Article

Selective Metal Cation Binding of Elastomeric Protein: Two Types of Binding Sites and Elastomeric Functions

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(Manuscript submitted February 14, 2003; accepted April 16, 2003)

Abstract

An elastomeric protein called elastin is fully responsible for sophisticated biological elasticity in mammalian tissues such as the aortal wall, ligament, lung, and skin. A key step in elastogenesis to establish the structural basis of elastomeric function, a self assembly of the precursor protein in extracellular space, can be mimicked by the temperature-dependent coacervation of an elastin-related polypeptide-water system. Characteristics of the two types of metal cation binding sites, carboxy oxygens of side amino acid residues and peptide backbone carbonyl oxygens, were examined in relation to the multiple biofunctionality as an extracellular matrix using biologically derived α -elastin and synthetic elastin model polypeptide. Nonspecific metal cation bindings to the carboxy groups of side amino acid residues cause simple salting-in and -out effects. Peptide backbone carbonyl oxygens of particular repeating peptide sequence, -Val-Pro-Gly-Val-Gly-, are specifically selective to Ca^{2+} ions and substantially rejective of other metal cations including alkali metal cations, Mg^{2+} , and Cu^{2+} ions. Ca^{2+} ion binding to peptide carbonyl groups of elastomeric protein induces destruction of a key structure for coacervate characteristics and elastomeric functions.

1 Introduction

Elastin, elastomeric protein widely found in all vertebrates, is the second major protein, next to collagen, in the biological system recognized as an important component of the extracellular matrix.[1,2] A schematic representation of the biosynthesis step of elastin proceeding in an extracellular space is shown as:

Tropoelastin, the precursor protein of elastin with a single-chain structure of amino acid residues of around 700-750, is released into extracellular space from the endoplasmic reticulum of cellular system by exocytosis mechanisms. Before enzymatic intra- and intermolecular cross-linking reaction catalyzed by copper-dependent lysyl oxidase, tropoelastin molecules are self-assembled with some regular configurations. The model process of elastogenesis can be represented in the following picture, in which soluble elastin-related polypeptides are self-assembled during the temperature-dependent coacervation.

The biological self-assembly processes of precursor protein in an extracellular crevice, as a key step in the elastogenesis to establish the structural basis of multiple extracellular matrix functions, can be mimicked by the temperature-dependent coacervation of elastin-related polypeptides, such as the tropoelastin, α -elastin, a hot oxalic acid fragmentation product of insoluble elastin with a molecular weight of around 70 kDa comparable to tropoelastin, and synthetic model polypeptides with specific repeating amino acid sequences.[1,2]

The elastin coacervate interactions with metal cations, especially Ca^{2+} ions, are closely related to the structural and functional bases of elastomeric proteins, as has been recognized generally that the high and specific affinities between calcium and abnormal elastin are observed in atherosclerotic aortas.

2 Experimental

2.1 Elastomeric Proteins and Temperature-Dependent Coacervation

α -Elastin isolated from bovine neck ligament and model polypeptide based on the specific repeating amino acid sequence of elastin, $\text{H}-(\text{Val-Pro-Gly-Val-Gly})_n\text{-Val-OMe}$ ($n \geq 40$), were used.[3] The pentapeptide repeating sequence is thought to be responsible to bring coacervate characteristics and elastomeric function of elastin.

2.2 Physicochemical Measurements on the Elastin Coacervate Characteristics

The static and dynamic light scattering measurements are useful to obtain insight of the preliminary and early stages of the elastin coacervate formation process.[4] NMR, ESR, and CD spectroscopy experiments were also employed to examine more direct molecular events with and without coexisting metal cations.[1]

2.3 Coacervate Layer as a Protein Liquid Ion-Exchange Membrane

The temperature-dependent liquid-liquid phase separation process of aqueous elastin-related polypeptide solution produces a separated transparent colorless macrocoacervate layer after the formation of dispersed microcoacervate droplets. Peptide-rich and viscous coacervate layer separated from upper layered peptide-poor

equilibrium solution is stable enough utilizing as a protein liquid membrane at around body temperature, 37°C.[5]

2.4 Phase Contrast Microscopy

Optical microscopic observations utilizing the video image enhancement facilities are employed as a useful method to investigate the phase separation behaviors in the late or advanced stages where the light scattering measurements occasionally encounter the technical difficulties. The sample solution was injected into the gap between two cover glasses with silicone rubber gasket, then the preparation was placed on the temperature-regulated stages of an inverted phase contrast microscope equipped with video recording facilities.

3 Results and Discussion

3.1 Phase Diagram for the Temperature-Dependent Coacervation of Elastomeric Protein-Water System

Binodal temperatures for the elastomeric protein-water system were estimated both from the temperature profiles of turbidity and observed numbers of microcoacervate droplets. Spinodal temperatures were estimated based on the forward scattering intensity data derived from an angular dependence of the scattering intensity of dynamic light scattering measurements. The correlation length of the concentration fluctuations of sample solution with the α -elastin concentration of 0.11 mg cm^{-3} , estimated by the Ornstein-Zernicke-Debye relation, divergently increased at around 21.5°C. In the case of the pentapeptide, H-(Val-Pro-Gly-Val-Gly)_n-Val-OMe, the lower-critical-solution-temperature (LCST) critical point of the liquid-liquid phase separation shifts up to 40-50°C and $25\text{-}50 \text{ mg cm}^{-3}$. The elastomeric protein-water systems characterized by the LCST phase diagram contrast markedly with other protein-water systems followed by the coacervate formation characterized by the upper-critical-solution-temperature (UCST) phase diagram, such as γ -crystallin, lysozyme, and cryoproteins.

3.2 Characterizations of Critical Self-Assembly Process in the Elastomeric Protein-Water System

At the critical concentration of bovine neck ligamental α -elastin, the value of hydrodynamic size of primary molecular assemblies, deduced from the light scattering measurements, decreased gradually and transiently with elevating temperature probably due to the exclusion process of water molecules from α -elastin aggregates. Microphase separation was initiated after abrupt increase in the hydrodynamic size of primary assembly as the temperature approached to the critical value. In these situations, the phase separated droplets were characterized as a particle with relatively large size and broad size distribution. As the α -elastin concentration deviated from the critical concentration, temperature profiles of the increasing hydrodynamic size tend to change to a slower time course and, when the concentration deviated far from the critical value, the increasing process of hydrodynamic size of the primary assemblies was disappeared and the microphase separation was rather initiated after a slow reduction of the size of primary assemblies. The off-critical slow reduction of hydrodynamic size of primary assemblies was reflected on the resultant phase separation of microdroplets with smaller size and narrower size distribution as compared with the coacervate droplets under the critical conditions.

3.3 Effects of Metal Cations on Critical Behavior in the Elastomeric Protein-Water System

The temperature-dependent phase separation mode itself indicates that a major driving force to assemble elastomeric protein and to induce phase separation is hydrophobic interactions. When α -elastin with charged amino acid residues was replaced with more hydrophobic polypentapeptide without charged sites, light scattering and microscopic experiments demonstrated results similar to the critical characteristics of the α -elastin-water system at 0.11 mg cm^{-3} . To further investigate the fast process with expanding self-assemblies and the slow process with contracting self-assemblies, effects of addition of metal chlorides on the temperature-dependent coacervation

of the elastomeric protein-water system were examined. In case of the α -elastin with charged amino acid residues, adding metal chlorides revealed usual salting-in and salting-out effects, since, for example, at low concentration of NaCl, 10^{-4} mol dm $^{-3}$, phase separation was suppressed, while at concentration above 10^{-2} mol dm $^{-3}$, phase separation was commonly enhanced. It can be expected that major contribution of adding metal chlorides is a sealed effect on electrostatic interactions between charged sites of polar amino acid residues.

3.4 Ca $^{2+}$ Selective Binding on Elastomeric Proteins in Coacervate State

It is probable that the glycine-rich amino acid composition and the helical or spiraling structure of the elastomeric protein, which are somewhat similar to ion transporting antibiotics and calcium-specific tooth enamel protein, reveal some sort of ionic selectivity.[1,3] Transmembrane potential responses for a series of metal chloride aqueous solutions divided by the α -elastin membrane were pH-sensitive, in which the mode of the ion transport process should turn from more anion-selective to more cation-selective characteristics with an increase in pH through the isoelectric point of elastomeric proteins. Only for Ca $^{2+}$ ions, the membrane potential responses across binary charged α -elastin membrane were pH-insensitive and comparable with those across noncharged polypentapeptide membrane. These results indicated that the mechanisms of major Ca $^{2+}$ ion transport are based on the specific and selective interactions with electrically neutral sites of elastin, such as peptide carbonyl oxygen on the polypentapeptide backbone chain.

3.5 Two Types of Metal Cation Binding Sites on Elastomeric Proteins

Direct observations of the selective cation binding on pentapeptide carbonyl oxygen can be detected by NMR spectroscopy utilizing ^{13}C -enriched polypentapeptide, (Gly 3 (5%)-Val 4 (4%)-Gly 5 (3%)-Val 1 (6%)-Pro 2 (1%)) $_n$, where the percentage values indicate the rates of ^{13}C enrichment for respective amino acid residues. There were almost no essential changes in the chemical shift of the five peptide carbonyl groups on the addition of NaCl and MgCl $_2$. In the presence of Ca $^{2+}$ ions, on the contrary, ^{13}C -NMR spectra changed significantly with downfield shifts of Gly $^3\text{C-O}$ and Gly $^5\text{C-O}$ carbonyl signals. According to the CD spectroscopy measurements, the Ca $^{2+}$ selective interactions induce modifications of pentapeptide conformation suggesting a degradation of β -spiral conformation. The significance of the pentapeptide sequence and its Ca $^{2+}$ ion selectivity is also indicated as a key to solving the structure-function correlations of the biological elasticity of elastin. During our extensive studies on the interactions between metal cations and elastomeric proteins, La $^{3+}$ ion proved to be the only metal cation capable of binding to pentapeptide carbonyl oxygens similar to the Ca $^{2+}$ ion.

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