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Inclusion complexes of GA_3 and the plant growth regulation activities

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ABSTRACT

Gibberellic acid (GA_3) is an important phytohormone that is applied in agriculture, nurseries, tissue culture, tea gardens, etc. However, it has some drawbacks such as potential hazardous effects on mammals and labile in the condition of a weak base or acid. In this study, the enhanced stability and bioavailability of GA_3 were achieved by forming the inclusion complexes of GA_3 with cyclodextrins (β- or γ-CD) and its derivative (HP-β-CD). In the preliminary plant growth regulation assay, GA_3/CDs displays superior bioactivity compared to pure GA_3 to help with the early seedling growth of cucumber and mung bean and the root growth of cucumber and mung bean, respectively. The results showed that there was a certain relationship between the inclusion ability, stability and bioactivity. The inclusion stability constants of gibberellin clathrate are consistent with the order of stabilities of the inclusion complex. Among these complexes, GA_3/HP-β-CD possess highest inclusion constant, and the binding ability of the HP-β-CD not only enhances the stability of gibberellic acid in the stability test but also plays a slow release role in the bioactivity assay. Therefore, the complex of GA_3 may be used as a promising plant growth regulator.

1. Introduction

The gibberellins (GAs) are a large group of highly functionalized diterpenoids which were discovered in Japan in an investigation of the "bakanae" disease of rice resulting from infection by the fungus [1]. As we now know, GAs are applied as plant hormones, which regulate plant growth and development, including seed germination, breaking winter dormancy, enzyme synthesis, reversal of dwarfism, the induction of stem growth, flowering, modification of flower sex expression, parthenocarpic development of fruit, fruit enlargement, inhibition of senescence, and so on [1–4]. Currently, 136 GAs has been isolated from plants, fungi, and bacteria, which are termed GA1 to GA136 in their order of discovery [5–7]. The gibberellins are conveniently divided into two subgroups, the larger of which is C19 gibberellins (gibberellic acid: GA_3; a typical representative), with most of the remaining having 20 carbons [8]. Gibberellic acid (GA_3) is a commercially important phytohormone, produced commercially in ton quantities by fermentation of the fungus *Gibberella fujikuroi*, which is gaining much more attention all over the world due to its effective use in agriculture, nurseries, tissue culture, tea gardens, etc. [9,10]. Chemically, GA_3 (Scheme 1) is a tetracyclic dihydroxy γ-lactonic acid containing two ethylene bonds and one free carboxylic acid group, with an empirical formula of $C_{19}H_{22}O_6$ [1].

Although GA_3 is used worldwide in agriculture, it has a potentially hazardous effect on mammals. In fact, GA_3 causes alarming toxicity to the mammalian systems [11], particularly in the breast, lung [12], kidney, and liver of adult mice. Moreover, GA_3 induced chromosomal aberrations in human lymphocytes and mice, carcinogenic effects in adult Swiss Albino mice, and sexual differentiation and some physical parameters in laboratory mice. Many reports indicated that GA_3 may induce oxidative stress [13], leading to the generation of free radicals and causing cell damage in many organs, including the heart, kidney, stomach and spleen of adult rats [14] and the kidney [15], and liver [16], as well as causing bone disorders [12], hematological disorders [17], neurotoxicity [18], and affecting thyroid function and plasma antioxidant status in adult rats and their progeny [19]. In addition, GA_3 is unstable in the condition of a weak base or acid (Scheme 2) and is rapidly isomerized by 0.01 M NaOH to lactone, while treatment with acid initially leads to mixtures of diene acids [1]. Therefore, the search for an efficient and nontoxic carrier for GA_3 has become important in order to improve its bioavailability in agricultural applications.

Cyclodextrins (CDs) are some of the most important nontoxic

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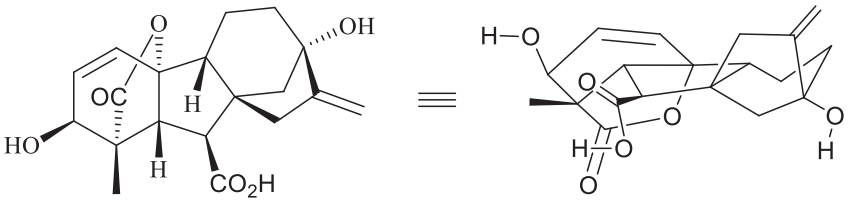
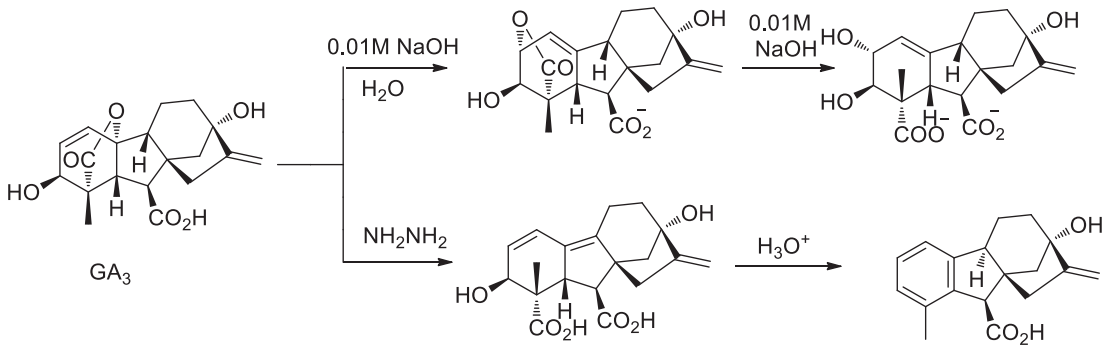


Scheme 1. The structure of GA_3.



Scheme 2. The reactions of GA_3 at acid or base condition.

carriers, which can form host-guest complexes with various polar and apolar "guest" molecules to improve their solubility, stability, and bioavailability; therefore, they are found to have a number of applications in a wide range of fields. It has been stated that agents were prepared with various components, which were composed of many subunits, including cyclodextrin and GA_3, and used for wheat, maize, and rice fields [20,21]. Despite the fact that the study of the binding behavior and ability is fundamental to understanding host-guest interactions, data on the binding behavior and ability are currently not available. Besides, the satisfactory results were obtained from one of our previous works that the water solubility and thermal stability of nimbin with distinctive structure and specific properties has been successfully improved in the inclusion complex with CDs [22].

The aim of this work was to synthesize and characterize some water-soluble inclusion complexes formed by GA_3 and cyclodextrins (β-, γ-CD) and the derivative 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). We were particularly interested in exploring the stabilization effect of CDs on GA_3 and the binding ability of the resulting inclusion complexes, which would provide a useful approach for obtaining novel GA_3-based phytohormone products with high pH stability, high bioavailability and low toxicity. Moreover, the biological effect of novel GA_3-based phytohormone products on the stimulation of plant growth has been determined.

2. Experimental

2.1. Materials

GA_3 (FW = 346, PC > 95%) was purified by flash column chromatography from material purchased in Yunnan Province, PR China. β-CD (FW = 1135), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, average molecular weight = 1380) and γ-CD (FW = 1297.15) were purchased from ABCR GmbH & Co. KG and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

2.2. Methods

2.2.1. Encapsulation of GA_3/β-CD, GA_3/γCD and GA_3/HP-β-CD complexes

GA_3 (0.02 mmol, 6.9 mg) and CD (0.01 mmol) were added to water (ca. 7 mL), and the mixture was stirred for 5 days at room temperature. The uncomplexed materials were removed by filtration. The filtrate was evaporated under reduced pressure to remove the solvent and dried in a vacuum to give the GA_3/CDs complexes. GA_3/β-CD complex (yield 89%): ^{1}H NMR (500 MHz, D_2O): δ 1.03–3.02 (m, 14H, some protons of GA_3), 3.37–3.98 (m, > 33H, H-2–6 of β-CD and some protons of GA_3), 4.86–5.0 (s, 7H, H-1 of β-CD), 5.78–5.79 (d, 1H, GA_3 proton), 6.29–6.31 (d, 1H, GA_3 proton); GA_3/γ-CD complex (yield 88%): ^{1}H NMR (500 MHz, D_2O): δ 1.13–3.11 (some protons of GA_3), 3.44–3.91 (H-2–6 of γ-CD and some protons of GA_3), 4.07–4.08 (some protons of GA_3), 4.97–5.14 (H-1 of γ-CD), 5.87–6.33 (some protons of GA_3); GA_3/HP-β-CD complex (yield 91%): ^{1}H NMR (500 MHz, D_2O): δ 1.11–3.18 (m, 39H, $-CH_3$ of HP-β-CD and some protons of GA_3), 3.55–4.13 (m, > 50H, H-2–6 and CH_2– and CH_3–2, 3, 6 of HP-β-CD and some protons of GA_3), 5.01–5.18 (s, 7H, H-1 of HP-β-CD), 5.94–5.96 (d, 1H, GA_3 proton), 6.46–6.47 (d, 1H, GA_3 proton).

2.2.2. Preparation of the GA_3/HP-β-CD physical mixture

The physical mixture, to test for possible inclusion, was performed by mixing the powders in a 1:1 molar ratio of GA_3 and HP-β-CD in an agate mortar.

2.2.3. ^{1}H NMR spectral titration

The ^{1}H NMR spectra measurements were carried out to determine the Ks values for the inclusion of various complexes with a Bruker Avance DRX spectrometer at 600 MHz and 298 K. D_2O was used in the spectral measurements. The concentration of GA_3 was kept constant at 1.50 mM while an appropriate amount of CDs was added with the final concentrations varied from 2.97×10^{-4}-3.20×10^{-4} to 4.50×10^{-3}-4.52×10^{-3} M (c (β-CD) = 3.20×10^{-4}, 6.50×10^{-4}, 8.90×10^{-4}, 1.03×10^{-3}, 1.23×10^{-3}, 1.58×10^{-3}, 2.31×10^{-3}, 2.93×10^{-3}, 3.79×10^{-3}, 4.50×10^{-3} M; c (HP-β-CD) = 2.97×10^{-4}, 6.27×10^{-4}, 9.20×10^{-4}, 1.08×10^{-3}, 1.24×10^{-3}, 1.50×10^{-3},