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Investigation of proton pump inhibitors binding with bovine serum albumin and their relationship to molecular structure

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ABSTRACT

The interactions of three proton pump inhibitors (PPIs), omeprazole, pantoprazole and ilaprazole with bovine serum albumin (BSA) have been investigated by fluorescence, synchronous fluorescence, ultraviolet–visible (UV–vis) and circular dichroism (CD). Various binding parameters have been calculated at various temperatures. The results indicated that omeprazole, pantoprazole and ilaprazole had a strong ability to quench the intrinsic fluorescence of BSA with static quenching mechanism, and the binding affinities were significantly affected by different substituents and polarities as the order ilaprazole > pantoprazole > omeprazole. The site marker competitive experiments indicated that the binding of omeprazole, pantoprazole and ilaprazole to BSA primarily took place in subdomain IIA. The results of thermodynamic parameters ΔG, ΔH and ΔS indicated that electrostatic interaction played a major role for PPIs–BSA association. The distance r between PPIs and BSA was evaluated according to the theory of Förster's energy transfer. The quantitative analysis of synchronous fluorescence and CD spectra showed the change in secondary structure of the BSA upon interaction with PPIs by a reduction of α-helix. All the above results many have relevant insight into the PPIs' availability and distribution.
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1. Introduction

Proton pump inhibitors (PPIs) of the substituted benzimidazole class are widely utilized for the treatment of gastro-esophageal reflux disease, as well as other acid-related disorders for about 25 years [1]. Omeprazole (Fig. 1) is the first of the PPI marketed in 1987 [2], and pantoprazole is the third PPI marketed in 1995 [3], while ilaprazole (named IY-81149 before), is a newly developed PPI [4] (Fig. 1). All the PPIs are effective and can be used safely. Clinical studies have revealed that there were no significant differences between omeprazole and pantoprazole in pharmacological activity [5–7]. However, ilaprazole produced a statistically greater and prolonged acid-suppressing effect than did omeprazole [8], which was attributed to the longer plasma half-life of ilaprazole compared with that of omeprazole [9]. Drugs in the body were correlated with their affinities toward serum albumin for their distribution, absorption, bioavailability and metabolism. Ghuman and coworkers have shown that the distribution, free concentration and the metabolism of various drugs could be significantly altered as a result of binding to serum albumin [10]. Moreover, the structures of the drug may affect the interaction with serum albumin [11]. Consequently, the binding study of drugs to serum albumin was of imperative and fundamental importance for drug development.

Serum albumin is the major soluble protein in the circulatory system, which has many physiological functions, such as maintaining the osmotic pressure and pH of blood and as carriers transporting and decomposing a great number of endogenous and exogenous compounds such as fatty acids, amino acids, drugs and pharmaceuticals [12]. The drug–serum albumin interaction plays a dominant role in drug disposition and efficacy. The bound drugs can act as a depot while unbound drugs produce the desired pharmacological effect. To study the interaction between drugs and serum albumin is not only important to provide salient information about the nature of drugs and pharmacokinetics, but it is also helpful to explain the relationship between the structures and functions of drugs. In this work, BSA has been selected as the protein model because of its structural homology with human serum albumin (HSA) [13]. Fluorescence spectroscopy is an appropriate method to clarify the interactions between compounds and serum albumin [14–19]. The interaction between omeprazole and bovine serum albumin (BSA) had been investigated by fluorescence quenching study [20]. Our previous investigations showed the binding of ilaprazole and their metabolites on BSA and declared that the existence of Cu^{2+} and Fe^{3+} decreased the binding affinity of ilaprazole with BSA [21,22]. However, the binding of pantoprazole on serum albumin is not

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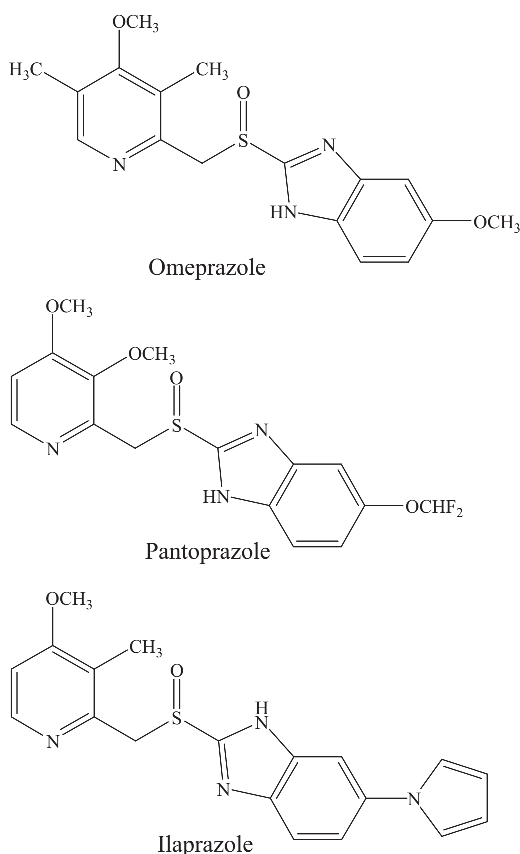

Fig. 1. Molecular structures of omeprazole, pantoprazole and ilaprazole.

yet clear, and few reports have focused on the binding sites, conformational change and structure–affinity relationship of PPIs binding with serum albumin on molecular biology level.

Herein, three PPIs, omeprazole, pantoprazole and ilaprazole, were studied for their affinities for BSA by fluorescence, synchronous fluorescence, UV–vis and CD in an attempt to characterize the chemical associations taking place.

2. Materials and methods

2.1. Chemicals and reagents

Omeprazole and pantoprazole with purity 99.5% were obtained commercially from Sigma Chemical Co. (St. Louis, MO, USA). Ilaprazole with purity 99.1% was provided by Livzon Pharmaceutical Group Inc. (Zhuhai, China), which signed a license agreement and got the patent from ILYANG Pharmaceutical Company Ltd. (Seoul, South Korea). Bovine serum albumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals such as buffer Tris with the purity more than 99.5%, NaCl, HCl, and ethanol were all of analytical purity and used without further purification. Water used in all experiments was doubly distilled water.

2.2. Instrumentations

All fluorescence spectra were recorded on an F-2000 spectrofluorimeter equipped with 1.0 cm quartz cells and a 150 W xenon lamp (Hitachi, Tokyo, Japan). An excitation wavelength of 280 nm was used. The excitation and emission slit width were both set at 2.5 nm. The UV spectra were obtained on a Perkin-Elmer Lambda 17 UV spectrophotometer with the wavelength range of 200–450 nm (Perkin Elmer Corp., Edison, NJ, USA). CD spectra were recorded with a Jasco-810 spectropolarimeter (Jasco, Japan) with the wavelength range of 200–240 nm using a 10 mm path length cell at 298 K. The weight measurements were performed on an AY-120 electronic analytic weighing scale with a resolution of 0.1 mg (Shimadzu, Japan). The pH value was measured in a pHs-3 digital pH meter (Shanghai, China).

2.3. Preparation of solutions

Tris-HCl buffer solution (0.1 mol L^{-1} Tris, pH 7.4) containing 0.1 mol L^{-1} NaCl was prepared to keep the pH value and maintain the ionic strength of the solution. The working solution of BSA (1×10^{-4} mol L^{-1}) was prepared by dissolving it in Tris-HCl buffer solution and stored in refrigerator at 4 °C prior to use. The omeprazole, pantoprazoel and ilaprazole stock solution (4×10^{-4} mol L^{-1}) was prepared by dissolving them in ethanol.

2.4. Procedures

300 μL of BSA solution were added to eleven 5 mL flasks, and then appropriate amounts of 4.0×10^{-4} mol L^{-1} omeprazole, pantoprazole or ilaprazole were added, and diluted to 5 mL with Tris-HCl buffer. The final concentrations of omeprazole, pantoprazole or ilaprazole were 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0 μmol L^{-1}. The resultant mixtures were then incubated at 298 K for 1.0 h. After 1.0 h incubation, the fluorescence emissions spectra were scanned in the range of 290–450 nm and the fluorescence intensity at 340 nm was measured. All the experiments were repeated in triplicate and found to be reproducible with the experimental error ($<1\%$).

3. Results and discussion

3.1. Fluorescence quenching of BSA

BSA has three linearly arranged, structurally distinct, homologus domains (I–III), and each domain is composed of two subdomains (A and B). The specific sites binding with BSA are sites I and II which are located in hydrophobic cavities in the IIA and IIIA subdomains [23]. When the fluorescence emission spectra of BSA are measured with a series of concentrations of quencher by fixing the excitation wavelength at 280 nm, the fluorescence emission peak of BSA at 340 nm gives the information of tryptophan and tyrosine residues [24]. Therefore, fluorescence quenching can be considered as a very sensitive way with potentiality to analyze the interactions between drugs and proteins.

When adding three PPIs, omeprazole, pantoprazole and ilaprazole to BSA solution, the fluorescence of BSA was quenched as shown in Fig. 2. It was observed that the fluorescence intensity of BSA dropped regularly with the increasing concentrations of PPIs, which indicated that the interaction had been happened between PPIs and BSA. The intensities of BSA fluorescence decreased slightly by adding omeprazole and pantoprazole, but decreased remarkably by adding ilaprazole. Weak slight blue shifts of the maximum emission wavelength (λ_{em}, 1–3 nm) of BSA fluorescence occurred, which suggested that the fluorescence chromophore of BSA was placed in a more hydrophobic environment by hydrophobic interactions of PPIs with BSA via hydrogen bonds. About 20.2%, 23.4% and 46.3% of the fluorescence intensities of BSA were quenched by adding 20 μmol L^{-1} omeprazole, pantoprazole and ilaprazole, respectively (calculated from Fig. 2). The extent of the fluorescence attenuation was in the order: ilaprazole > pantoprazole > omeprazole, which indicated that the

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