=[cordycepin]/([cordycepin]+[CDs]), [cordycepin]+[CDs]=4.3×10^{-5} M) is systematically varied from large to small. The maximum amount of the complex cordycepin should occur at the stoichiometric ratio from 0.0 to 1.0.

2.4. Solubility study

A solubility study was carried out according to a previously reported method. In brief, excess amount of the inclusion complex was added to deionised water (2 mL, ca. pH 7.0) to ensure the solution reached saturation; then, the suspension was stirred continuously at 25 °C for 24 h. After equilibrium was attained, the solution was filtered using a 0.45 mm cellulose acetate membrane. The filtrate was evaporated under reduced pressure to dryness and the residue was assessed using the weighing method.

2.5. Characterisation of inclusion complexes

FTIR spectra of cordycepin, CDs, the inclusion complexes and the physical mixtures were monitored using a KBr disk with a PerkinElmer Spectrum 100 FTIR Spectrometer (PerkinElmer, USA).

Differential scanning calorimetry (DSC) analysis was performed using a differential scanning calorimeter (2960 SDT V3.0F instrument). Samples of approximately 4–5 mg (cordycepin, CDs, the physical mixtures and the inclusion complexes) were placed separately in flat-bottomed aluminium pans and heated at a rate of 10 °C/min from room temperature to 400 °C under a dynamic nitrogen atmosphere.

The particle shape and surface features of the samples (cordycepin, CDs, the physical mixtures and the inclusion complexes) were determined and imaged using SEM (FEI Quanta 200, Holland).

The XRD data of cordycepin, CDs, the physical mixtures and the inclusion complexes were obtained at ambient temperature using a Rigaku TTRIII Rotating Target diffractometer with Cu Kα radiation (40 kV, 100 mA). All samples were measured in the 2θ angle range 3–50° with a scan rate of 5°/min and a step size of 0.02°.

^{1}H NMR spectra were recorded at 25 °C using a Bruker Avance DRX spectrometer at 500 MHz. CDs, cordycepin and the cordycepin/CDs complexes were dissolved in D_2O solution to be tested. It is well known that CD molecules adopt the conformation of a torus, in which the H-3 and H-5 protons are located inside the cavity, whereas H-2 and H-4 are on the outside. The H-6 protons of the primary alcohol group are on the narrower side, while H-1 lies on in the glycosidic bond plane of the CDs (Fig. 1B). It is a generally accepted fact that, when an aromatic guest is included in the CD cavity, the signals of the host inner protons (H-3 and H-5) suffer a significant up-field shift as a consequence of the change in their chemical environment. Therefore, the shift deformation of the H-3 and H-5 proton peaks is highly sensitive to the formation of inclusion complexes. The ^{1}H shifts of the guest may be different,