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Interaction of alcohols with serum LDL An infrared study

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This article is dedicated to the memory of Xabi who left us so early.

Abstract

The interaction of low molecular weight alcohols with low density lipoprotein (LDL) has been studied using amide I band-fitting, thermal profiling and two-dimensional infrared correlation spectroscopy (2D-IR). At 0.3 M alcohol, no changes in secondary structure are observed. In the presence of 1 M alcohol, ethanol and propanol decreases protein denaturation temperature and produces changes in the amide I thermal profiles of protein components and in the lipid bands. The 2D-IR synchronous map corresponding to protein or lipid component at 20–37 °C suggests differences in the presence of propanol. The asynchronous map corresponding to the lipid component indicates changes in bandwidth, compatible with a more fluid environment. In the 37–80 °C temperature range the thermal profile is different in the presence of propanol, both for the lipid and protein components. The results presented show that when alcohols affect the protein component, the lipid spectrum also varies pointing to an effect on the lipid-protein interaction.

Keywords: LDL; Alcohol; Infrared spectroscopy; 2D-IR; Protein–lipid interaction; Structure

1. Introduction

Human serum low density lipoprotein (LDL) is a major carrier of serum cholesterol in humans. It is described as a spherical particle containing a hydrophobic core of cholesteryl esters and triglycerides surrounded by an amphipatic monolayer of phospholipid and cholesterol in which a single molecule of apoB is located. ApoB is a hydrophobic protein containing 4536 residues. The structure of LDL has been studied previously by infrared spectroscopy (IR) showing that secondary structure at 37 °C is 24% α-helix, 23% β-sheet, 6% β-turns, 24% unordered structure 24% β-strands and is affected by lipid transitions occurring in the particle lipid core (Bañuelos et al., 1995). ApoB contains a band located around 1617 cm^{-1} that is unusual in proteins and has been attributed to extended chains (β-strands) embedded in the monolayer (Bañuelos et al., 1995). This

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fraction is also resistant to proteolysis (Goormaghtigh et al., 1993).

Alcohols can be found in bloodstream where they can form macromolecular assemblies. Abusive drinkers can survive with alcohol levels up to 0.3 M with a serum LDL concentration of 1.2 μM. Besides, short-chain alcohols have an anaesthetic effect that increases with chain length (Chiou et al., 2005). It is not well defined how anaesthetic exert its action. Alcohol action has been suggested to be due to an interaction with either the lipid or the protein phase. ESR studies using spin labelling of the lipid phase (Kveder et al., 1997), of the lysines of apo-B (Kveder et al., 1998) or of both lipid and protein phases (Kveder et al., 2000) suggest that alcohol would influence lipoproteins through the lipid interphase, modulating their packing and promoting conformational changes in apoB-100.

Infrared spectroscopy has become a widely used tool in the study of protein structure (Barth and Zscherp, 2002; Tamm and Tatulian, 1997; Vigano et al., 2000) Structural analysis usually implies a mathematical approach in order to extract the information contained in the composite bands, designated in IR spectroscopy as 'amide bands', arising from proteins. Commonly used methods of analysis involve narrowing the intrinsic bandwidths to visualize the overlapping band components and then decomposing the original band contour into these components by means of an iterative process. The various components are finally assigned to protein or subunit structural features (Arrondo and Goñi, 1999). External perturbations such as temperature are commonly used to obtain a deeper insight into protein structure by means of infrared spectroscopy. Thermal profiles have often been used to study conformational changes in proteins (Arrondo et al., 1993). Recently, the use of two-dimensional correlation spectroscopy (2D-IR) has been proposed. In this procedure the spectra before and after an external perturbation are correlated, to increase the amount of information obtained from the infrared spectrum (Noda et al., 2000). Proteins are a good target for this method, since changes induced by temperature (Fabian et al., 1999), by the presence of lipids (Shanmukh and Dluhy, 2004; Torrecillas et al., 2004) or other external ligands (Pastrana-Rios, 2001) can be studied in more detail than with the conventional infrared approach.

In the present work IR spectroscopy has been used to study the interaction of low molecular weight alcohols with human serum LDL. The combined approach of band decomposition, thermal profiles and 2D-IR spectroscopy has been used in order to determine the interaction of low molecular weight alcohols with serum LDL.

2. Materials and methods

2.1. Sample preparation

Blood (300 ml) from the blood bank was poured over 15 ml of concentrated buffer containing EDTA (1 M Tris-HCl, pH 7.4, 1 g/l EDTA, 2 g/l chloramphenicol). Plasma was immediately separated at 4 °C by repetitive low-speed centrifugation. Low-density lipoprotein was obtained by sequential floating ultracentrifugation in a Kontron Centricon T2080 ultracentrifuge using a TFT 50.38 rotor at 48,000 rpm for 20 h at 7 °C according to the modified method of Jürgens et al. (Jürgens et al., 1987). Potassium bromide was used to adjust the serum densities to yield the LDL fraction. LDL was isolated at $d = 1.019–1.050$ g/ml by two repetitive centrifugations. The concentration of EDTA was kept constant (1 g/l) through all preparative steps to prevent lipoprotein peroxidation. Lipoproteins were dialysed overnight in the dark against 0.1 M Tris–HCl, pH 7.4, containing 1 g/l EDTA, then concentrated by pressure dialysis or Amicon MDS-1 system and subsequently filtered through a 0.45 μm filter. Isolated lipoproteins were stored overlayed with N_2 at 4 °C in the dark. Prior to IR measurements the samples were liophylised and reconstituted in a D_2O buffer. The amide I spectrum after liophylisation was alike the one obtained previously by dyalisis (Bañuelos et al., 1995) or speed-vac concentration (Chehin et al., 2001). The protein content of LDL was determined according to Lowry with bovine serum albumin as a standard.

The alcohol effect was studied by dissolving liophylised LDL in a D_2O solution at the desired alcohol concentration. Two alcohol concentration were used, 0.3 M at the physiological upper limit concentration and 1 M which produces more accused effects without variation in the two apo-B100 amide I characteristic bands at 1617 and 1693 cm^{-1} indicating that the particle has not been disrupted (Bañuelos et al., 1995). Methanol, ethanol propanol (*n*- and *iso*-) were assayed.

2.2. Infrared studies

The samples were recorded in a Nicolet Magna IR 550 spectrometer equipped with a MCT detector using a demountable liquid cell (Harrick Scientific, Ossining, NY) with calcium fluoride windows and 50 μm spacers. A tungsten-copper thermocouple was placed directly onto the window and the cell placed in a thermostatized cell mount. Thermal analysis was performed by heating continuously in the range of 15–75 °C with a heating rate of 1 °C/min. Spectra were collected by using a rapid scan