Elastic Fiber Assembly and Organization

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The elastic fiber consists of two morphologically distinct components: amorphous appearing elastin and 10-12 nm fibers called microfibrils. Microfibrils provide a scaffolding for the deposition of elastin and serve to bind tropoelastin and hold it in register for the covalent crosslinking that forms the functional polymer. Our understanding of the composition of microfibrils remains incomplete. Recently two microfibrillar proteins, fibrillin and microfibril associated glycoprotein (MAGP), have been cloned and sequenced. To begin to understand which of these molecules are important for elastic fiber assembly, we studied the propensity of purified elastic fiber proteins to interact one with another.

Tropoelastin, MAGP, and fibrillin-1 were purified from elastin-rich tissues or from a baculoviral expression system. Wells of a microtiter plate were coated with tropoelastin and MAGP or fibrillin was added in the liquid phase. After incubation for several hours, the liquid phase was removed and binding of MAGP or fibrillin to tropoelastin was quantified using protein-specific antibodies. In a second set of experiments, antibodies to different regions of tropoelastin were included in the incubation buffers with MAGP or fibrillin. Using this in vitro binding assay, we found that MAGP but not fibrillin-1 specifically bound to tropoelastin. Binding to tropoelastin was divalent-cation independent and was completely blocked by reduction and alkylation (denaturation) of either protein, suggesting that native structure is important for this interaction.

To determine which part of tropoelastin was responsible for interactions with MAGP, antibodies specific for three distinct regions of tropoelastin were tested for their ability to inhibit binding between tropoelastin and MAGP. Antibodies specific for sequences in the middle of the tropoelastin molecule and near the amino terminus had no effect on binding, whereas an antibody raised against tropoelastin's carboxy terminus inhibited interaction between the two proteins. Further evidence that the carboxy-terminal domain contains the MAGP binding site was obtained by showing that MAGP did not bind to reduced and alkylated tropoelastin. Since the only cysteine residues in tropoelastin are in the carboxy-terminal domain, binding of MAGP must be targeted to this site.

An important role for the carboxy-terminus of elastin in fiber assembly has been suggested by the high degree of conservation of this domain relative to other domains in the molecule and by the observation that tropoelastin molecules that lack this domain do not assemble into fibers. This region has been shown to contain a clustering of lysine and arginine residues that form an unique, positively charged pocket--ideal for interacting directly with the negatively charged MAGP molecule.

In conclusion, interactions between tropoelastin and microfibrils are important in organizing a mature elastic fiber. MAGP appears to be the protein in microfibrils that binds to tropoelastin and holds it in register for the crosslinking reactions that are necessary for forming a functional elastic polymer. The carboxy-terminus of tropoelastin is the region of the molecule that binds to MAGP.