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Mechanical, structural, and dynamical modifications of cholesterol exposed porcine aortic elastin

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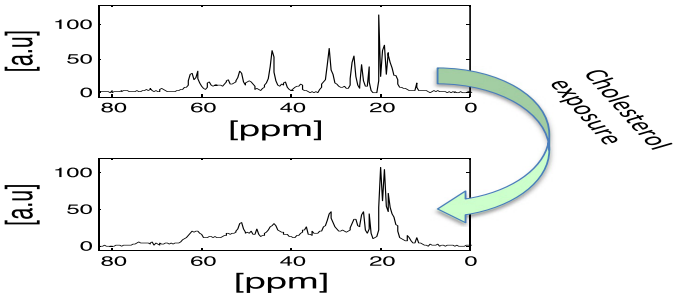
HIGHLIGHTS

- Cholesterol treated elastin swells and reduces stiffness relative to controls.
- ^{13}C NMR measurements point to increase in mobility following cholesterol exposure.
- Cholesterol treatment of elastin leads to changes in water populations.
- MD simulations were performed on a short elastin repeat, VPGVG.
- Simulations show exposure to cholesterol reduces the retractive entropic force.

GRAPHICAL ABSTRACT



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ABSTRACT

Elastin is a protein of the extracellular matrix that contributes significantly to the elasticity of connective tissues. In this study, we examine dynamical and structural modifications of aortic elastin exposed to cholesterol by NMR spectroscopic and relaxation methodologies. Macroscopic measurements are also presented and reveal that cholesterol treatment may cause a decrease in the stiffness of tissue. ^{2}H NMR relaxation techniques revealed differences between the relative populations of water that correlate with the swelling of the tissue following cholesterol exposure. ^{13}C magic-angle-spinning NMR spectroscopy and relaxation methods indicate that cholesterol treated aortic elastin is more mobile than control samples. Molecular dynamics simulations on a short elastin repeat VPGVG in the presence of cholesterol are used to investigate the energetic and entropic contributions to the retractive force, in comparison to the same peptide in water. Peptide stiffness is observed to reduce following cholesterol exposure due to a decrease in the entropic force.

1. Introduction

Elastin is a protein in the extracellular matrix that provides mechanical properties of elasticity and extensibility to connective tissues, including arteries [1]. These characteristics allow arteries to accommodate deformations encountered during physiological functions and maintain a steady pressure for regular blood flow throughout the body [2,3]. Elastin contains hydrophobic domains, which make it attractive for interactions with ligands such as cholesterol [4]. The lipid content of vascular arteries includes cholesterol and is known to increase with age and with various pathological

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conditions [5,6], which may lead to arterial stiffening [7,8]. Biochemical analyses of arterial elastin (from human atherosclerotic subjects) have shown that the lipids accumulating on elastic membranes of atherosclerotic lesions are associated with elastin even in early atherosclerosis. In mild and moderate atherosclerosis changes in elastin are limited to the plaque areas of the arterial intimae while the composition of elastin in the adjoining intimae and midiae were observed to remain unaltered. However, in severe atherosclerosis the lipid accumulation appears to spread into the intimae adjacent to plaque as well as into the midiae below the plaques involving elastin in the elastic membranes of these areas [9,10]. As elastin is the primary site within the vascular wall for degenerative disorders, it is often hypothesized that changes in elastin's biochemical environment, such as exposure to various lipids, may be an important contributor to arterial stiffening [11,12].

Elastin consists of hydrophobic regions that are rich in glycine, proline, and valine as well as cross-linking domains, comprised of desmosine and isodesmosine, that are often surrounded by polyalanine [13,14]. To understand the mechanism of elasticity of this protein, several models have been proposed based on short repeating motifs. In recent years, the repeating motif $(VPGVG)_n$, which repeats $n = 9$ times in human elastin [15], has received a great deal of attention. Luan et al. proposed the librational entropy mechanism (LEM) using both experimental and theoretical studies on the polypentapeptide VPGVG. According to this model the motif VPGVG is characterized by type II β-turns whose amplitude of libration is reduced upon extension. The changes in the amplitude of libration give rise to a decrease in entropy resulting in an elastomeric force [16]. Hong and coworkers performed a number of studies on this elastic mimetic polypeptide. In one of their studies, proline and glycine isotropic and anisotropic chemical shifts indicated the presence of type II β-turns [17]. In a following study, they reported the dynamics of an elastin-mimetic protein, $[(VPGVG)_4(VPGKG)]_{39}$, as a function of hydration by solid state NMR spectroscopy. It was shown that water induces segmental motion and that the polypeptide exhibits large amplitude dynamical characteristics at and above 30% hydration levels [18].

Environmental factors such as the degree of hydration, polarity of the solvent, and temperature are known to affect the dynamical characteristic of this remarkable protein. ^{13}C NMR studies of calf ligamentum nuchae, by Torchia et al., showed that elastin consists of highly mobile chains [19]. A following study supported this finding with relaxation data which indicated that there is high mobility in this protein while the molecular motion is restricted in the alanine-rich regions [20]. Gotte and coworkers proposed that elastin may behave like rubber under certain conditions; however, this property can be lost upon complete dehydration. Kumashiro et al. observed the ^{13}C NMR spectra in elastin with different hydration levels at various temperatures. Samples with high hydration levels were observed to exhibit highly mobile characteristics; however, rigid solid-like characteristics are observed at low hydration levels and at temperatures below the freezing point of water [21]. Recent studies of the effects of a solvent such as glycerol by Demuth et al. have demonstrated that the protein mobility may be governed by solvent viscosity and showed evidence for a strong coupling between protein fluctuations and solvent dynamics [22].

Lillie and Gosline showed that biochemical solvents have an important effect on the elastic and viscoelastic behavior of elastin. An increased tensile storage and loss modulus was observed and the tissue became stiffer when treated with glucose [23]. In another study, they showed that the hydration of the elastin network decreased at low sodium dodecyl sulfate (SDS) levels and increased at higher SDS levels [4]. Our recent work probed microscopic dynamical and structural modifications of porcine aortic elastin exposed to glucose, using solid-state spectroscopic and relaxation methodologies. Additionally, macroscopic stress strain tests were performed and revealed that glucose treated samples were mechanically stiffer than the same tissue without glucose treatment. Results from ^{13}C magic-angle spinning NMR methods indicated that glucose exposure gives rise to reduced mobility throughout the protein. Additionally, two dimensional ^2H T_1-T_2 experiments indicated that the tumbling motion of water in these samples were similar; however, differences were observed between the relative populations of water across the samples. Simulations were also performed to probe the effects of glucose on elastin and showed that stiffening arises from an energetic contribution to the retractive force in the hydrophobic VPGVG domains of the protein [24].

In this work we report on the changes in macroscopic properties of elastin following exposure to cholesterol. Elastin may exhibit a decrease in stiffness following cholesterol treatment, and dimensional changes are observed which suggest micro-structural alterations. We study the dynamical and structural modifications of cholesterol treated porcine aortic elastin compared with untreated samples, using ^{13}C magic angle spinning spectroscopy and relaxation NMR methodologies. In addition, the dynamics and populations of water in untreated as well as cholesterol treated samples were investigated using 2D ^2H T_1-T_2 NMR techniques. Lastly, we present results from molecular dynamics simulations on the repeating motif VPGVG in cholesterol solution and in water to give insight into structural and dynamical alterations which may contribute to changes in the proteins' elastomeric characteristics.

2. Materials and methods

2.1. Sample preparation

Fresh porcine thoracic aorta was collected from a local abattoir. Porcine aorta was chosen because of its availability and anatomical similarity to humans [25]. The sample was prepared within 24 h of acquisition to minimize the natural deterioration of the artery and its constituents. The artery was cleaned of blood, fat, and adherent tissues. The cleaned artery was then carefully cut open along the longitudinal direction and then into square pieces with dimensions of about 20 mm × 20 mm. Samples were then washed with distilled water. Elastin was isolated from the tissue by cyanogen bromide (CNBr) treatment [26]. Arterial samples were submerged in 50 mg/mL CNBr solution in 70% formic acid. For every square cm of tissue 8 mL of solution was used. After 19 h of treatment at room temperature and constant stirring, the samples were stirred in the same solution for 1 h at 60 °C, followed by 5 min of boiling to inactivate CNBr. The purified samples were transferred to 1× phosphate buffered saline (PBS) solution (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4) prior to experiments. Throughout this work, purified elastin in 1× PBS solution is referred to as the 'untreated' sample and that treated with cholesterol (as described above) is denoted as the 'cholesterol treated' sample.

2.2. Preparation of cholesterol treated elastin

A stock solution of cholesterol (Matreya LLC) (100 μg/mL) in 1% taurodeoxycholic acid, sodium salt (Santa Cruz Biotechnology) was prepared by following the procedure developed by Hornebeck and Partridge [27]. For cholesterol treatment of elastin, taurodeoxycholic acid, sodium salt was used to dissolve cholesterol in order to form an optically clear solution of 1% taurodeoxycholic acid solution because cholesterol is insoluble in water. 100 mL of 1% aqueous taurodeoxycholic acid, sodium salt solution was mixed with 1% solution of cholesterol dissolved in ethanol (1 mL) under constant stirring for 3–4 days. The isolated elastin samples were placed in beakers filled with the prepared stock solutions. The treated samples were incubated under constant magnetic stirring at 37 °C for 7 days. The