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Binding interaction of isoxsuprine hydrochloride and levothyroxine to milk β-lactoglobulin; from the perspective of comparison

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ABSTRACT

Isoxsuprine hydrochloride (ISO) and levothyroxine (LEV) are medicines which can be utilized alone or simultaneously by pregnant women. The purpose of this work is to investigate the separate and simultaneous interaction of ISO and LEV with β-LG. The results showed that both drugs can bind to β-LG; the static quenching was suggested for fluorescence quenching mechanism of β-LG.The values of binding constants ($K_{\beta\text{-LG-ISO}} = 2.69 \times 10^4\ M^{-1}$, $K_{\beta\text{-LG-LEV}} = 0.54 \times 10^3\ M^{-1}$ and $K_{\beta\text{-LG–ISO–LEV}} = 2.18 \times 10^3\ M^{-1}$ at 310 K) suggested that ISO has stronger binding affinity toward β-LG than LEV and affinity of β-LG to LEV is increased in the presence of ISO while the presence of LEV has no significant effect on the affinity of protein to ISO. Thermodynamic parameters showed that the binding of LEV to β-LG are hydrogen bonding and Van der Waals forces but the formation of β-LG–ISO is hydrophobic associations. The results of FT-IR and UV–visible measurements indicated that the binding of both drugs to β-LG may induce conformational changes of protein. *In silico* molecular docking analyses confirmed that ISO and LEV binds to residues located at site I and site II of β-LG, respectively.

1. Introduction

Proteins are the important molecules in human life and the major targets of all medicines in organisms [1,2]. It has been revealed that the distribution, free concentration, and the metabolism of a variety of drugs may be affected by drug–protein interactions. Also, this interaction can influence the stability and toxicity of drugs during the chemotherapeutic process [3]. β-lactoglobulin (β-LG) has formed the center of many research activities for years. This soluble globular protein is the most abundant protein of milk whey, and displays functional properties useful in the food industry and in advanced oral drug delivery [4]. β-LG is a member of the lipocalin family with a wide range of applications such as an antioxidant [5], retinol [6] and vitamin D [7] transport protein [8]. So, this protein can serve as a potential transport and depot protein, particularly for hydrophobic molecules. It contains 162 amino acids with a molecular mass of 18.3 kDa and has an isoelectric point of around 5.2 [9–11]. It is largely dimeric at ambient temperature and at a pH equal to or higher than 7 and monomeric below pH 3.

The evidence shows the binding of a drug to carrier proteins can influence simultaneous binding of other drugs [2]. This occurs, probably due to dislocation in the binding site by another drug that has a higher affinity to protein or the modification of the protein structure, resulting in the change (increase or decrease) of the affinity of the carrier protein toward the drugs [12]. The simultaneous presence of two drugs for interaction with carrier protein may lead to a decrease in binding and therefore, enhances the concentration of a free biologically active part of one or both drugs [13]. Surely if amount of drug is decreased, the side effects will be less.

Levothyroxine (LEV, Fig. 1), also known as L-thyroxine, is a manufactured form of the thyroid hormone, thyroxine. It is used to treat hypothyroidism, a condition where the thyroid gland does not produce enough thyroid hormone. LEV is also used to treat congenital hypothyroidism (cretinism) and goiter (enlarged thyroid gland). This drug is also used with surgery and radioactive iodine therapy to treat thyroid cancer. In fact, treating hypothyroidism is essential during pregnancy. There are foods and other substances that can interfere with absorption of LEV. Examples include calcium and iron supplements taken within four hours of levothyroxine [14]. A study of eight women suggested that coffee may interfere with the intestinal absorption of LEV, though at a level less than eating bran [15,16]. Certain other substances can cause adverse effects that may be severe. Combination of LEV with ketamine may cause hypertension and tachycardia. Also, tricyclic and tetracyclic antidepressants increase its toxicity [17]. Although obstetricians try to minimize the number of medications women take during pregnancy, LEV is safe for pregnant women with hypothyroidism.

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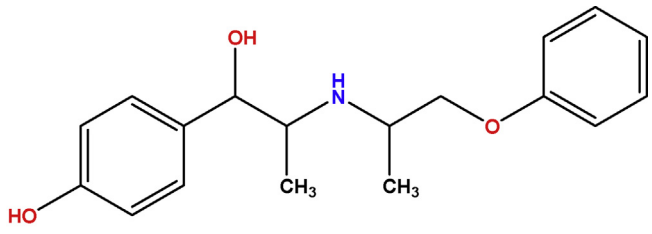
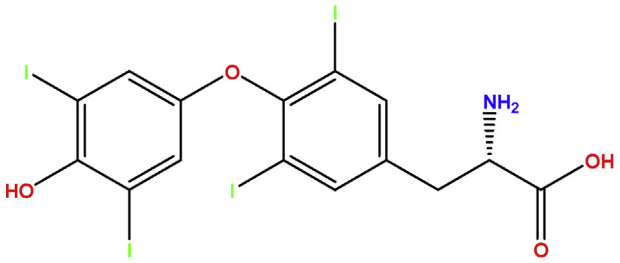


Isoxsuprine hydrochloride



Levothyroxine

Fig. 1. Molecular structure of isoxsuprine hydrochloride and levothyroxine.

Isoxsuprine hydrochloride (ISO, Fig. 1) is a drug used as a vasodilator [18] in humans and equines. This drug is a β_2 adrenoceptor agonist that causes direct relaxation of uterine and vascular smooth muscle via β_2 receptors. A number of large clinical trials have shown the therapeutic efficacy of ISO in patients at risk of preterm labor [19,20] and risk of abortion [21]. During pregnancy, especially for women with hypothyroidism these two drugs may be used simultaneously by pregnant women. The goal of this study is to investigate the interaction between above drugs (ISO and LEV) and β-lactoglobulin in the absence and presences of another drug and compare the results of these two modes with each other. In this comparison, we try to analyze the tendency of each of drugs (ISO and LEV) to the transporting protein β-LG separately or simultaneously by multi-spectroscopic and molecular modeling techniques.

As binding of drug-protein be strengthened, the amount of drug that reaches to the target organ is increased. So, if ISO could be changed the affinity of levothyroxine to β-LG, this can be exploited to revise the dose of medicine consumption for LEV by pregnant women.

2. Materials and methods

2.1. Materials

Bovine milk β-lactoglobulin (β-LG ~ 90%), Tris-HCl buffer, levothyroxine and isoxsuprine hydrochloride were purchased from Sigma. All other materials and reagents obtained from Sigma-Aldrich Co, and were of the analytical grade. The concentration of protein was determined spectrophotometrically using a molar absorptivity of 17600 M^{-1} cm^{-1} at 280 nm. Binding experiments were carried out in Tris–HCl buffer (10 mM) containing NaCl (10 mM) at pH 7.4.

2.2. Methods

2.2.1. UV–vis studies

The UV–vis measurements of β-LG were recorded in the range of 200–400 nm at 298 K after incubation of the protein sample with these drugs for 5 min, on a JASCO UV/Vis-7850 double-beam spec-

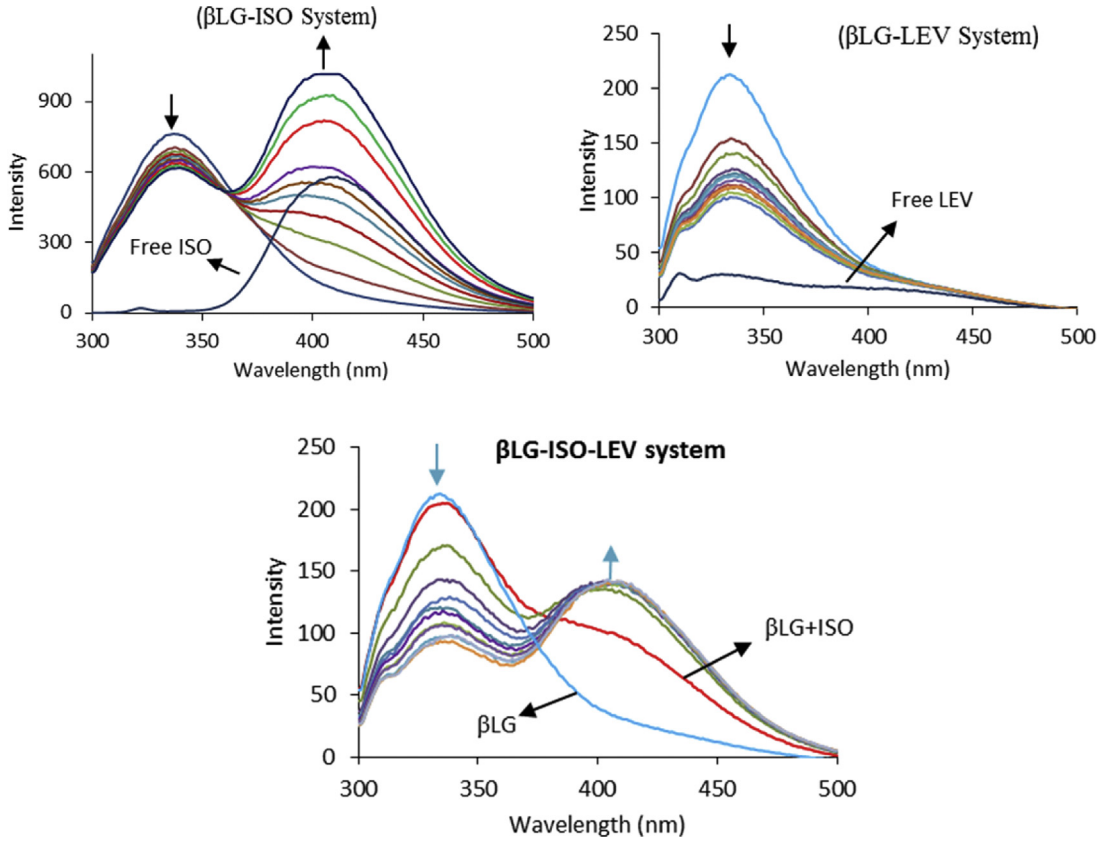


Fig. 2. Fluorescence spectra of β-LG (2.5×10^{-6} M) with increasing concentration of drugs ($0 - 1.7 \times 10^{-6}$ M) at pH 7.4.