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NMR investigation of magnesium chelation and cation-induced signal shift effect of testosterone

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ABSTRACT

We have previously reported that testosterone (Tes) is able to interact with magnesium chloride dissolved in methanol. In this study, we have applied ^{1}H and ^{13}C NMR spectroscopies to a series of Tes solutions containing Mg^{2+} at various concentrations. High-resolution ^{13}C NMR spectra of Tes/Mg^{2+} revealed well-resolved ^{13}C signals, and the intensities of those arising from C3, C5, C16, and C17 decreased linearly with increasing Mg^{2+} concentration. The magnitude of the chelation affinity could be deduced from the slopes of the ^{13}C intensity variations; typically, the greater the slope the higher the chelation affinity. The results revealed Tes/Mg^{2+} chelation to be mediated by the oxygen atom attached to C3 in ring A, and the hydroxyl group attached to C17 in ring D. With regard to the chelation specificity, we showed that Tes chelates Mg^{2+}, but not Ca^{2+} or Zn^{2+}. We also explored the cation-induced signal shift effects of Tes in the presence of Mg^{2+}, Ca^{2+}, or Zn^{2+}. We demonstrate that high-resolution ^{13}C NMR spectroscopy provides a better probe than ^{1}H NMR for the detection of cation chelation and cation-induced signal shift effects for steroid compounds such as Tes.

1. Introduction

Steroids are divided into different categories according to their biological functions in animals, i.e. androgens, estrogens, progestins, glucocorticoids, and mineraloorticoids [1–4]. Both naturally occurring steroids and synthetic analogues are well known to act as hormones, regulating many biological processes, including maturation, reproduction and development of the gonad [5,6], maintenance of blood volume and electrolyte concentration [7], and synthesis of bone and muscle [8,9]. Although these compounds are structurally similar, consisting of a relatively rigid fused ring skeleton and a flexible side chain, their biological functions are diverse. One of the members of the androgens is testosterone (Tes), which is the most important male sex steroid in humans, mediating the development of sexual characteristics and function. Around 95% of Tes is synthesized by Leydig cells in the testes, and the remaining 5% is derived from peripheral adrenal androgen conversion [10]. In men, a progressive decrease in Tes level is often associated with ageing, leading to complex clinical syndromes [11–14]. Regarding steroidal activity, a bio-chromatographic analysis has been developed to measure thermodynamic parameters and the effect of magnesium (Mg^{2+}) on the binding of Tes to sex hormone-binding globulin (SHBG) over a wide temperature range [15]. It was demonstrated that, in the biological Mg^{2+} concentration domain, there is an uncompetitive inhibition effect of Mg^{2+} on Tes–SHBG binding, which leads to an enhancement of bioavailable Tes since it is not tightly bound to SHBG [15].

Mg^{2+} is well known to play important and essential roles in physiological processes of the brain, heart, and skeletal muscle in humans [16]. The United States Food and Nutrition Board recommends a daily intake of 420 mg for men and 320 mg for women [17,18]. An adult contains 22–26 g of magnesium [19], of which 60% is present in the skeleton, 39% is intracellular (20% in skeletal muscle), and 1% is extracellular [20]. In 1926, Leroy demonstrated that Mg^{2+} is essential for life in mice [21]. As for humans, Mg^{2+} deficiency in plasma was first reported by Hirschfelder and Haury in 1934 [22]. Since then, Mg^{2+} has been used for the treatment of a variety of diseases, including migraine, cardiovascular disease, and diabetes.

Solution NMR spectroscopy is a powerful analytical technique that allows one to characterize chemical environments and molecular interactions not only in macromolecules, but also in small molecules such as steroids [23–26]. Given the fact that NMR has the capability to determine structural information at atomic resolution, it is useful for investigating molecule–ligand interactions. Variations in chemical environment surrounding the nuclei of

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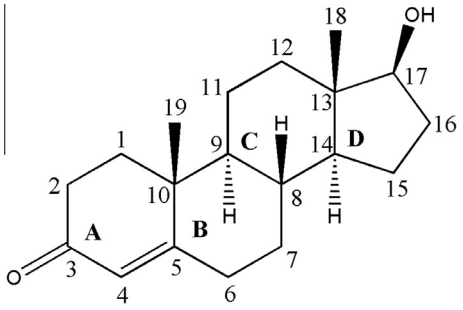

Scheme 1. Molecular structure of testosterone (Tes).

interest can be deduced from differences in chemical shifts. Among various NMR-active nuclei, ^{13}C represents an especially sensitive probe with a wide spectral range of more than 200 ppm, which greatly facilitates the analysis of steroid/cation interactions. In the present study, we aim to uncover the Mg^{2+} chelation effect of Tes *in vitro* by solution NMR spectroscopy. To this end, one- and two-dimensional solution ^{1}H and ^{13}C NMR spectroscopies have been applied to Tes (Scheme 1) in the absence and presence of Mg^{2+} in methanol. It was found that ^{13}C NMR provides a more sensitive probe for the detection of Mg^{2+} chelation than ^{1}H NMR. The chelation effect has been analyzed on the basis of intensity variations of the ^{13}C resonances. It was found that the ^{13}C intensities of the signals of C3, C5, C16, and C17 decreased linearly in the presence of increasing concentrations of Mg^{2+}. Our data suggest that the oxygen atom attached to C3 and the hydroxyl group attached to C17 constitute two Mg^{2+} chelating sites. In addition, we also studied cation-induced changes in chemical shift in ^{1}H and ^{13}C spectroscopies, whereby the largest changes were found for the signals of C3, C5, C14, C16, and C17.

2. Methods

2.1. Sample preparation

Tes and $MgCl_2$ were purchased from Sigma–Aldrich (St. Louis, MO, USA). These compounds were dried by lyophilization for 3 h to completely remove moisture. Approximately 3 mg of Tes was dissolved in 1 mL of anhydrous d_4-methanol. A series of Tes/$MgCl_2$

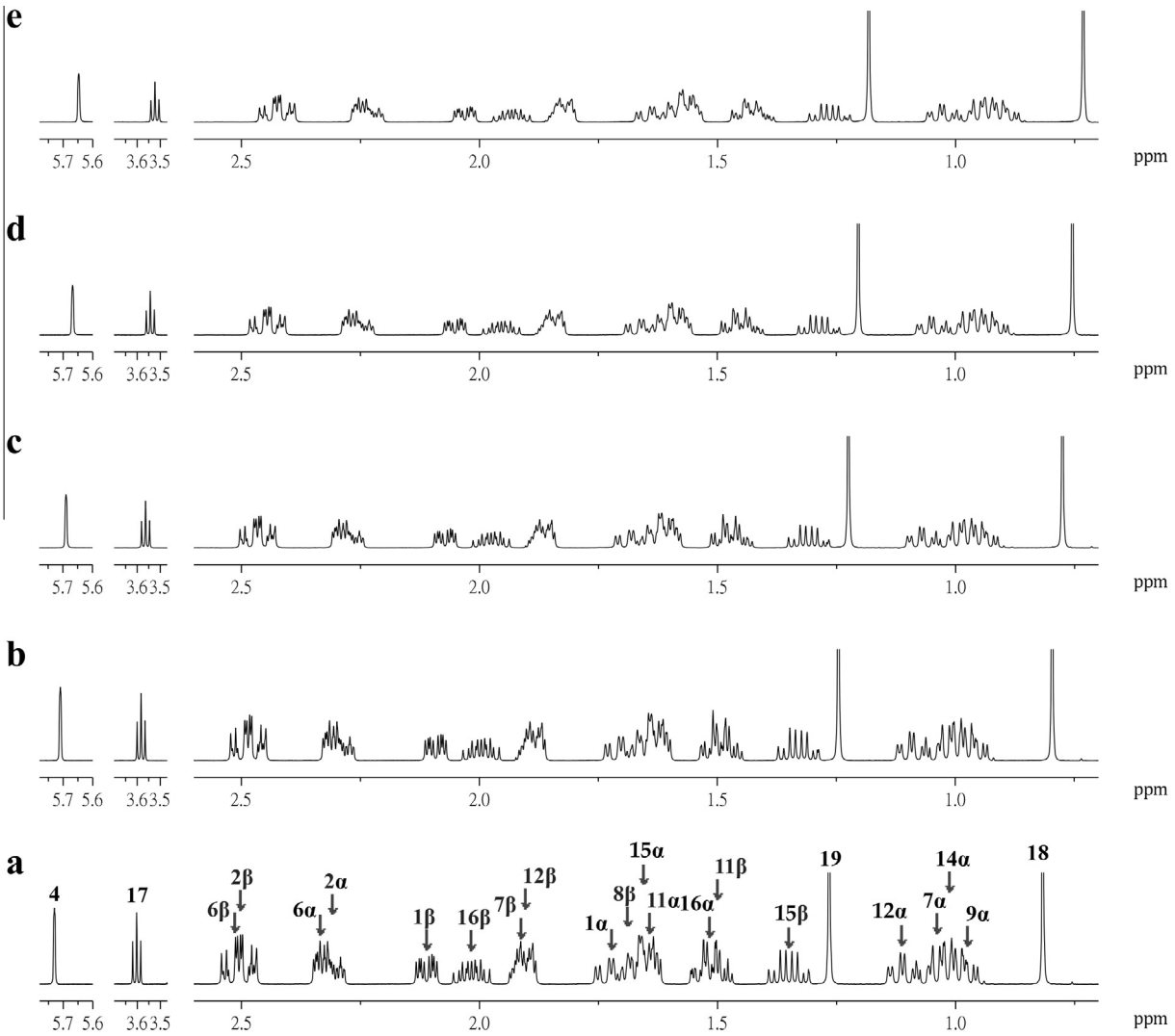

Fig. 1. Solution ^{1}H NMR analyses of Tes/Mg^{2+} mixtures. ^{1}H NMR spectra at five different Tes/Mg^{2+} molar ratios: (a) 1:0, (b) 1:5, (c) 1:10, (d) 1:15, (e) 1:20. As shown, there were systematic upfield shifts for the ^{1}H resonances as a function of Mg^{2+} concentration. In addition, a line-broadening effect was observed in the Mg^{2+} titration NMR spectra. The ^{1}H chemical shift assignments of the Tes/Mg^{2+} mixtures are indicated (see also Table 1). For better clarity, these spectra are presented in three different ranges displayed on different scales: δ = 0.70–2.60, 3.47–3.70, and 5.60–5.78 ppm.