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Gas phase fragmentation mechanisms of protonated testosterone as revealed by chemical dynamics simulations

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ABSTRACT

We have studied the fragmentation mechanisms leading to ions produced by collision-induced dissociation of protonated testosterone in the gas phase. At this aim we have used QM + MM chemical dynamics simulations with semi-empirical Hamiltonian for the description of testosterone ion fragmentation. Results show that MSINDO method is able to correctly produce the typical peaks obtained experimentally for protonated testosterone. Simulations also provide mechanisms. In particular we discussed those providing the typical testosterone peaks, *m*/*z* 97, 109 and 123 and compare with what suggested experimentally. Finally, we rationalized the appearance of different peaks in terms of their dynamical behavior. This study shows for the first time that chemical dynamics can be used to rationalize steroid gas phase fragmentation, thus paving the way for using this approach as complementary tool in doping detection.

1. Introduction

Testosterone is one of the male hormones (androgens) that is made and secreted in testicles. This natural anabolic hormone can promote the development of male reproductive organs and secondary sexual characteristics, and the increase of bone density, muscle mass, and power [1–3]. There are many synthetic anabolic hormones including testosterone which can be used for medical purposes [4–6]. However, they can also be used for short-term increase of athletes' muscle power and, if used for a long time, they can cause serious side effects such as mood disturbances (anxiety, depression, suicide, etc.), prostate cancer, cardiovascular diseases (high blood pressure, heart attack, etc.), and growth defects [7–9]. Therefore, in many athletic events, the use of anabolic steroids are strictly prohibited and in order to protect athletes from the misuse of these steroids, the World Anti-Doping Agency (WADA) was established [10,11], and studies on the techniques for the detection of steroids in the urine and blood are continuously performed [12–14].

Usually, gas-chromatography/mass spectrometry (GC–MS) had been used for doping tests on body fluids such as urine or blood [15,16]. Liquid-chromatography/mass spectrometry (LC–MS) with electrospray ionization (ESI) is able to make the tests faster and more accurate [17–19]. While the technique to analyze and detect anabolic steroids is being developed, many attempts are continuously tried to synthesize new anabolic steroids that cannot be detected by existing doping tests [20,21]. To detect unknown synthetic steroids, studies on the relationships between the structures of various steroids and the CID patterns have been reported [22–25]. Since testosterone is the only natural steroid which is the basis of all steroids and synthetic analogues, determining the structures and dissociation mechanisms of the ESI–MS/MS products would provide the basis for the detection of new synthetic steroids and help to develop and improve doping control analysis. However, ESI–MS/MS experiments alone can only provide the masses of product ions. Structures and dissociation pathways of the product ions can only be guessed from extra experiments such as isotope labeling and infrared multi-photon dissociation (IRMPD), as reported by different experimental groups for testosterone [22,26–28].

Testosterone, shown in Scheme 1 with the usual nomenclature of cycles and atom numbers, is characterized in ESI–MS/MS by three main peaks: *m*/*z* 97, 109 and 123. Williams et al. were not able to fully rationalize the fragmentation mechanism leading to *m*/*z* 97, that is the most abundant peak. Their experiments suggest that

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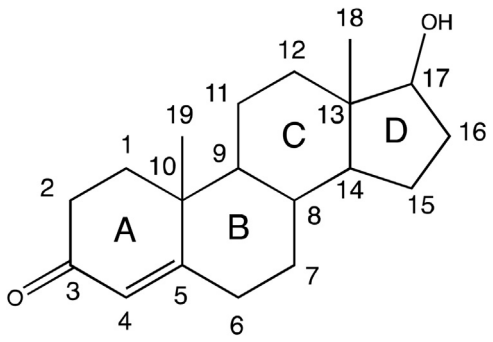

Scheme 1. Structure of testosterone with the associate nomenclature for cycles and atom numbers.

this fragment is "composed of all the A ring carbon atoms, except for C-5, all the ring A hydrogen atoms, C-19 and its three hydrogen atoms, the 3-one oxygen and a solvent proton" [26]. Pozo et al. used LC–MS/MS and isotopic labeling to propose the fragmentation mechanism shown in Scheme 2-a [27] for the formation of the same *m*/*z* 97 ion. More recently, Thevis et al. coupled ESI–MS/MS with IRMPD to propose two mechanisms, one that is the same as Pozo et al., and an alternative one, shown in Scheme 2-b [28]. Williams et al. proposed two fragmentation mechanisms for ions *m*/*z* 109 and 123, as shown in Scheme 3 [26]. Note that for *m*/*z* 123 two different reaction pathways were suggested. ESI–MS/MS experiments can only suggest mechanisms, in particular using isotopic labeling from which it is possible to know from which part of the molecule the atoms of the products come from, but the time evolution of the molecular structure from reactants to products cannot be directly detected. Coupling ESI–MS/MS with IRMPD can give more detailed information on the product structure. This last approach is very powerful, but it is very expensive and almost impossible to do on a daily basis as needed, for example, in the field of doping detection. This is where theoretical chemistry can play an important role.

To understand the mechanisms of testosterone gas phase MS/MS reactivity and the structure of the associated products we have used collisional chemical dynamics simulations [29]. This approach, modeling the collisional events similarly to what occurs in experiments, was able to characterize fragmentation mechanisms of different molecules, from small organic molecules to uracil, sugars and peptides [30–37]. In the present work we have applied to the case of protonated testosterone in order to elucidate the reaction mechanisms leading to the most occurring fragments and their corresponding structures.

Table 1
Proton affinities of protonated testosterone as obtained from different semi-empirical methods and B3LYP calculations (two basis sets). PACO corresponds to the [testosterone-COH]$^+$ structure, while PAOH to the [testosterone-OH_2]$^+$ one. The energy difference between the two isomers ($\Delta E_{CO\text{-}OH}$) is also shown.

	PA_{CO}	PA_{OH}	ΔE_{COOH}
PM3	177.45	151.77	−25.68
PM6	150.98	137.75	−13.23
RM1	163.84	141.43	−22.41
B3LYP/6-31G(d)	218.21	191.62	−26.59
B3LYP/6–311 + G(d, p)	215.91	189.88	−26.03
MSINDO	255.63	217.13	−38.50

2. Computational details

Testosterone can be protonated on two possible sites: carbonyl, [testosterone-COH]$^+$, and hydroxyl, [testosterone-OH_2]$^+$. The geometries of both tautomers were optimized at MSINDO [38,39] level of theory and also with other semi-empirical Hamiltonians (PM3 [40], PM6 [41], and RM1 [42]) and B3LYP [43,44] (with 6–31G(d) and 6–311 + G(d,p) basis sets).

The optimized structures were used as reference structures for the chemical dynamics of collisions. The system is composed by the ion, [testosterone-H]$^+$, and the projectile, here Ar. The total interaction potential is expressed as:

$$V = V_{ion} + V_{Ar-ion} \quad (1)$$

where V_{ion} is the intramolecular potential of [testosterone-H]$^+$, here treated at both PM3 and MSINDO level of theory. The system is too big to have an ensemble of B3LYP/6-31G(d) trajectories long enough for a sufficient sampling of reactivity.

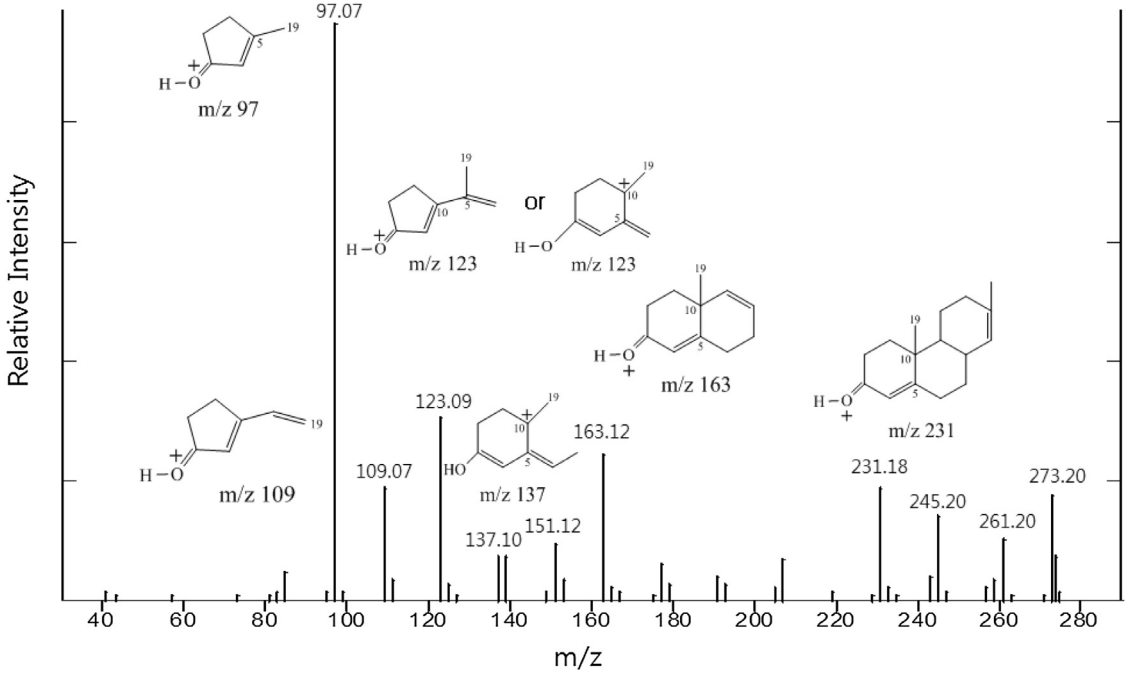

Fig. 1. MSINDO fragmentation spectrum of [testosterone-H]$^+$ as obtained by CID chemical dynamics simulations.

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