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Binding interactions of niclosamide with serum proteins

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ABSTRACT

A study of the binding of niclosamide (NC) to serum proteins such as human serum albumin, hemoglobin, and globulin was carried out using fluorescence and UV-visible spectroscopy. Interactions between NC and these proteins were estimated by Stern–Volmer and van't Hoff equations. The binding constants and the thermodynamic parameters, ΔH, ΔS, and ΔG at different temperatures were also determined by using these equations. Data showed that NC may exhibit a static quenching mechanism with all proteins. The thermodynamic parameters were calculated. Data showed that van der Waals interactions and hydrogen bonds are the main forces for human serum albumin and hemoglobin. Globulin, however, bound to NC via hydrophobic interaction. The spectral changes of synchronous fluorescence suggested that both the microenvironment of NC and the conformation of the proteins changed in relation to their concentrations during NC's binding.

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1. Introduction

Niclosamide (5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide; Fig. 1.) belongs to the family of medicines called anthelmintic drugs, which are effective against most tapeworms [1,2]. Its action against tapeworm species is to uncouple oxidative phosphorylation, block glucose uptake, and inhibit respiration in anaerobic ATP production [3,4]. Niclosamide is a US Food and Drug Administration approved compound that has been used in humans for 40 years [5]. It is commercially known as Bayer 73 in Germany or bayluscide or niclocide in Canada and the United States [6,7]. It is well tolerated by rats with an acute oral toxicity. A significant decrease in hemoglobin concentration occurs when niclosamide is given to male and female rats at a concentration of 5 g/kg/day for four weeks [6–8]. Niclosamide is effective against severe acute respiratory syndrome virus [9]. It also has antineoplastic activity and anti-anthrax toxin properties [10,11]. Niclosamide induces LC3-positive autophagosomes and inhibits the Wnt/Frizzled pathway and mTOR signaling [12–14].

Niclosamide's interaction with proteins and enzymes explains its mechanism of action in the human body. Thermodynamic parameters are especially helpful in identifying the binding mechanism of a drug to any protein. Serum proteins are the major transport protein in human body. Albumin is a serum protein widely used to explain the binding mechanism of a small molecule to a protein. Serum proteins are capable of

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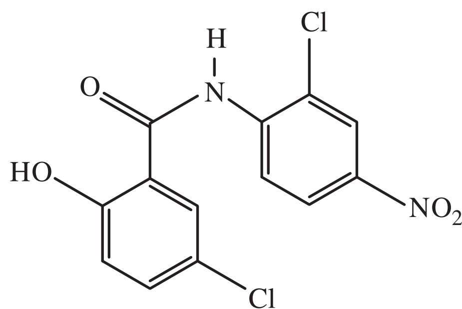

Fig. 1 – Structure of niclosamide.

reversibly binding many endogenous and exogenous drugs [15,16]. They may aid in selective delivery of the drugs to a target tissue, organ, or tumor region, thus delivering drugs into the cell. Most recently, a large number of studies on the drug–protein interaction have been reported using techniques including fluorescence, Fourier transform infrared spectroscopy, circular dichoism spectroscopy, and nuclear magnetic resonance [17–21]. It is necessary to determine binding parameters during destabilization by these methods for drug–protein systems.

Fluorescence serves as a sensitive method indicating alterations of the fluorophore environment, thus resulting in plenty of useful information [21]. Many drugs are not fluorescent but proteins have a fluorescent character that results from the protein's tyrptophan and tyrosine residues [22–24]. This property allows the monitoring of alterations of the protein during binding. Due to fluorescence quenching, binding can be analyzed through reduction of fluorescence intensity or the shifting of the emission wavelength of the protein upon the addition of the drug [16].

In this study, the binding mechanism of niclosamide to several proteins including human serum albumin, globulin, and hemoglobin is investigated using fluorescence and UV-visible spectroscopy. The nature of the drug's binding mechanism to all proteins was described by using Stern–Volmer and van't Hoff equations. Synchronous fluorescence was also used for all proteins to determine environmental changes corresponding to the interactions. The aim of this work was to estimate interactions of niclosamide with serum proteins.

2. Materials and methods

2.1. Materials

Niclosamide, HCl, NaOH, hemoglobin, globulin, and albumin from human serum (97–99%), and Tris (hydroxymethyl) aminomethane were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents and chemicals were of molecular and analytical grade. The ultra pure water was purified using the Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

Absorption spectra were recorded on a Shimadzu-1700 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) using a 1 cm quartz cell. Fluorescence spectra were scanned by a Perkin Elmer LS-55 spectrofluorometer (Perkin Elmer, Waltham, MA, USA) equipped with a xenon lamp source and a 1 cm quartz cell.

2.3. Fluorescence spectroscopy

Niclosamide was prepared as 1mM of stock solution in a water-dimethylformamide solvent system (V:V, 90:10%) for all experiments. Then, 1 mg mL^{-1} of protein solution was prepared by directly dissolving the protein in a Tris buffer including 20mM of Tris at pH, 7.4. All aqueous solutions used in testing contained 10% (v/v) dimethylformamide for all measurements. An appropriate volume of niclosamide solution was added to the proteins ranging from 1 μg to 20 μg and mixed for 10 minutes at room temperature. The fluorescence spectra from 200 nm to 500 nm were scanned by a spectrofluorometer using a 500 nm scan speed and 10 nm slit in a quartz cell.

An appropriate volume of protein (10 μg) was mixed with different amounts of niclosamide between 1 μM mL^{-1} and 80 μM mL^{-1} and then, diluted to 2.0 mL with Tris buffer. The fluorescence spectra of the solutions at different temperatures were collected at the excitation wavelength of 280 nm.

2.4. Synchronous fluorescence spectroscopy

Appropriate concentrations of proteins in Tris buffer were mixed with different concentrations of niclosamide. The emission spectrum was recorded with $\Delta\lambda = 15$ nm or $\Delta\lambda = 60$ nm for all niclosamide–protein complexes.

2.5. Absorption of niclosamide with protein

Appropriate concentrations of all proteins in 20mM of Tris buffer (pH:7.4) were added to different amounts of niclosamide between 1 μM and 80 μM. The absorption spectra of the niclosamide–protein complexes were collected by using the Shimadzu 1700 UV-visible spectrophotometer.

3. Results and discussion

3.1. Fluorescence quenching of proteins

Protein emits strong fluorescence peaks at 280 nm and 342 nm at excitation and emission wavelengths, respectively. The quenching effect of niclosamide on proteins' intrinsic fluorescence was studied for three proteins. The presence of niclosamide led to a decrease in the fluorescence intensity of human serum albumin (HSA) with a slight blue shift as increasing amounts of the drug were added (Fig. 2A). The intensity of fluorescence of both hemoglobin and globulin decreased with a slight red shift, suggesting that niclosamide interacts with these proteins (Fig. 2B and C).

3.2. Synchronous fluorescence of proteins

Proteins are composed of 20 amino acids in different sequences. The fluorescence of proteins results mainly from two

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