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Muscle Ultrastructural Changes by Lysosomal Enzymes

2. Scanning Electron Microscopic Studies

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Lysosomal Enzyme에 依한 筋肉組織의 變化

第2報 Scanning Electron Microscopy에 依한 考察

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Abstract

Surface ultrastructural changes in endomysial connective tissue, sarcolemma and transverse ridges of bovine *psoas* muscle produced by leukocyte lysosomal enzymes *in vitro* at different pH (pH 7.0 and 4.0), temperature (37 and 4°C) and time interval (12, 24 hours at 37°C and 36, 168 hours at 4°C) were studied by scanning electron microscope. Muscle incubated with leukocyte lysosomal enzymes at pH 7.0 produced severe degradation of endomysial and sarcolemmal connective tissue and transverse ridges but at pH 4.0 endomysial and sarcolemmal structures remain moderately stable and tranverse ridges are very stable even after 24 hours incubation at 37°C and 7 days incubation at 4°C.

Introduction

The results of the studies on the ultrastructural changes in bovine *psoas* musule caused by the treatment of porcine leukocyte lysosomal enzymes *in vitro* were presented in the previews paper(Cho *et al* 1978).

This is the results of the scanning electron microscopic studies on the degradation on endomysial and sarcolemmal connective tissue of bovine *psoas* muscle caused by the *in vitro* action of porcine leukocyte lysosomal hydrolases.

Materials and Methods

Muscle Tissue and Enzyme Source

Bovine *psoas* muscle was obtained from the university abattoir immediately after slaughter. The muscle was cut in strips of size 3×5×7mm. Porcine blood served as the source for leukocyte lysosomal enzymes and the hydrolases were isolated as the procedure of

Venugopal (1970). The lyophilized enzyme was dissolved in suitable buffers (1mg/ml), pH 4.0 and 7.0 and the muscle strips were treated as the procedure described on the previous paper (Cho *et al* 1978).

Scanning Electron Microscopy (SEM)

After incubation with enzyme solutions, the muscle strips were washed once with appropriate buffer and fixed in 1.25% glutaraldehyde, buffered with 0.07M Na_2HPO_4, 0.041M Na_2HPO_4 and 0.043M NaCl, for 4 hours and in 1% osmium tetroxide for 4 hours. The fixed muscle strips were dehydrated first in ethanol (20, 40, 60, 80, 90 and 100%×3) then in amyl acetate (20, 40, 60, 80% in absolute ethanol and finally in anhydrous amyl acetate×3). Residual amyl acetate in the sample was removed by critical point dehydration in liquid CO_2. The dehydrated muscle strips were then glued to aluminum slabs with silver paste, coated with gold (100Å thickness) and examined under JEOL-JSM-S1 scanning electron microscope at 10KV as an accelerating voltage.

Results and Discussion

Bovine *psoas* muscle contains less collagen than other muscles and the ultrastructure of the fiber surface can be observed directly by scanning electron microscopy(SEM). Muscle fiber is enclosed by endomysium and sarcolemma. Sarcolemma is now defined as a multilayered complex consisting of three components; the collagen fibrils, amorphous basement membrane and the plasma membrane(Franzini-Armstrong, 1973). Leukocyte lysosomal enzyme preparation contain a wide variety of hydrolases which include β-glucuronidase, cathepsin D, collagenase, elastase, cathepsin B_1 (possessing collagenolytic activity) and other cathepsins. All these enzymes are capable of degrading connective tissue mucoproteins and all except first one degrade myofibrillar proteins. Interpretation of several structures which appear in SEM micrographs of muscle is still controversial and there is poor agreement among leading investigators. Since SEM images surfaces, cutting of any kind on the muscle surfaces produce surface artifacts and this makes the interpretation still more difficult. Muscle is sensitive to dehydration and tissue shrinkage cannot be avoided. Despite of these limitations, we attempt here to describe our SEM micrographs of muscle strips treated with porcine leukocyte lysosomal enzymes. Porcine enzymes were used in this study because large amounts of porcine leukocytes are more easily isolated than bovine leukocytes from the respective bloods and there is little differences in the *in vitro* proteolytic activity between porcine and bovine leukocyte lysosomal preparations (Venugopal, 1970).

The SEM-micrograph of pre-rigor muscle (1a) fixed within 1 hour postmortem shows intact fibers with endomysial connective tissue and sarcolemma. The ramified materials could be collagen from perimysium. Myofibrils are seen where the muscle strip has been broken. Enlargement of this area(1b) shows the underlying myofibrils in some detail. Raised structures are seen where the Z-lines are expected. These structures are tubular and continuous across the fiber and seem to correspond in terms of location to the transverse ridges (T-tubules). This SEM is similar to those described by Eino and Stanley (1973a,b). Micrograph of muscle strip (1c) incubated in buffer pH 7.0 at 37°C for 12 hours do not show any appreciable difference from fresh muscle. Enlarged micrograph (1d) shows a small reduction in sarcomere length compared to that of fresh muscle (1b). Proteolytic degradation of endomysium and sarcolemma caused by incubation of the muscle strip in the enzyme solution is noticed in SEM (1e). Endomycium is pulled apart exposing the sarcolemma and deep horizontal fissions are seen due to the break up of sarcolemma. Enlarged micrograph (1f) shows distinct degradation of transverse ridges. It is suggestive that after the breakdown of sarcolemma by collagenase type of protease and other cathepsins from our enzyme preparation diffuse into the transverse ridges and act on Z-line materials. The effect of 37°C incubation for 24 hours at pH 7.0 is shown in 2a. Endomysium seems to be broken partially and the sarcolemma also is torn in some areas exposing the myofibrils. Enlargement of this area (2b) reveals the similarity with the structure of fresh muscle. Enzyme treated muscle (2c, d, and e) show the extent of proteolytic degradation. Sarcolemma and endomycium are completely

stripped revealing the myofibrils. Enlargement show the complete degradation of Z-line material. Exposure of muscle strips to buffer, pH 4.0, at 37°C for 12 hours affects only the endomysial fibers (3a). Clumping of fibers along with some drops are noticed. The striated structure could be seen under the intact sarcolemma. The striations could be the transverse ridges, which looks like elevated structures. The highly magnified micrographs (3b) show the elevated transverse ridges more clearly and these conceal the Z-line structures. Other characteristic features such as M-line or H-zone are not recognized. Incubation of muscle strips in enzyme preparations at pH 4.0 for 12 hours does not affect the muscle to any extent since the control and enzyme treated muscle are quite similar in appearance. However incubation for 24 hours affects the muscle strips somewhat and the lysosomal proteases break up the endomycium (4a).

Enlargement of the area shows partial breaking of sarcolemma exposing the fibrils. The transverse ridges straddling the Z-line appear to be intact. Exposure of muscle strips to lysosomal enzymes in a buffer, pH 7.0 at 4°C for 36 hours results in the degradation of both endomysium and sarcolemma exposing the myofibrils. Partial degradation of Z-line material is observed (4f) in the enlarged micrograph. Protein material at the junction of A-I band is also partially degraded. In some of the myofibrils, the M-line has disappeared suggesting proteolytic breakdown. Micrographs (5a, b, c, d) depict the effect of 7 days treatment of the muscle strips with lysosomal enzyme in a buffer pH 7.0 at 4°C. Control muscle samples depict partial degradation of endomysium and sarcolemma exposing the myofibrils and this could be due to any residual neutral proteases present in the muscle.

Some Z-line material has been degraded in some of the myofibrils. Fragmentation of sarcolemma is seen with the enzyme treated samples. Z-line material is completely degraded with some elongation of the myofibrils. Micrographs (5e, f, and 6a, b) depict the effect of 36 hours incubation of muscle strips in lysosomal enzyme solutions at pH 4.0 and at 4°C. Control samples show partial degradation of sarcolemma and endomysium and slight cracks or fissures on the muscle surface. Enzyme treated samples (6a, b, 6e, f) also depict the same characteristic. SEM micrographs (5c, d, e and f) depict the 7 day treatment of muscle strips with lysosomal enzyme solution, pH 4.0 and at 4°C. Both control and enzyme treated samples show degradation of connective tissue membranes. Enlargement of the micrographs do not show any further degradation in the myofibrillar structure.

要 約

脈白血球 Lysosomal효소를 pH (7.0, 4.0), 온도 (37°C, 4°C) 및 처리기간(37°C에서 12 24시간, 4°C에서 36 168시간)을 달리하여 處理한 牛의 腰筋組織의 變化에 對하여 endomysial connective tissue, sarcolemma 및 transverse ridge등 筋섬유 表面組織의 變化를 SEM으로 관찰한 바, 처리溫度에 關係없이 pH 7.0에서는 endomysial connective tissue, sarcolenna 및 transverse ridge의 分解를 나타내어 이 효소의 높은 力價를 보였으나, pH4.0에서는 이들 表面組織에 變化가 없었으며 特히 transverse ridge는 37°C에서 24시간, 그리고 4°C에서 7일간 處理하여도 變化를 나타내지 않아 安定됨을 보였다.

References

Benugopal, B. Ph.D Dissertation. Univ. of Missuri, Columbia, Mo. U.S.A. (1970).

Cho M.J., Tae Gyu Yoon and Milton E. Bailey. *Korean J. Food Sci. & Tech.* **10**(1), (1978).

Eino, M.F. and M.J. Stanley. *J. Food Sci.* **38**, 45. (1973a).

Eino, M.F. and M.J. Stanley. *J. Food Sci.* **38**, 51. (1973b).

Franzini-Armstrong, C. The structure and function of muscle. Vol. II. pp.532 G. H. Bourne, ed. Academic Press, New York. (1973).

Figure 1, 2, 3, 4, 5 and 6 Scanning Electron Micrographs of Bovine Psoas Muscle

Fig. 1. 1a (X 390) & 1b (X 9, 700): Pre-rigor muscle

1c (X 1, 190) & 1d (X 7, 100): Control muscle incubated without leukocyte lysosomal e nzymes in phosphate buffer (pH 7.0) at 37°C for 12 hours.

1e (X 1, 280) & 1f (X 12, 100): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 37°C for 12 hours.

Fig. 2. 2a (X 400) & 2b (X 8, 900): Control muscle indubated without leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 37°C for 24hours.

2c (X 400), 2d (X 1, 100) & 2e (X 71, 120): Enzyme-treated muscle incubated with leukocyte lysosmal enzymes in phosphate buffer (pH 7.0) at 37°C for 24 hours.

Fig. 3. 3a (X 1, 230) & 3b (X 12, 310): Control muscle incubated without leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 37°C for 12 hours.

3c (X 1, 220) & 3d (X 4, 580): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 37°C for 12 hours.

3e (X 1, 310) & 3f (14, 500): Control muscle incubated without leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 37°C for 24 hours.

Fig. 4. 4a (X 12, 500) & 4b (X 5, 450): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in acetate buffer (pH4.0) at 37°C for 24 hours.

4c (X 11, 500) & 4d (X 8, 600): Control muscle incubated without leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 4°C for 36 hours

4e (X 1, 200) & 4f (X 18, 890): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 4°C for 36 hours.

Fig. 5. 5a (X 3, 500) & 5b (X 9, 900): Control muscle incubated without leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 4°C for 168 hours.

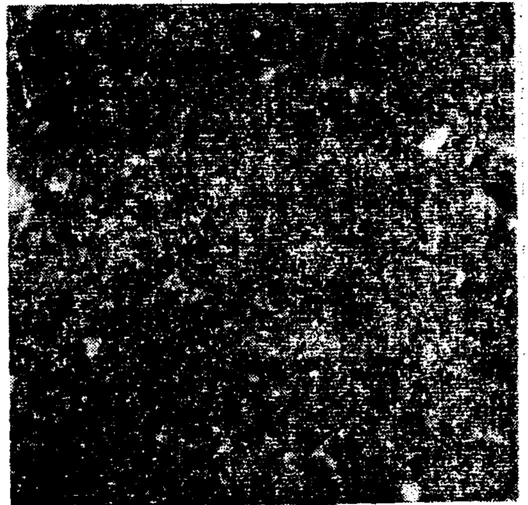
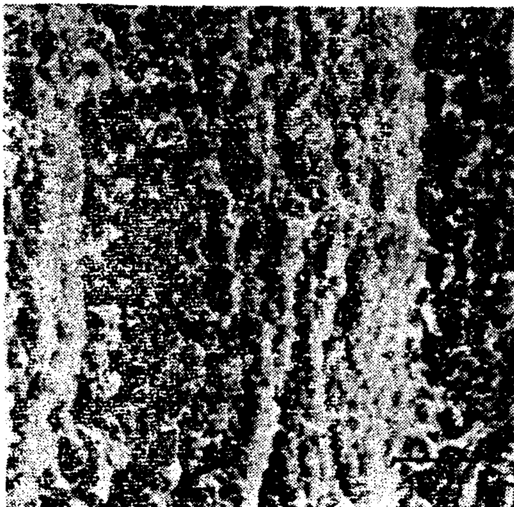
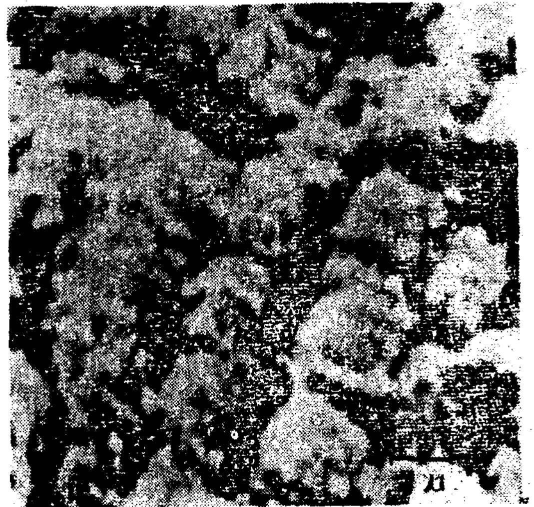
5c (X 1, 200) & 5d (X 10, 260): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in phosphate buffer (pH 7.0) at 4°C for 168 hours.

5e (X 1, 280) & 5f (X 8, 100): Control muscle incubated without leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 4°C for 36 hours

Fig. 6. 6a (X 1, 300) & 6b (X 1, 900): Enzyme-treated muscle incubated leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 4°C for 36 hours.

6c (X 1, 250) & 6d (X 5, 850): Control muscle incubated without leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 4°C for 168 hours.

6e (X 1, 620) & 6f (X 8, 020): Enzyme-treated muscle incubated with leukocyte lysosomal enzymes in acetate buffer (pH 4.0) at 4°C for 168 hours.

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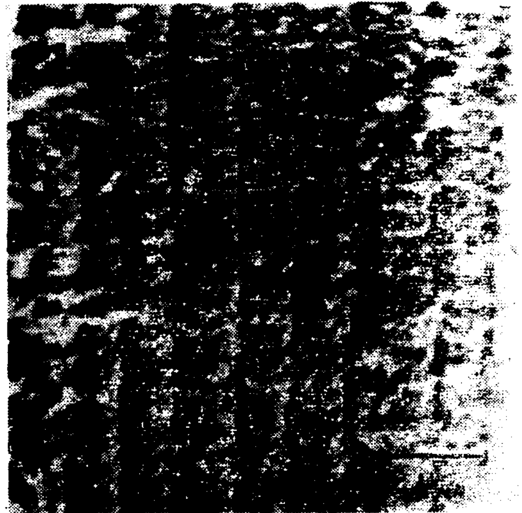

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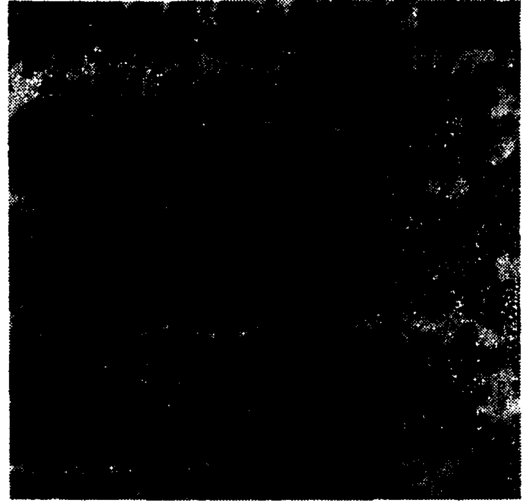
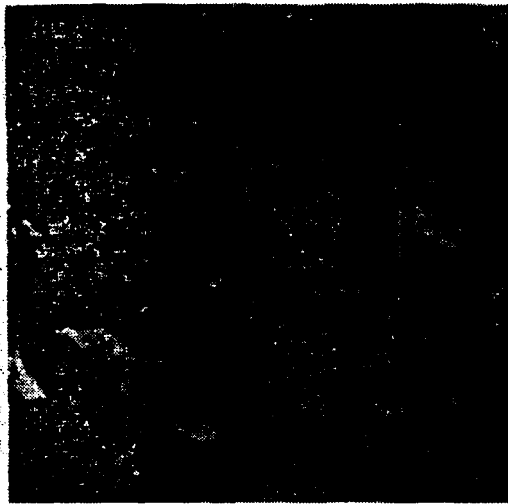

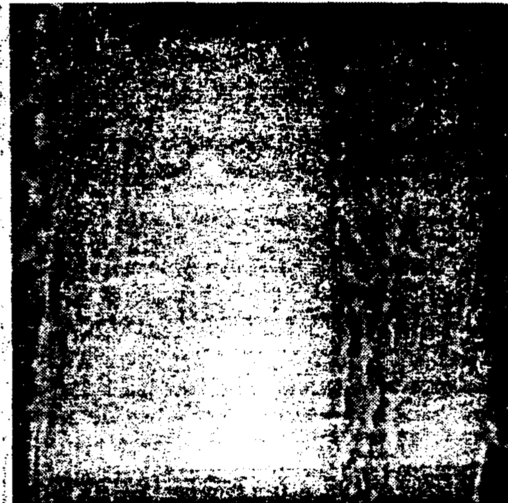
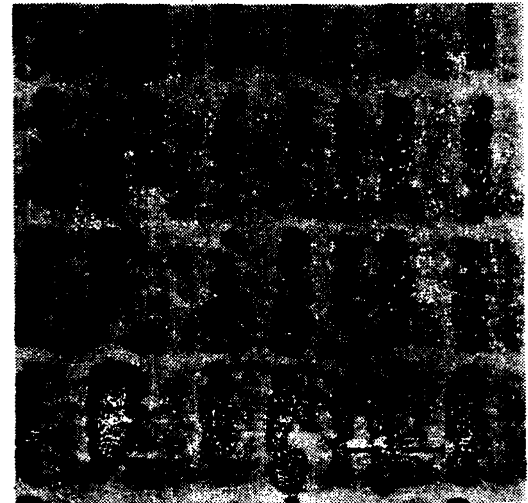

4a

4c