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ScienceDirect
Chemistry and Physics of Lipids 148 (2007) 105–111
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

^{1}H NMR investigation on interaction between ibuprofen and lipoproteins

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Received 22 November 2006; received in revised form 27 April 2007; accepted 27 April 2007
Available online 10 May 2007

Abstract

A large number of studies indicate that oxidative modification of plasma lipoproteins, especially low-density lipoprotein (LDL), is a critical factor in initiation and progression of atherosclerosis. We have previously found that ibuprofen (IBP), a potential antioxidant drug to inhibit LDL oxidation, interacted with lipoproteins in intact human plasma. In the present study, we compare the binding affinities of IBP to LDL and HDL (high-density lipoprotein) by ^{1}H NMR spectroscopy. When IBP is added into the HDL and LDL samples, the $-N^+(CH_3)_3$ moieties of phosphatidylcholine (PC) and sphingomyelin (SM) in lipoprotein particles experience the chemical shift up-field drift. Intermolecular cross-peaks observed in NOESY spectra imply that there are direct interactions between ibuprofen and lipoproteins at both hydrophobic and hydrophilic (ionic) regions. These interactions are likely to be important in the solubility of ibuprofen into lipoprotein particles. Ibuprofen has higher impact on the PC and SM head group ($-N^+(CH_3)_3$) and $-(CH_2)_n-$ group in HDL than that in LDL. This could be explained by either IBP has higher binding affinity to HDL than to LDL, or IBP induces orientation of the phospholipid head group at the surface of the lipoprotein particles.

Keywords: NMR; Lipoprotein; Ibuprofen; Antioxidant; Interaction; Affinity

1. Introduction

As the key components in regulation of lipid transport in circulation, lipoproteins are generally classified by their size and density as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Brown and Goldstein, 1986). They have a spherical structure with a hydrophobic core of nonpolar triglyceride (TG) and cholesteryl esters (CE) surrounded by an amphipathic surface of apolipoproteins, cholesterol and phospholipids, predominantly phosphatidylcholine (PC) and sphingomyelin (SM) (Hevonoja et al., 2000). HDL and LDL are two of the most abundant and important lipoprotein fractions, and are responsible for cholesterol reversible transportation in blood. It is well established that LDL cholesterol (LDL-C) has positive correlation with atherosclerosis (Cullen and Assmann, 1997), while increasing data shows that HDL cholesterol (HDL-C) is in the con-

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doi:10.1016/j.chemphyslip.2007.04.012

trary (Barter et al., 2003). A large number of studies indicate that oxidative modification of LDL plays a key role in initiation and progression of atherosclerosis (Witztum and Steinberg, 1991; Colles et al., 2001). It has been reported that many antioxidants could inhibit oxidation of LDL in several experimental models (Huang et al., 1999; Zapolska-Downar et al., 1999; Naderi et al., 2003; Upston et al., 2003; Gonen et al., 2005; Tavridou et al., 2006). Most studies were carried out in vitro with isolated LDL focusing on antioxidant efficiency to protect LDL (Zapolska-Downar et al., 1999; Naderi et al., 2003). In order to understand mechanism of the protection, it is essential to study antioxidant–lipoprotein interaction at molecular and atomic resolutions.

Nuclear magnetic resonance (NMR) spectroscopy can provide information at both molecule and atomic levels under physiological or 'near-physiological' conditions. As a unique tool, NMR has been widely used to investigate drug–protein interaction, and to derive information of binding site and affinity, conformation and dynamics in a noninvasive manner. Such information is included in NMR observable parameters, such as chemical shift (Medek et al., 2000; McCoy and Wyss, 2002; Cui et al., 2003; Yang et al., 2004), relaxation time (Liu et al., 1997a; Veglia et al., 1998; Delfini et al., 2000; Bai et al., 2005), diffusion coefficient (Hajduk et al., 1997; Liu et al., 1997a,b; Luo et al., 1999; Yang et al., 2004), and nuclear Overhauser effects (NOE) (Meyer et al., 1997; Chen and Shapiro, 1998). Another significant advantage of NMR is that magnitude of a NMR signal, regardless of its chemical shift, is proportional only to the number of nuclei responsible for the signal. This makes ^{1}H NMR a sensitive and useful tool for semi-quantitative and quantitative analysis.

Ibuprofen (IBP) is a well-known nonsteroidal anti-inflammatory drug and could act as antioxidant to inhibit oxidation of LDL in a high dose-dependent manner over the concentration range 0.1–2.0 mM (100 μg/mL LDL) (Zapolska-Downar et al., 1999, 2000). In our previous work, we found that IBP caused chemical shift up-field drifts for the protons of $-N^+(CH_3)_3$ moieties of phosphatidylcholine and sphingomyelin, olefinic chains from unsaturated lipids in lipoprotein particles in intact human blood plasma (Yang et al., 2004). Diffusion coefficient measurements suggested that the protons that experienced chemical shift changes were from relatively small and rapidly diffusing lipoprotein particles. From that, we primarily presumed IBP might interact with lipoproteins in plasma (Yang et al., 2004). In this article, we provide evidences that electronic and hydrophobic interactions are likely to be important in the solubility of ibuprofen into lipoprotein particles. We also show that ibuprofen has higher binding affinity to HDL than to LDL.

2. Materials and methods

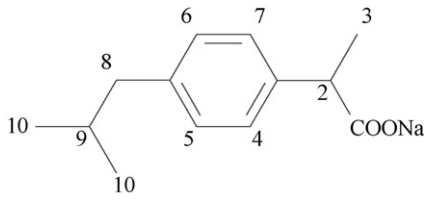
2.1. Materials

Ibuprofen sodium salt was purchased from Sigma (Poole, Dorset, UK). Na_2EDTA, KBr, NaCl were from Shanghai Chemical Reagent Corporation (China). All chemicals were used without further purification. For convenience, the numbering systems and molecular structure of IBP are shown in Scheme 1.

2.2. Isolation of HDL and LDL

Vein blood was taken from a fasted female subject. The sample was moved into a tube containing 0.1% EDTA and centrifuged at 3000 rpm for 20 min to obtain plasma. HDL and LDL were isolated by one-step density gradient ultracentrifugation (Chung et al., 1980). After adjusting density to 1.30 g/mL by adding solid KBr, the plasma sample was distributed into polycarbonate centrifuge tubes. The density gradient solution (1.006 g/mL) was layered over the density-adjusted plasma. The tubes were ultracentrifuged with a Beckman Ti 80 rotor at 50,000 rpm for 350 min at 4 °C. Lipoproteins were dialyzed extensively at 4 °C against phosphate buffer, pH 7.0, containing, e.g., 137 mM NaCl, 2.7 mM KC1, 10 μM Na_2EDTA, 0.01 M Na-phosphate, resulting in fractions of HDL and LDL. The lipoprotein samples were stored at 4 °C until required for NMR analysis.

The PAGE-SDS analysis of isolated HDL and LDL fractions indicated absence of significant contamination with plasma proteins and other lipoprotein fractions. Protein concentrations were measure using modification of the Lowry procedure (Lowry et al., 1951) with bovine serum albumin as a standard. The protein concentrations of HDL and LDL samples are 0.39 and 0.31 g/L, respectively.



Scheme 1. The structure and numbering systems of ibuprofen.