TECHNICAL NOTE

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Comparison of Postmortem Autolysis in Cardiac and Skeletal Muscle

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ABSTRACT: To understand the mechanism in postmortem autolysis better, processes in the postmortem degradation of myofibril proteins in the presence of protease inhibitors were studied. Male Wistar rats were given injections of the carboxyl-, thiol-, and serine-protease inhibitors, pepstatin, Ep-475[L-transepoxysuccinyl-leucylamide(3-methyl) butane; E-64-C], and chymostatin, via the femoral vein. Control rats were similarly treated with saline. Then, myofibril proteins were isolated from their cardiac and femoral muscles and from those of control animals at various times after death, and degradation of these myofibril proteins with time was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

In cardiac muscle, α-actinin was degraded rapidly, followed by the heavy chain of myosin and light chain of myosin (L2). Actin and the light chain of mosin (L1) were degraded slowly. The degradations of the heavy chain of myosin, α-actinin, tropomyosin and L2 after 14 days were not inhibited by pepstatin, but were inhibited by Ep-475 and chymostatin.

In skeletal muscle, L1 and L2 were degraded rapidly, followed by the heavy chain of myosin and α-actinin. Actin was degraded slowly and was still unchanged 2 weeks after death. The degradations of protein components were inhibited by pepstatin, Ep-475 and chymostatin. These results indicated that after death the components of myofibrils are degraded by various proteases at various rates depending on their properties or structures. This degradation is fundamentally the same in cardiac and skeletal muscles, but inhibitors have somewhat different effects on the postmortem degradation processes after death in the two types of muscle.

KEYWORDS: pathology and biology, postmortem autolysis, protease, cardiac and skeletal muscle

It is relevant to study the mechanism of postmortem autolysis in forensic science, and there is still much left to be learned about the postmortem degradation of protein. There has been substantial progress in studies on intracellular proteases and their inhibitors [*1*–

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6], which are thought to be physiologically important in turnover of intracellular proteins. In this study, we compared postmortem autolysis of cardiac and skeletal muscle of rats in relation with the activity of these proteases.

The postmortem degradation of myofibril proteins in cardiac and femoral muscle were examined at weekly intervals by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Materials and Methods

Male Wistar strain rats weighing 250 to 300 g were given a 10% solution of sodium pentobarbital intraperitoneally followed by an injection of saline only (group I, control) or 20 mg of the protease inhibitor pepstatin (group II), Ep-475 (group III) or chymostatin (group IV) via the femoral vein. Pepstatin, Ep-475 and chymostatin were obtained from the Protein Research Foundation, Japan. The rats were decapitated 20 min after the injection, and placed in a room with a constant temperature of 20°C and humidity of 50%. Myofibrillar structural proteins were isolated by the method of Sugita et al. [7]. Small pieces of cardiac and femoral muscle were sampled once every 7 days, and homogenized for 60 s with 0.1M NaCl-5mM $NaHCO_3$ in the cold in a homogenizer. The homogenate was centrifuged for 20 min at 10 000 g and the precipitate was washed four times with the same solution, and then once with 0.3 mM $NaHCO_3$. The protein concentration of the sample in 0.3 mM $NaHCO_3$ was determined by the Lowry method [8], with bovine albumin as a standard. The sample was then mixed with an equal volume of 2%(w/v) SDS containing 50%(v/v) glycerol, 10%(v/v) 2-mercaptoethanol, and 0.05%(w/v) bromophenol blue, and boiled for 3 min. A sample containing 50 μg of protein was subjected to electrophoresis in SDS-polyacrylamide gel by the method of Laemmli [9], except that slab gel containing 7.5% acrylamide was used. The concentrations of myofibril proteins in the electrophoretograms were measured with a densitometer, type DMU-33C (Toyo Kagaku Sangyo Co., Ltd.).

Results

The electrophoretograms of myofibril proteins from control cardiac and femoral muscles (group I) and those treated with Ep-475 (group III) and chymostatin (group IV) are shown in Figs. 1, 2 and 3, respectively.

In group I, the bands of the heavy chain of myosin, α-actinin and the light chain of myosin (L2) in cardiac muscles and the heavy chain of myosin and tropomyosin in femoral muscles were decreased in density 7 days after death. But, the light chains of myosin (L1 and L2) in femoral muscle were degraded rapidly and their bands had almost disappeared after 7 days. α-Actinin was degraded more rapidly in cardiac muscle than in femoral muscle. The heavy chain of myosin, α-actinin and L2 in cardiac muscle and the heavy chain of myosin and α-actinin in femoral muscle had almost disappeared after 14 days. Tropomyosin in femoral muscle had almost disappeared after 21 days (Fig. 1).

In group II, pepstatin had almost no inhibitory effect on postmortem degradation of myofibrils in cardiac muscle, but in femoral muscle it had an inhibitory effect on the degradation of the heavy chain of myosin, L1 and L2 in 14 days and α-actinin, actin and tropmyosin in 21 days (Fig. 2).

In group III, Ep-475 inhibited the postmortem degradation of myofibrils (Fig. 3). The heavy chain of myosin, α-actinin, tropomyosin and L2 in cardiac muscle and the heavy chain of myosin, α-actinin, L1 and L2 in femoral muscle were not degraded after 14 days. Ep-475 inhibited the degradations of α-actinin and tropomyosin in 21 days more in femoral muscle than in cardiac muscle. Thus Ep-475 inhibited the degradations of almost all components of myofibril proteins in cardiac and femoral muscles.

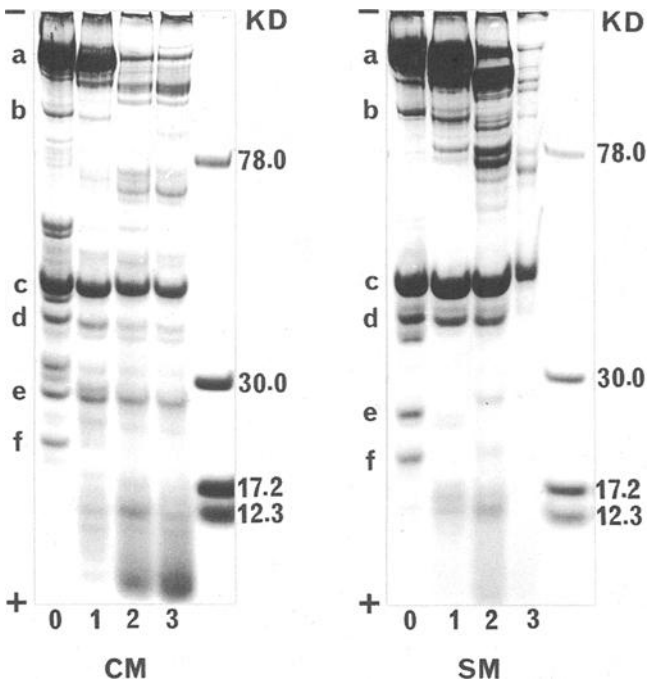

FIG. 1—*Sodium dodecyl sulfate(SDS)-polyacrylamide gel electrophoretograms of myofibril proteins of control muscles (group I). a, myosin; b, α-actinin; c, actin; d, tropomyosin; e, f, light chains of myosin. Numbers 1 to 3 indicated weeks after death. CM, cardiac muscle; SM, skeletal muscle.*

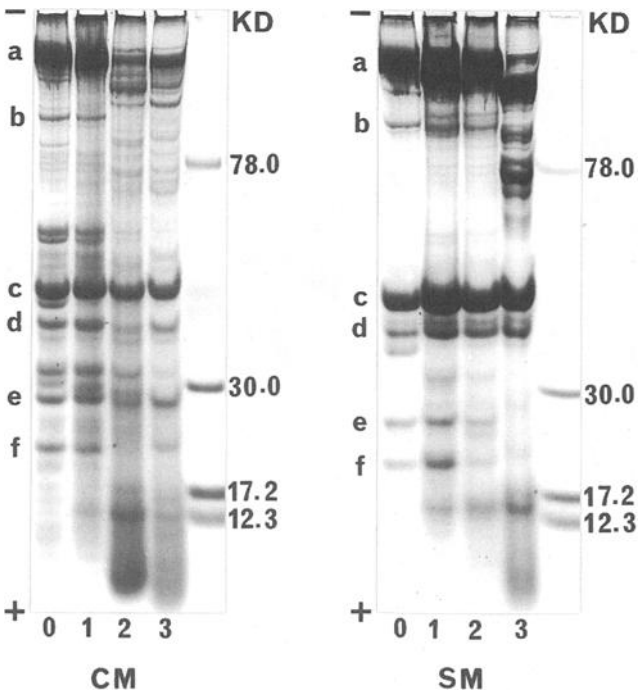

FIG. 2—*SDS-polyacrylamide gel electrophoretograms of myofibril proteins of muscles treated with pepstatin(group II). The symbols a, b, c, d, e, f, CM and SM and numbers 1 to 3 are defined in Fig. 1.*

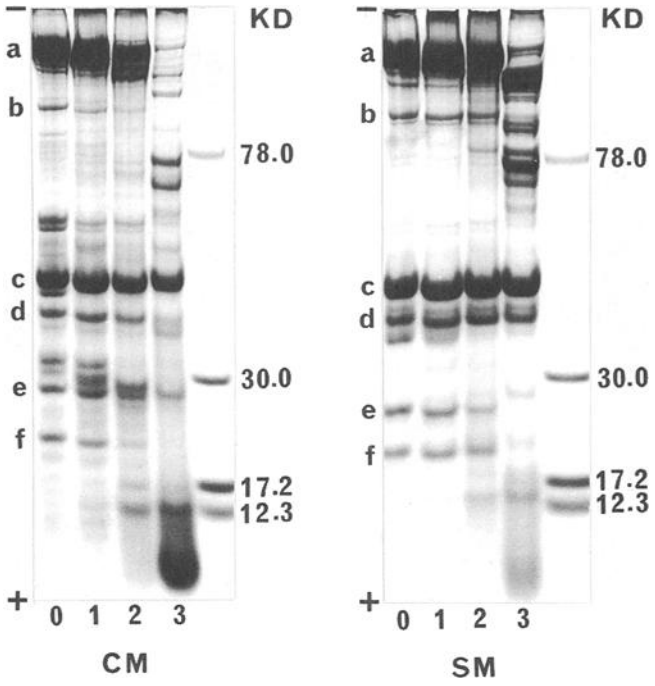

FIG. 3—*SDS-polyacrylamide gel electrophoretograms of myofibril proteins of muscles treated with Ep-475(group III). The symbols and numbers are defined in Fig. 1.*

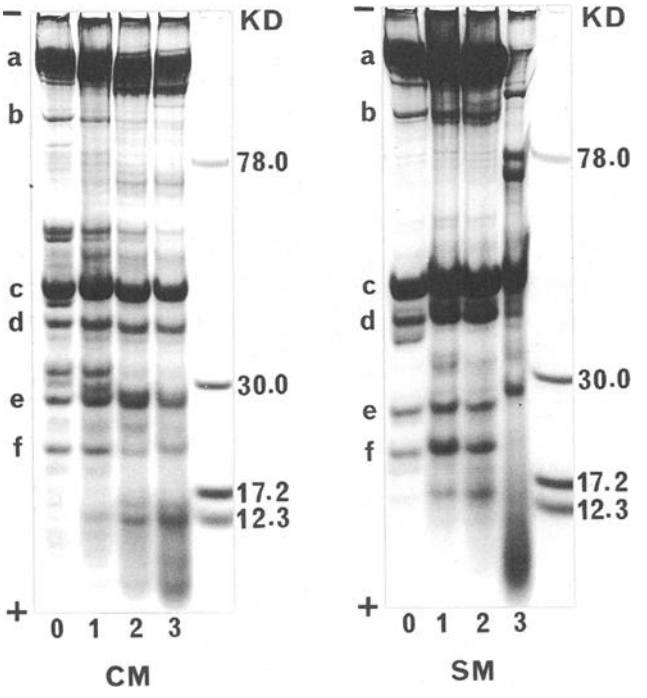

FIG. 4—*SDS-polyacrylamide gel electrophoretograms of myofibril proteins of muscles treated with chymostatin(group IV). The symbols and numbers are defined in Fig. 1.*

TABLE 1—*Comparison of changes in intensities of bands on SDS-PAGE in each experimental group.*

Myofibril proteins	Exp. group	Cardiac M. Days after death 0	7	14	21	Skeletal M. Days after death 0	7	14	21
Heavy chain of myosin	I	+4	+3	±	−	+4	+3	±	−
	II	+4	+4	±	+1	+4	+4	+4	±
	III	+4	+3	+2	−	+4	+4	+4	±
	IV	+4	+4	+3	+3	+4	+4	+4	±
α-Actinin	I	+4	+2	−	−	+4	+4	±	±
	II	+4	+4	±	−	+4	+4	+4	+4
	III	+4	+3	+3	−	+4	+3	+4	+2
	IV	+4	+4	+1	+2	+4	+4	+4	±
Actin	I	+4	+4	+3	+3	+4	+4	+4	+2
	II	+4	+4	+4	+2	+4	+4	+4	+4
	III	+4	+4	+4	+3	+4	+4	+4	+4
	IV	+4	+4	+4	+4	+4	+4	+4	+4
Tropomyosin	I	+4	+4	+2	+2	+4	+3	+4	±
	II	+4	+4	+2	+2	+4	+4	+4	+4
	III	+4	+4	+4	+1	+4	+4	+4	+3
	IV	+4	+4	+4	+4	+4	+4	+4	+3
Light chain of myosin (L_1)	I	+4	+4	+4	+4	+4	−	−	−
	II	+4	+4	+4	+4	+4	+4	+4	−
	III	+4	+4	+3	+4	+4	+3	+3	−
	IV	+4	+4	+4	+4	+4	+4	+4	−
(L_2)	I	+4	+3	−	−	+4	−	−	−
	II	+4	+4	−	+3	+4	+4	+4	−
	III	+4	+4	+3	−	+4	+4	+4	−
	IV	+4	+4	+4	+4	+4	+4	+4	−

NOTE: Intensity of band relative to that initially: +4, slight if any decrease (81–100%); +3, 61–80%; +2, 41–60%; +1, 21–40%; ±, 11–20%; −, 0–10%.

In group IV, the degradation of cardiac muscle was strongly inhibited by chymostatin, and actin and tropomyosin remained almost undegraded for 21 days after death. In femoral muscle, all components remained completely undegraded for 14 days after death. The degradations of almost all components of myofibril proteins in the 21 days were inhibited more in cardiac muscle than in femoral muscle by chymostatin. The inhibitory effect of chymostatin on the degradations of all components in cardiac muscle was stronger than those of the other inhibitors (Fig. 4). Table 1 summarizes the postmortem changes over time of various myofibril proteins measured by densitometry and shown relative to the initial levels of the proteins.

Discussion

It is unclear how intracellular proteins are digested by postmortem autolysis. In this study we investigated the postmortem degradation of components of myofibril proteins in heart and skeletal muscle in the presence of protease inhibitors. Intracellular proteins are digested by intracellular proteases, such as lysosomal cathepsins [*1–7*]. There may be a specific mechanism for release of cathepsins from the lysosomes into the cytoplasm in vivo, but after death cathepsins may leak into the cytoplasm due to postmortem degeneration and/or increase in permeability of the lysosomal membrane. Takeichi et al. [*10*] have examined the mechanism of postmortem autolysis of rat skeletal muscle using

the carboxyl, thiol and serine protease inhibitors, pepstatin, Ep-475 and chymostatin. They reported that the postmortem degradations of almost all myofibril proteins in skeletal muscle were inhibited more by chymostatin than by Ep-475. They also measured the cathepsins B and L activities and mast cell serine protease activities one week after death, finding that in muscle treated with Ep-475, the activity of thiol protease was strongly inhibited but that of serine protease was only slightly inhibited, and that in muscle treated with chymostatin, thiol and serine proteases both showed very low activity.

In this study we found that the postmortem degradations of almost all myofibril proteins in heart muscle were inhibited more by chymostatin than by Ep-475, compared with the effects of these enzymes in skeletal muscle, but were scarcely inhibited by pepstatin. This finding suggests that extracellular chymotrypsinlike proteases, such as chymase from mast cells [*11*], cathepsin G from neutrophils [*12*], an unidentified protease from macrophages and intracellular chymotrypsinlike proteases, such as the proteasome [*13–15*], may play a major role in the postmortem degradation of protein in heart muscle. Cathepsins B, H, and L released from lysosomes may also play some role in the degradation. Cathepsin D may leak into the cytoplasm due to increase in permeability of the lysosomal membrane, but its activity is presumably weaker than those of other cathepsins because its optimal pH is 3 to 4, and the pH value of skeletal muscle does not decrease below 5.8 after death [*10*].

Further studies are needed to identify the protease that is mainly responsible for postmortem degradation of proteins in these muscles.

Acknowledgments

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