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Protein-binding properties of a designed steroidal lactam compound

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ABSTRACT

Introducing amide bonds into a steroid nucleus or its side chain may reduce the acute toxicity and enhance the pharmaceutical activity. In this work, a designed steroidal amide compound, named 3β-hydroxy-17-aza-D-homo-5-androsten-17-one (HAAO), was synthesized and identified. The interactions between HAAO and human serum albumin (HSA) were studied by multiple spectroscopic methods and molecular modeling procedures. It was found that HAAO locates in Sudlow's site I in subdomain IIA of HSA molecules, relying on hydrogen bonds and van der Waals power to form HAAO–HSA complexes at ground state. The number of binding sites, binding constants, enthalpy change (ΔH^{θ}), Gibbs free energy change (ΔG^{θ}) and entropy change (ΔS^{θ}) were calculated at different temperatures based on fluorescence quenching theory and classical thermodynamic equation. The percentages content of the HSA's secondary structures in presence of HAAO were detected by circular dichroism (CD) spectra and compared with those in no presence of HAAO. In addition, the experimental results of both binding site and conformational change were further confirmed by molecular modeling investigation, in which more details of the binding were visually unfolded. The information provided by the study may be useful for designing novel chemotherapeutic drugs and be helpful both in the early stages of drug discovery and in clinical practice.

1. Introduction

Steroids are a class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived there from, which are playing important roles in life activities of animals and plants for their good performance in cell penetration [1–3]. For example, sex steroids including androgens, estrogens and progestogens produce sex differences and support reproduction, glucocorticoids regulate metabolism and immune function, mineralocorticoids help maintain blood volume and control renal excretion of electrolytes, and anabolic steroids interact with androgen receptors to increase muscle and bone synthesis [4–6]. However, the applications of natural steroids were greatly restricted because of their limited amount and low content in spite of the excellent bioactivity. So introducing specified functional groups into the core or the side chains of natural steroids to obtain new compounds with higher activity or some special function has become a focus of research on the steroidal chemistry in recent years [7]. The introduced functional groups include heteroatom, hydroxyl, sulfhydryl, ester, oximido, hydrazone, acylamino, etc. [8,9]. In particular, in the decade of the 2000s, considerable interest was generated in steroidal derivates with acylamino (—NH—CO—) in their molecular structures among chemists, pharmacologists, biologists and life scientists [10]. On one hand, plenty of literatures indicated that many synthesized steroidal amide compounds exhibited excellent bioactivity in anti-tumor, antibacterial, inhibition of 5α-reductase, and so on [11,12]. On the other hand, only a handful of steroidal amides, such as estramustine phosphate sodium, dutasteride and finasteride, have already been successfully used in clinic. One of the most important reasons for this phenomenon is that the molecular mechanism of pharmacological action of steroidal amides is unclear.

Drug–plasma-protein binding is critically involved in drug pharmacokinetics (i.e., absorption, distribution, metabolism, and elimination) and pharmacodynamics (pharmacological effects) [13]. It is therefore highly important to estimate drug-binding ability to macromolecules in the early stages of drug discovery [14]. Human serum albumin (HSA) is the most abundant protein in human blood plasma with ascribed drug-binding and transport properties [15], which can bind a wide range of endogenous and exogenous compounds and serves as an important carrier for an extraordinarily broad range of drugs [16–18]. In present work, HSA was selected as a model to detect the drug–protein binding at the molecular level.

The aim of this paper was (1) to synthesize 3β-hydroxy-17-aza-D-homo-5-androsten-17-one (HAAO), a steroidal amide compound which has obvious effects on human cervical carcinoma and human liver carcinoma; (2) to investigate the interaction between HAAO and HSA under simulated physiological conditions (ionic strength = 0.1 mol L^{-1}; pH 7.40 ± 0.01). In order to attain these objectives, multiple spectroscopy methods, molecular probe and molecular modeling techniques were employed.

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2. Experimental

2.1. Materials

HSA was purchased from Sigma (USA, 99%). Warfarin and ibuprofen were obtained from Hubei Biocause Pharmaceutical Co., Ltd. (Hubei, China) with the purity no less than 99.7%. 3β-hydroxy-5-androsten-17-one, Ac_2O, pyridine, NH_2OH, $SOCl_2$, THF, Tris, HCl, NaCl were purchased from Shanghai Chemical Reagent Company (China). 3β-hydroxy-17-aza-D-homo-5-androsten-17-one (HAAO) had a purity of no less than 99.5%. All other chemicals were of analytical grade. Stock solutions of HSA (10^{-5} mol L^{-1}), HAAO (2×10^{-3} mol L^{-1}), NaCl (0.5 mol L^{-1}) and Tris–HCl buffer (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} HCl) of pH 7.40 ± 0.01 were prepared by directly dissolving the original reagents. Water used to prepare solutions was double-distilled.

2.2. Instrumental methods

All fluorescence spectra were recorded at four different temperatures on LS-55 spectrofluorometer (Perkin-Elmer, America) equipped with 1.0 cm quartz cells and a thermostatic bath. To obtain smooth emission spectra with moderate intensity between 260 and 400 nm, the widths of the excitation slit and the emission slit were set to 15 and 4.5 nm with the scanning speed at 1000 nm/min. An excitation wavelength of 290 nm was chosen and appropriate blanks corresponding to the buffer were subtracted to correct the background. CD measurements were performed at 310 K on a J-810 Spectropolarimeter (Jasco, Japan) equipped with 1.0 cm quartz cells over a wavelength range of 250–200 nm and under constant nitrogen flush at a scanning speed of 200 nm/min. IR spectra were obtained by a Nicolet380 FI-IR infrared spectrometer (Thermo Electron, America). Nuclear magnetic resonance spectra were measured on a FX-90Q (JEOL, Japan) instrument. LC–MS were carried on a TSQ Quantum Access MAX (Thermo Fisher, America) instrument. The weight measurements were performed with an AY-120 electronic analytic weighing scale (Shimadzu, Japan). All pH measurements were made with a pHS-3 digital pH-meter (Shanghai, China).

2.3. Molecular modeling

Molecular modeling calculations were carried out using Sybyl8.1. The crystal structure of HSA was from Brookhaven Proteins Data Bank (PDB) database (entry codes: 1h9z for site I, 2bxg for site II). All the ligands and water molecules were removed before the analysis. H atoms were added and the biopolymer was charged using AMBER7 FF99 method. The site of the protein was defined with ligand (A/WRR2001 for site I, A/IBP2001 for site II); the principal regions of ligand binding to HSA were analyzed by Sybyl 8.1 software. The structure of HAAO was generated by sybyl8.1 package and the molecule was optimized (energy minimization) using Tripos Force Field after being charged with Gasteiger and Marsili method. The docking mode of HAAO with HSA was conducted by a Surflex-Dock program in Sybyl 8.1 package. The original ligand was redocked into HSA by the same procedures and the results were close to the crystal structure, which verify the docking program works well.

2.4. Synthesis of HAAO

Using 3β-hydroxy-5-androsten-17-one as a starting material, according to the literature by Huang et al. [12], HAAO was synthesized in four steps and the procedure for its preparation was shown in Fig. 1. Yield 76%, mp 168–171 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.02 (3H, s, 19-CH_3), 1.18 (3H, s, 18-CH_3), 2.34–2.31 (1H, m, C_{16}–H), 2.40–2.34 (1H, m, C_8–H), 2.47 (1H, ddd, J = 18.6, 7.2, 1.2, C_{16}–H), 3.57–3.50 (1H, m, C_3–H), 5.38 (1H, t, J = 3.0, C_6–H), 5.67 (1H, s, –NH). ESI-MS m/z: 306 $(M + 1)^+$.

3. Results and discussion

3.1. Fluorescence spectra

Fluorescence is the process of photon emission as a result of the return of an electron in a higher energy orbital back to a lower orbit, which in the life sciences is used generally as a non-destructive way of tracking or analysis of biological molecules that can either give intrinsic fluorescence themselves or be "labeled" with some extrinsic fluorophore [19,20]. A variety of molecular interactions can result in quenching, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching. The intrinsic fluorescence of HSA molecule was generated by aromatic amino acid residues in its structure, especially the tryptophan residues [21].

According to experimental procedures, the fluorescence spectra of HSA as well as HSA–HAAO systems were recorded (Fig. 2). As shown in Fig. 2, the fluorescence of HSA around 350 nm regularly decreased with the increasing amount of HAAO, indicating that HAAO interacted with HSA and acted as a fluorescence quencher.

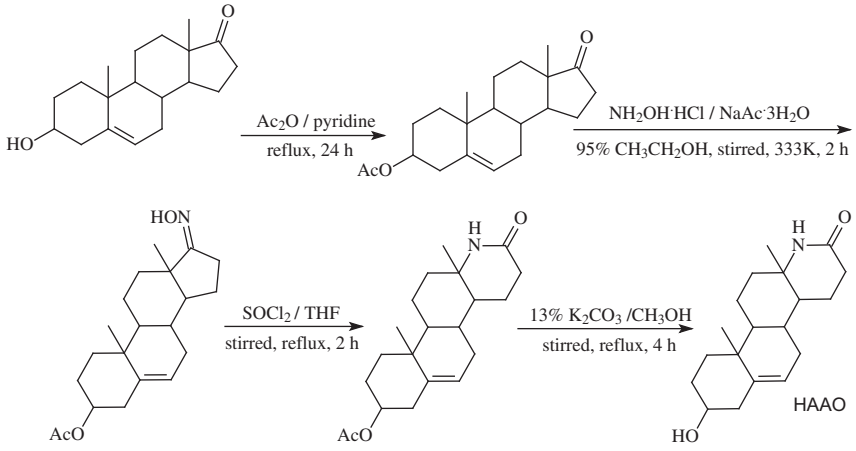

Fig. 1. Procedure for the preparation of HAAO.