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Experimental and computational modeling of interaction of kolaviron-kolaflavanone with aldehyde dehydrogenase

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ABSTRACT

Aldehyde dehydrogenases (ALDHs) are a diverse family of enzymes that catalyze the $NAD(P)^+$-dependent detoxification of toxic aldehyde compounds. ALDHs are also involved in non-enzymatic ligand binding to endobiotics and xenobiotics. Here, the enzyme crucial non-canonical and non-catalytic interaction with kolaflavanone, a component of kolaviron, and a major bioflavonoid isolated from *Garcinia kola* (Bitter kola) was characterized by various spectroscopic and *in silico* approaches under stimulated physiological condition. Kolaflavanone quenched the intrinsic fluorescence of ALDH in a concentration dependent manner with effective quenching constant (K_{sv}) of 1.14×10^3 L.mol^{-1} at 25 °C. The enzyme has one binding site for kolaflavanone with a binding constant (K_a) of 2.57×10^4 L.mol^{-1} and effective Forster resonance energy transfer (FRET) of 4.87 nm. The bonding process was enthalpically driven. The reaction was not spontaneous and was predominantly characterized by Van der Waals forces and hydrogen bond. The flavonoid bonding slightly perturbed the secondary and tertiary structures of ALDH that was 'tryptophan-gated'. The interaction was regulated by both diffusion and ionic strength. Molecular docking showed the binding of kolaflavanone was at the active site of ALDH and the participation of some amino acid residues in the complex formation with −9.6 kcal mol^{-1} binding energy. The profiles of atomic fluctuations indicated the rigidity of the ligand-binding site during the simulation. With these, ALDH as a subtle nanoparticle determinant of kolaviron bioavailability and efficacy is hereby proposed.

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1. Introduction

Aldehyde dehydrogenase ALDHs (*E.C. 1.2.1.3*) are multigene family of $NAD(P)^+$-dependents group of structurally and functionally related ubiquitously distributed enzymes involved in the specific and irreversible oxidation of a broad spectrum of aliphatic and aromatic aldehydes to their corresponding less toxic carboxylic acids [1–5]. They are important component of phase I detoxification system and occurs throughout all phyla [1,6] and exhibit functional polymorphisms among racial populations with tissue-specific distributions, subcellular location, and substrate specificity [7]. The human genome contains more than 19 functional genes for aldehyde dehydrogenases and all showing sequence similarity of 60% or more [6,8,9]. The three-dimensional structure of ALDHs have similar fold despite the low overall identity amongst the sequences of different isoforms [10,11]. ALDH homobiopolymers variant is composed of two or four polypeptides of 50–55kDa, and made up of catalytic site, ligand-competent (LC) cleft, and oligomerization domain [10,12]. This superfamily has multiple functions and is involved in a wide range of physiologic, biologic and pharmacologic processes in prokaryotic and eukaryotic cells. Their diversity provides the capability of detoxifying a very broad range of exogenous and endogenous aldehydic compounds. ALDHs support cellular homeostasis; and have both metabolic and regulatory roles in cancerous cells [1]. ALDH polymorphism and expression have been implicated in several diseased conditions [14–16] and alcohol-associated pathology [17]. ALDH up-regulation, in yeast, is an appropriate molecular response to environmental and chemical stress [18].

The extended biological function, beside detoxification from aldehyde-induced cytotoxicity, of ALDH isoenzyme is not detailed and haphazardly documented. Evidently, ALDH has been reported to be involved in pseudo-ligandin properties both for non-aldehydic endobiotics and xenobiotics [5,19]. The non-canonical binding properties to some hormones and other small molecules have been sketchy [2,15,20]. This is not unconnected to the ligand competent binding cleft which hosts the chemically diverse

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ligands. This extended function, probably linked to detoxification function, might be implicated in intracellular uptake and transport of hydrophobic non-substrate compounds. They might serve to prevent the accumulation of these otherwise hydrophobic non-substrate compounds within the cell when their concentration becomes overwhelming for ALDH catalytic detoxification. Catalytic and ligand complexing properties (ligandin), though connected and difficultly inseparable, are important for detoxification mechanism [5,19]. ALDH catalytic mechanisms of detoxification have been investigated extensively, however, its non-catalytic binding function is becoming more evident.

ALDH has been linked as a multidrug resistant efflux transporter (MDR) to drugs [21]. MDR are major challenges in the chemotherapeutic treatment. Increased aldehyde dehydrogenase activity (ALDH) has been demonstrated to be a mechanism of cytostatic drug resistance during cancer treatment; and, an indicator of patient poor survival [13,22]. Therefore, an ALDH specific, competitive type (reversible) inhibitor not requiring enzymatic activation would be preferred for *in vivo* inhibition of ALDH related pathophysiologically associated diseases.

Kolaviron, a bioflavonoid complex of *Garcinia* bioflavonoid (GB1), Garcinia bioflavonoid 2 (GB2) and kolaflavanone, is a major phytochemical from the seeds of *Garcinia kola* otherwise known as bitter kola [23–25]. The seed has oral masticatory appeal in subtropical countries [26] and has become important phyto-compound dietary supplements. Despite its pharma-nutritional interface there is no recommended daily allowance (RDA) for kolaviron bioflavonoids complex. Its consumption has been linked to reduction of some chronic diseases and infections incidences. Kolaviron bioflavonoids complex has gained significant research appeal and is an effective antioxidant and inhibits lipid peroxidation and possess very high therapeutic potentials has been shown to exhibit many pharmacological actions. It has anti-atherogenic, anti-diabetic, anti-proliferative, anticancer, anti diabetes, antibacterial, antiviral and anti-inflammatory properties [27–32]. There are neither symptoms nor deficiencies of the bioflavonoids; and, are treated by eukaryotic cells as xenobiotics [33]. Consequently, there is likelihood of low bioavailability and poor efficacy. Flavonoids bioavailability for therapeutic is affected by phase I and II bio-transforming enzymes and phase III transporter enzymes. Bioflavonoids inhibit many unrelated enzymes [34] and modify the catalytic, kinetics and thermodynamic parameters of the enzyme.

Flavonoids-protein interactions studies play a substantial part in the hunt for novel molecules which are to interact with a selected disease-relevant target [35]. The mode of potential interaction gives information about the flavonoid effectiveness and selectivity. Lately, possible target molecules and not the mode of interaction with targets constituents has been an important focus of current pharmacological research of kolaviron bioflavonoids complex [36–39]. Here, the mode of this potential drug candidate interaction with target/carrier protein was sought. We report the binding interaction of kolaviron–kolaflavanone with ALDH by multiple spectroscopic methods under physiological conditions and molecular modeling to understanding the thermodynamics of its interaction *in vivo.*

2. Materials and methods

2.1. Materials

Baker's yeast Aldehyde dehydrogenase (ALDH) was purchased from Millipore EMD Millipore Corporation, Billerica, MA, USA and used without further purification and its molecular weight was assumed to be 200,000 Da. Tris, NAD^+, $NADP^+$, MES, HEPES, MOPS, dimethylsulfoxide, were products of Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA. Bradford reagent and kit were products of Bio-Rad, Palo Alto, CA, USA. All other chemicals were commercial products of reagent/analytical grade. Unless otherwise stated all solutions were prepared with distilled-deionized Milli-Q H_20 (Millipore). The pH measurements were carried out with a Sartorius PP-50 standardized pH meter (Germany). All glass Ostwald viscometer (VWR, USA) was used to measure the intrinsic and extrinsic relative viscosity. ALDH protein concentrations were measured by Bradford method [40]. Protein sample, ligand solutions and buffers were filtered through a Millipore membrane filter (0.45 μm syringe filters) immediately before use.

2.2. Extraction of kolaviron

Garcinia kola seeds were purchased from a local vendor in Akure, Nigeria. Kolaviron was isolated, explicitly, as described elsewhere [26,29]. Briefly, the powdered seeds were extracted with light petroleum ether (bp 40–60 °C) in a soxhlet for 24 h. The defatted dried product was repacked and extracted with acetone. The extract was concentrated and diluted twice its volume with water and extracted with ethylacetate. The concentrated ethylacetate yielded a golden yellow solid termed kolaviron. The purity and identity of kolaviron was determined by subjecting it to thin-layer chromatography (TLC) using silica gel GF 254-coated plates and solvent mixture of methanol and chloroform in a ratio of 1:4 (v/v). The separation revealed the presence of three bands which were viewed under UV light at a wavelength of 254 nm with RF values of 0.48, 0.71 and 0.76 [41]. Purity of isolated kolaviron was ≥96%. During this research work, Kolaflavanone concentrate, based on its abundance and FT-IR identification, was isolated from TLC, reconstituted and re-purified. Kolaviron-kolaflavanone stock solution was prepared in double distilled ethanol or dimethyl sulfoxide (DMSO).

2.3. Fluorescence quenching measurements

All fluorescence spectra were recorded on an F-4500 spectrophotometer (Hitachi, Tokyo, Japan) with a 10 mm quartz cell and a 150 W xenon lamp on it. The fluorometer is equipped with a refrigerated circulating water bath (Pharmacia Biotech) and interfaced with HP Window XP Computer. The Hitachi FL solution software controlled the whole system. The photomultiplier tube voltage and scanning speed were set at 950 V and 1200 nm min^{-1}, respectively. The excitation and emission slit widths were all set at 5.0 nm. The response time was set at 0.01sec. The wavelength of the excitation was set at 280 nm, and the emission wavelength scans ranged from 300 to 500 nm. A 2.0 mL solution containing ALDH (0.250 μM) in 25 mM Tris–HCl pH 7.4 containing 0.1 M NaCl was titrated manually by successive addition of 6.25 μM ethanol stock solution of kolaflavanone to final concentration of 56.25 μM. The solution of kolaflavanone was added dropwise to the ALDH solution with constant stirring to ensure homogenous solution. 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. Tyrosine residue contribution to the bulk protein intrinsic fluorescence was negligible.

The Synchronous fluorescence (SF) of ALDH in the absence and presence of different concentrations of the same ligand was monitored by scanning the samples in the wavelength range of 280–320 and 310–370 nm while keeping a difference between excitation and emission wavelengths ($\Delta\lambda$) of 15 and 60 nm, for Tyrosine and Tryptophan residues, respectively. To decrease the

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