Superfast Myosin Protein Isolation

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ABSTRACT

The role of DNA in cells is to carry a code that can be translated to make proteins; it is the specific combination of proteins that make cells function properly. Muscle cells' DNA codes for an important group of contractile proteins, actin and myosin. The speed of contraction depends upon the type of myosin that a fiber contains; myosin is classified generally as either 'fast' or 'slow'. Several recent studies conducted on rattlesnake tailshaker muscles suggest the presence of 'superfast' myosin, enabling very rapid contraction. The presence of 'superfast' myosin in the songbird syringeal muscles (responsible for song production) has also been suggested through physiological studies. Typically, immunohistochemical mechanisms involving antibody use, are used to identify both 'fast' and 'slow' myosin. However, no such antibody to superfast myosin exists. Therefore, a study was initiated to generate a 'superfast' antibody, involving two approaches. The first employs the common molecular technique, RT-PCR, on RNA isolated from rattlesnake tailshaker muscles. Degenerate oligonucleotide primers made toward myosin proteins were used to amplify the enzymatic region of myosin. This region can be cloned for production of a protein fragment to which an antibody can be made. An alternative biochemical process requires specific amplification of the myosin DNA sequence. Without a proper elastic scaffold, vascular tissues fail to function appropriately. This is evidenced in patients who suffer abdominal aortic aneurysms (AAA), a condition marked by the swelling and ultimate rupture of an abdominal segment of the aorta. AAs occur in 5 to 7 percent of individuals over 60 years of age and account for 15,000 deaths per year in the U.S. (Radvany, 2004) Any information obtained in this study could be of help in identifying the cause of this and other elastic extracellular matrix disorders.

Fig 1. Diagram showing the location of the syrinx in a bird's body.

Fig 2. Large scale animation of the syrinx

Fig 4. The product of the RT reaction was subjected to PCR for specific amplification of the myosin DNA sequence.

Fig 3. Agarose gel showing the myosin PCR product. Arrowed bands were cut and isolated from the gel for cloning and sequencing.

TCTGGCCAAAGCGGTCATGAGAAGATGTTCCCTGGAGATTCTTGAGAAAGTTGTCGCAAAATGCTCCTGAGTAGTGGTGGTCTGAGTACATCGGGTCCAACAGAGTCTTGGACAGAGTGGCCTGCACATCGAGCTCATTGAGAAGCCTATGGGCATCTT
GGCTGCTGAGTGGGAATGGCATTGACTTCGGGATGGAACCTTGACCAATTCGGTGGGTGCTCTGGCCAAAGCCGTCTACGAGAAGATGTTCCTGTGGTTTCAAGATGTTCCTGTGGAGATTCAAGATGTTCCTGTGGACAGAGTGGCCTGCACATCGAGCTCATTGAGAAGCCTATGGGCATCTTGGCTGAGAAGCCTATGGGCATCTTGGCTGAGAAGCCTATGGGCATCTT

Fig 6. A portion of the DNA sequence of human fast myosin, which was used to make primers (in red) for the PCR reaction.