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Stereoselective interactions of lactic acid enantiomers with HSA: Spectroscopy and docking application

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ABSTRACT

Lactic acid enantiomers, normally found in fermented food, are absorbed into the blood and interact with plasma carrier protein human serum albumin (HSA). Unveiling the effect on the function and structure of HSA during chiral interaction can give a better understanding of the different distribution activities of the two enantiomers. Multi-spectroscopic methods and molecular modelling techniques are used to study the interactions between lactic acid enantiomers and HSA. Time-resolved and steady-state fluorescence spectra manifest that the fluorescence quenching mechanism is mainly static in type, due to complex formation. Binding interactions, deduced by thermodynamic calculation, agree with the docking prediction. Docking results and kinetic constants represent chiral-recognizing discriminations consistently. The bindings of lactic acid enantiomers lead to some microenvironmental and slight conformational changes of HSA as shown by circular dichroism (CD), synchronous and three-dimensional fluorescence spectra. This investigation may yield useful information about the possible toxicity risk of lactic acid enantiomers to human health.

1. Introduction

Lactic acid affects the rheological and sensory qualities of milk and gives good storage properties to fermented products. As a nutrient, it provides 3.6 kcal/g or 15.2 kJ/g of energy (Alm, 1982). Lactic acid is chiral, consisting of two optical enantiomers (Fig. 1). One is L-(+)-lactic acid (L-Lac) and the other is D-(−)-lactic acid (D-Lac). Lactic acid enantiomers are characteristic in all fermented dairy products (wines, sake and milk products) which are generated by both homo- and heterofermentative microbes. In fermented milk (Alm, 1982), the amount of total lactic acid is about 0.6–1.2%, and L-Lac is the major enantiomer formed. The amount of D-Lac is about 0–10% of the total lactic acid in acidophilus milk. In yogurt, about 40% of the total lactic acid is D-enantiomer.

Physiological experiments in man and animals showed that lactic acid enantiomers were absorbed from the human intestinal tract (Duran, Van Biervliet, Kamerling, & Wadman, 1977). L-Lac can promote calcic absorption. However, the rate of metabolism of the D-enantiomer was considerably lower than that of L-Lac (Flemström, 1971). However, after intake of large quantities of D-Lac, enhanced Ca^{2+} was secreted in the urine (Alm, 1982). Restricted consumption of products which contain a high concentration of D-Lac is worth advocating. Infant formulae containing D- or DL mixture should be avoided (Organization, 1974).

It is known that ligand–protein interactions affect the distribution, free concentration, and metabolism of various small molecules in the bloodstream. When lactic acid enantiomers are absorbed into the blood, they may bind to plasma proteins and subsequently change the structure and function of the protein. Yet, no reports have so far examined the chiral effects of lactic acid enantiomers at the molecular level, and the possible effect on plasma proteins is still poorly understood. Nutrition and safety of foods are concerns around the world, and thus information is needed to fill in this gap.

HSA is the major protein component of human blood plasma. The physiological and pharmacological functions of HSA are maintaining osmotic pressure of blood, buffering pH, and serving in the

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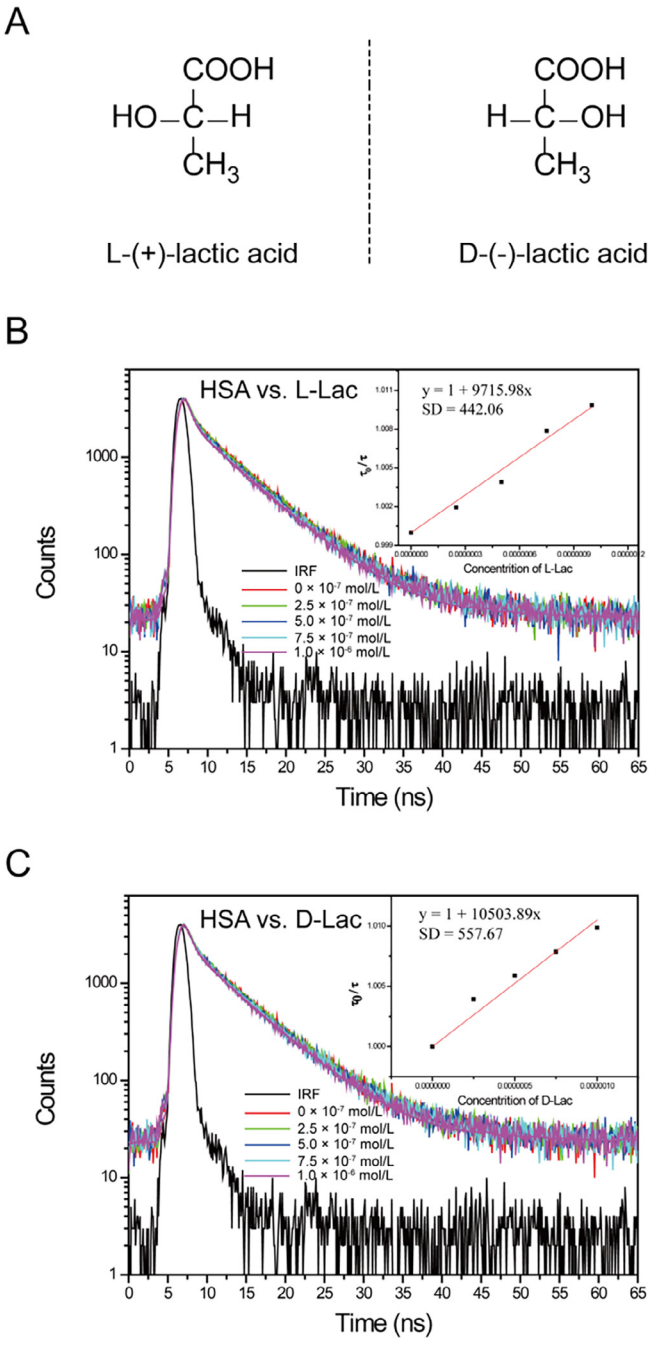

Fig. 1. (A) Structures of lactic acid isomers and fluorescence intensity decay of HSA (1.0×10^{-6} M) in presence of increasing (B) L-Lac and (C) D-Lac. Inset: dynamic SV constant $K_{SV'}$ regression. IRF: instrument response function.

transportation and distribution of a variety of nutrients and medicines. HSA is a monomeric protein which contains 585 residues. It contains 3 structurally similar α-helical domains (I–III), and each domain can be divided into subdomains A (containing 6 α-helices) and B (containing 4 α-helices) (He & Carter, 1992). Drugs or compounds mainly bind to one of the two primary binding sites on the protein, known as Sudlow's sites I (warfarin binding site) and II (benzodiazepine binding site).

Exploring the interaction mechanism of lactic acid enantiomers with HSA can give a better understanding of the different distribution activities of the two enantiomers. Because of their high sensitivity, rapidity, reproducibility, and convenience, spectroscopic approaches have become useful techniques for study of protein–ligand interactions (Cao et al., 2018; Zhang, Wang, & Pan, 2012). The measurements can be carried out under physiological conditions to better mimic *in vivo* interaction environments (Mu et al., 2018; Zhang, Zhuang, Tong, & Liu, 2013). Multi-spectroscopic approaches, such as time-resolved fluorescence, steady-state fluorescence quenching, three-dimensional (3D) fluorescence, synchronous fluorescence and CD spectroscopy, were used to determine the binding characteristics, the main acting forces and conformational changes during chiral binding. The molecular docking technique was also employed to further elucidate chiral binding. This method is easily applicable to find out the preferred orientation of one or more molecules on the active sites of proteins (Liu et al., 2018). In the meantime, the binding affinity may be predicted, using the preferred orientation. Molecular docking has been successfully employed to further elucidate the mechanism of chiral binding, because it has the ability to predict the optimal conformations between small molecules and the binding sites of protein (Alonso, Bliznyuk, & Gready, 2006). Spectra and docking results may provide basic data for clarifying the stereoselective binding mechanisms of L-Lac/D-Lac with HSA and may be helpful for human health and food safety.

2. Materials and methods

2.1. Materials

L-(+)-Lactic acid (90% in water) and D-(−)-lactic acid (89–91%) were purchased from Adamas Reagent, Ltd. (Shanghai, China). HSA (96–99%) was obtained from Sigma-Solarbio Co. (Beijing, China). Other reagents and chemicals used in this research were all of analytical grade or higher level. Deionized water (18.2 MΩ) was prepared using a Milli-Q water purification system (Millipore, Bedford, MA). Tris–HCl buffer solution (0.1 M NaCl, 0.1 M Tris, pH 7.40) was used in fluorescence measurement, and phosphate buffer (0.02 M, pH 7.40) was used in circular dichroism measurements.

2.2. Steady-state fluorescence measurements

Steady-state fluorescence spectra measurements were performed on a Cary Eclipse Spectrofluorimeter (Varian, USA) equipped with CARY Temperature Controller (−10–110 °C). Excitation wavelength of 280 nm was used for fluorescence emission spectra measurement. Fluorescence titrations were performed by keeping the fixed concentration of HSA (4.30×10^{-7} M) while titrating by successive additions of L-Lac or D-Lac at temperatures of 293 K, 301 K and 310 K. The lactic acid concentrations after each titration were 1.28, 2.56, 3.84, 5.12, 6.40, 7.68, 8.96, 10.2, 11.5×10^{-7} M, respectively. The synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength interval (Δλ) at 15 and 60 nm (298 K). The 3D fluorescence spectra of L, D-Lac (1.15×10^{-6} M), HSA (4.30×10^{-7} M) and mixtures of HSA-Lac enantiomer were obtained in an excitation wavelength range from 200 nm to 320 nm at 5 nm increments at temperature of 298 K, and emission wavelength range from 280 nm to 480 nm and recorded.

2.3. Time-resolved fluorescence experiments

A FLS980 system (Edinburgh Instruments) equipped with a 290.80 nm picosecond pulsed light emitting diode was used for time-resolved fluorescence decays measurement. The emission monochromator was fitted to 346 nm with slits of 10 nm. The fluorescence decay curves were recorded over 100 ns up to a peak count of 4000 (1024 channels) at 301 K. The decay curves of HSA (1.0×10^{-6} M) were measured with 0, 2.5, 5.0, 7.5, 10×10^{-7} M lactic acid isomers. the intensity decays were fitted using the FAST software package (Edinburgh Instruments).