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Interaction of Aldehyde dehydrogenase with acetaminophen as examined by spectroscopies and molecular docking

Ayodele O. Kolawole

Department of Biochemistry, The Federal University of Technology, Akure, Nigeria

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ABSTRACT

The interaction of acetaminophen, a non-substrate anionic ligand, with Aldehyde Dehydrogenase was studied by fluorescence, UV–Vis absorption, and circular dichroism spectroscopies under simulated physiological conditions. The fluorescence spectra and data generated showed that acetaminophen binding to ALDH is purely dynamic quenching mechanism. The acetaminophen-ALDH is kinetically rapid reversible interaction with a binding constant, K_a, of 4.91×10^3 L mol^{-1}. There was an existence of second binding site of ALDH for acetaminophen at saturating acetaminophen concentration. The binding sites were non-cooperative. The thermodynamic parameters obtained suggest that Van der Waal force and hydrogen bonding played a major role in the binding of acetaminophen to ALDH. The interaction caused perturbation of the ALDH structures with an obvious reduction in the α-helix. The binding distance of 4.43 nm was obtained between Acetaminophen and ALDH. Using Ficoll 400 as macro-viscosogen and glycerol as micro-viscosogen, Stoke-Einstein empirical plot demonstrated that acetaminophen-ALDH binding was diffusion controlled. Molecular docking showed the participation of some amino acids in the complex formation with −5.3 kcal binding energy. With these, ALDH might not an excipient detoxifier of acetaminophen but could be involved in its pegylation/encapsulation.

1. Introduction

Aldehyde dehydrogenases (ALDH; EC 1.2.1.3) are short-chain dehydrogenases/reductases (SDR) superfamily containing NAD(P)$^+$-dependent enzymes that catalyse the irreversible dehydrogenation of a wide range of endogenous and exogenous aldehydes to their corresponding less toxic carboxylic acids [1–3]. ALDHs are widely distributed in prokaryotic and eukaryotic cells and play important roles in detoxification of toxic and reactive aliphatic and aromatic aldehydes formed during the metabolism of alcohols, amino acids, carbohydrates, lipids, biogenic amines, vitamins and steroids [4]. Currently, there are 19 known members of the ALDH superfamily [5,6]. ALDHs functional and physiological properties have been studied extensively and are involved in the maintenance of cellular homeostasis, modulate cell proliferation, differentiation, survival and cellular response to oxidative stress [1,7,8]. ALDHs play essential role in the metabolic pathways that are critical for cell development and response to environmental changes [9].

ALDHs are homo-biopolymers composed of two or four polypeptides of 50–55 kDa, and made up of N-terminal NAD$^+$-binding domain, a catalytic domain and an oligomerisation domain [10,11]. Aldehyde dehydrogenases kinetic mechanism is literarily an ordered sequential kinetic mechanism with NAD(P)$^+$ binding first, followed by the aldehyde [12–14]. In some cases, it is random kinetic mechanism with preference for initial binding of NAD(P)$^+$ [15]. The ternary complex forms thio-hemiacetal intermediate which is transformed to thioester by giving its hydride ion to NAD(P)$^+$. Eventually, the thioester is hydrolysed by a water molecule to carboxylic acid. The sequential dissociation of carboxylic acid and NADH, which is the rate-limiting step, ends the reaction [14,16].

ALDHs exhibit additional, non-enzymatic functions, the non-catalytic binding properties for endobiotics, some hormones and other small molecules [1,17]. It is 'housekeeping' functions linked with detoxification. This is associated with the ubiquitous, ample and constitutively expressed properties of the enzyme. These ligand binding properties might be connected to protective function through the sequestration of metabolites. They conceivably serve to prevent the accumulation or minimize potentially toxic free endobiotics and xenobiotics or involved in the uptake and transport of hydrophobic non-substrate prior to its detoxification. Catalytic and ligand complexing properties (ligandin) are important for detoxification mechanism [1] and there is connection in both [17]. Although ALDH catalytic mechanisms of detoxification have been investigated extensively, however, relatively little is known about its non-catalytic binding function.

Acetaminophen (N-acetyl-p-aminophenol, AAP) (Fig. 1) is a medically important, low cost, readily available and commonly used over

E-mail address: aokolawole@futa.edu.ng.

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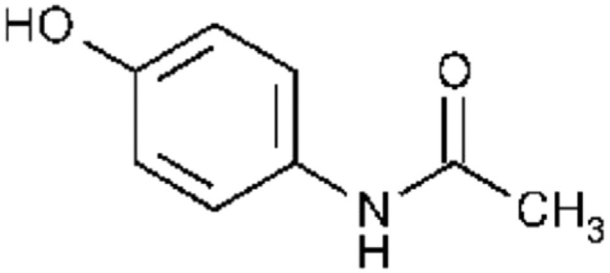

Fig. 1. Structure of Acetaminophen.

the counter analgesic and antipyretic drug [18,19]. Acetaminophen monotherapy is efficient and is safer than Aspirin and Ibuprofen [20]. The efficacy and tolerability in individual condition is warranted [18]. The mechanism of analgesic action of acetaminophen is complex and its action of medicament has not been completely understood [20]. At therapeutic doses, acetaminophen is safe drug but not devoid of side effects [18] and suggest the possibility of acetaminophen exerting other specific biological effects [21]. High dosages, in humans and experimental animals, lead to necrosis, nephrotoxicity, and extra hepatic lesions [22]. Nevertheless, it is grossly abused in Nigeria and it has been blamed for the rising cases of heart attacks, stroke and early death [23,24]. The negative effect of Acetaminophen on the antioxidant defense enzyme system has been documented [23]. The interaction of acetaminophen with Human Serum Albumin (HSA) was previously investigated [25]. The authors detailed the biochemical and biophysical data illustrating the relevance of HSA to the acetaminophen pharmacokinetics. However, in a pathogenic state of human serum albumin, lower albumin concentration and weaker drug–protein interaction can result in the increase of drug concentration in the blood and lead to toxicity [26,27]. More worrisome, is the use of acetaminophen with alcoholic beverages [21,28].

The link between Aldehyde dehydrogenase and Acetaminophen metabolism is becoming increasingly imaginable [7,21]. ALDH has been identified as a major acetaminophen-binding protein [28]; and was down regulated in mouse liver exposed to high dosage of acetaminophen [29]. However, the affinity and interaction mechanism of acetaminophen to ALDH still remain uncharted. The effect of the complexation on ALDH structure and conformation is yet to be elucidated.

Several spectroscopic techniques, as powerful tools, have been used to study the interaction between drugs and proteins. They allow non-intrusive measurements of substances in low concentration under physiological conditions [30]. Fluorescence technique is the simplest method to study the interaction of drugs/ligands and bio-macromolecules because it has the advantage of high sensitivity, rapidity and ease of implementation [31,32]. It is an important method to sense changes in the local microenvironment of fluorescent chromophore [33] and help understand the biopolymer's binding mechanisms to drugs and provide clues to the nature of the binding phenomenon [34,35]. The information on the acetaminophen-ALDH binding mode, the binding constant and the effects of acetaminophen complexation on the protein structure is obscured. In the present work, the binding of Acetaminophen to ALDH was studied under physiological conditions by spectroscopic techniques. The quenching mechanism between Acetaminophen and ALDH with regards stoichiometric and thermodynamic of ligand binding and consequently the effect on the protein conformation were investigated at molecular level. In addition, the effects of pH and viscosity of Acetaminophen -ALDH complex were also examined. All these were complimented by *in silico* analysis and molecular docking.

2. Materials and methods

2.1. Materials

Aldehyde dehydrogenase (ALDH; molecular weight 200,000 Da) was obtained from Sigma-Aldrich Fine Chemicals, USA and was used without further purification. Acetaminophen concentrate (≥99% purity,) was a generous gift from Deshalom Pharmaceuticals Nig. Ltd., Ilesha, Nigeria. All reagents were of analytical grade unless specialized. All solutions were prepared with double distilled water. Acetaminophen stock solution was prepared in analytical grade double distilled ethanol. All glass Ostwald viscometer (VWR, USA) was used to measure the intrinsic and extrinsic relative viscosity. ALDH protein concentration was measured using Bradford method. Protein sample, ligand solutions and buffers were filtered through a Millipore membrane filter (0.45 μm membrane filter) immediately before use. The pH was checked with a Sartorius PP-50 standardized pH meter (Germany).

2.2. Methods

2.2.1. Fluorescence spectra

All fluorescence spectra were measured with a Hitachi F-4500 Fluorescence Spectrophotometer (Hitachi Ltd., Tokyo, Japan) equipped with a refrigerated circulating water bath (Pharmacia Biotech) and interfaced with HP Window XP Computer. The equipment was furnished with a 150 W Xenon lamp and a 1 cm quartz cell. The spectra were recorded in the wavelength range of 300–500 nm upon excitation at 280 nm when ALDH samples were titrated with acetaminophen. Both excitation and emission bandwidths were set on 5 nm with a scan speed at 900 nm/min. The response time was set to 2 s with a high sensitivity signal. Titrations were performed manually by using trace syringes. A 2.0 mL solution containing an appropriate concentration of ALDH (0.120 μM) in 25 mM Tris–HCl pH 7.4 containing 0.1 M NaCl was titrated manually by successive additions of ethanol stock solution of Acetaminophen to a very saturating concentrations of 125 μM. The final ethanol concentrations never exceeded 1% (v/v), and all fluorescence readings were corrected for the dilution effect. The presence of this volume of ethanol in the assay mixtures had no effect on the fluorescence measurements. Also, respective blanks of the buffer were used for the correction of all fluorescence spectra.

Synchronous fluorescence spectroscopy (SFS) was used to study the environment of amino acid residues. It involves the measurement of any shift, to reflect the changes of polarity around the chromophore molecule, in the emission maximum on addition of ligand molecules. Synchronous fluorescence spectra of solutions prepared as above were measured on the same fluorescence spectrophotometer. The excitation wavelength (λex) was set at 280 nm. The excitation and emission slit widths were set at 5.0 nm. The D-value (Δλ) between the excitation and emission wavelengths was set at 15 or 60 nm. PMT voltage was 700 V.

2.2.2. UV–Visible absorption spectroscopy

All absorbance spectra and equilibrium ligand binding experiments were measured in 25 mM Tris-HCl buffer, pH 7.4, at 25 °C using Shimadzu double beam UV-Visible spectrophotometer (UV-1800) equipped with a Pharmacia refrigerating circulator for temperature control (25 ± 0.1 °C) unless otherwise stated. The scan speed and slit of absorbance (λ_{abs}) were set to medium and 1.0 nm respectively. The spectra were recorded between 200–500 nm. A 1.0 mL solution of 0.120 μM ALDH was titrated with successive addition of acetaminophen. Circular dichroism (CD) measurements were made on a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature under constant nitrogen flush. The Circular dichroism (CD) spectra were measured from 190 to 240 nm at a scan speed of 200 nm/min. Each result was the average of the three scans.

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