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ORIGINAL ARTICLE

In-gel detection of esterase-like albumin activity: Characterization of esterase-free sera albumin and its putative role as non-invasive biomarker of hepatic fibrosis

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Abstract Albumin is a globular and un-glycosylated multifunctional plasma protein and thus correlated with several human diseases. Owing to esterase contamination, albumin levels are usually misleading. In this study, we propose methodical accuracy for albumin estimation taking healthy and fibrotic rats. Liver fibrosis in rats was generated by *N*′-Nitrosodimethylamine (NDMA) (10 mg/kg body weight) within three weeks followed by its confirmation through H&E and immunohistochemical staining for α-SMA expression. Animal sera were screened by native polyacrylamide gel electrophoresis (native-PAGE) (7.5%). In-gel esterase-like albumin activity was detected using α- and β-naphthyl acetate (5.58×10^{-3} mM; pH 7.5) as substrate. Sera albumin was purified from unstained PA gel-slices through electroelution. Subsequent to conformation of albumin purity by its molecular weight determination using SDS–PAGE (10%) and peptide mass fingerprinting by MALDI-TOF-MS, samples were treated with different concentrations of urea. Urea-treated albumins were screened for esterase activity, conformational change and, albumin levels by immunoblotting. Our results demonstrate that esterase-like albumin activity in rat sera albumin is located in domain-III. The esterase-like activity remains detectable up to 4 M urea, which diminishes with increasing urea concentrations. Further, immunoblotting of urea-treated albumin samples displays a significant decline in purified protein bands, indicating hypoalbuminemia during

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hepatic fibrosis in rats. In conclusion, the present approach of albumin separation and estimation is of potential interest and may be recommended for diagnostic purposes.

1. Introduction

Albumin is a 65–67 kD globular, un-glycosylated and the most abundant multifunctional plasma protein in mammals. It is involved in many vital physiological functions like maintenance of plasma oncotic pressure and pH and, regulation of the transport of fluids across the capillary membrane etc. Besides, the protein is a major carrier of endogenous molecules, metabolites, vitamins, fatty acids, bilirubin, drugs and other xenobiotics into the blood. Moreover, albumin has been reported as the most important antioxidant substance in plasma (Roche et al., 2008).

Published literature on human serum albumin (HSA) suggests that it contains at least two distinct binding sites; site-I for the binding of bulky heterocyclic molecule with centrally located negative charge (like a large number of drugs) and site-II, which binds aromatic carboxylic acids with hydrophobic centers (Sudlow et al., 1975, 1976). An interesting attribute of HSA is that it also possess esterase-like activity with p-nitrophenol (Means and Bender, 1975), α- & β-naphthyl acetate (Casida and Augustinsson, 1959; Morikawa et al., 1979; Ahmad et al., 2012), nicotinate esters (Salvi et al., 1997), aspirin (Rainsford et al., 1980), ketoprofen glucuronide (Dubois-Presle et al., 1995), carprofen acylglucuronide (Georges et al., 2000), cyclophosphamide (Kwon et al., 1987) and a large number of long and short chain fatty acid esters (Tove, 1962). This esterase-like activity of sera albumin has been endorsed to the presence of arginine and tyrosine residues at 410 and 411 positions (Watanabe et al., 2000). This has been confirmed that esterase-like activity of sera albumin is inhibited by various drugs and actually the acetylation of lysine residues results in the inhibition of esterase-like activity of sera albumin (Lockridge et al., 2008).

Structural and functional impairments in albumin are attributed to various pathophysiological conditions like diabetes (Doweiko and Bistrain, 1994), osteoarthritis (Ahmad et al., 2011a) and advanced liver diseases (Zoli et al., 1991; Sugimura et al., 1994). Due to esterase–albumin complex formation in these pathologies, use of albumin as non-invasive marker may be misleading. However, in many of the liver diseases plasma levels of albumin are reported to decline due to its reduced synthesis and oxidative modifications that result in its altered binding with bilirubin (Masood et al., 2002). One of the major limitations to the clinical use of direct markers of liver fibrosis is that they are not routinely same in all settings. As a result, simple and less expensive/cheap markers are needed to be used more extensively in the clinical practice.

A reliable marker for hepatic fibrosis must fulfill the following criteria: (a) ability to quantify total mass of fibrous tissue in the liver; (b) ability to evaluate whether the liver is in a profibrogenic or anti-fibrogenic state and, (c) be sensitive enough to determine the response of the liver to the treatments designed to combat fibrosis. Like many other pathologies, there has been a debate on albumin levels in liver fibrosis also, where several researchers have reported an increase as well as decrease in the levels of albumin during liver fibrosis (Rothschild et al., 1969; Panduro et al., 1990; Masood et al., 2002; Sakaida et al., 2004; George, 2006). The discrepancy in quantifying the exact levels of albumin may be attributed to esterase–albumin complex and the sensitivity of site-I and -II to bind with various molecules. Although there are other methods available for the detection of liver fibrosis such as liver biopsy examinations, transient elastography and several laboratory tests, but still exists scarcity of non-invasive supportive biomarker. Therefore, discovery of non-invasive biomarkers to detect liver fibrosis shall be a priority. In the present study, we demonstrate a methodical procedure for albumin separation and purification free from esterase contamination in a mammalian model of liver fibrosis utilizing the following procedural steps: (1) In-gel detection of esterase-like albumin activity, (2) purification and further confirmation of rat sera albumin by electroelution and SDS–PAGE followed by MS analysis respectively, (3) inhibition of esterase-like activity of purified albumin by urea and, (4) confirmation of sera albumin by spectroscopy and later by immunoblotting to show its accurate levels in healthy and fibrotic rats.

2. Materials and methods

2.1. Chemical

Acrylamide, bis-acrylamide, ammonium persulfate (APS), TEMED, and *N*′-Nitrosodimethylamine (NDMA), were procured from Sigma–Aldrich. α- and β-naphthyl acetate, urea and Tris buffer were purchased from SRL, India, goat anti-mouse IgG-HRP conjugated antibody was purchased from CALTAG Laboratories, Bangkok. α-smooth muscle actin (α-SMA) antibodies were obtained from Trend Bio-products Pvt. Ltd., India. All the other chemicals and reagents used were of analytical grade.

2.2. Care and maintenance of animals

Healthy adult male albino rats *Rattus norvegicus* of Wistar strain, weighing around 145 ± 10 g were used in the present study. The animals were housed in well aerated polycarbonate cages (12 h: 12 h = light: dark period) with proper humane care and were fed regularly with commercial, sterilized diet (Ashirwad Industries Pvt. Ltd., Mohali, Punjab, India) and water available *ad libitum*. They were acclimatized for a week before taking them for the treatment. All the experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.3. Induction of hepatic fibrosis

Fibrosis was induced essentially as described previously (Ahmad et al., 2009). Briefly, the animals were divided into

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