

Effect of Vitamin E-deficiency on the Activity of Some Lysosomal and Non-lysosomal Proteases in Rabbit Muscles

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Abstract. The activity of different cathepsins and neutral proteinases was measured in normal and vitamin E-deficient rabbit muscles using specific substrates. Among the changes of enzyme activities in dystrophy caused by vitamin E-deficiency the increase in the activity of cathepsin B is the most striking. The activity of cathepsin H, both in the fast and slow muscles and that of MMP-ase in the slow muscle remains practically unchanged. Activities of other proteases significantly increase. The change in the activity of proteolytic enzymes in striated muscle of vitamin E-deficient rabbits seems to be selective. As a rule the increase in the activity is higher in fast than in slow muscles.

Key words: Vitamin E deficient rabbits—Skeletal muscle—Proteases

Introduction

The increase in protein and nucleic acid turnover in dystrophy caused by vitamin E-deficiency has been known for a long time (Young and Dinning 1951; Dinning et al. 1955, 1956; Antoni et al. 1957).

Enhanced synthesis of certain enzymes has been assumed in vitamin E-deficiency and attributed to the effect of vitamin E as a repressor (Olson and Carpenter 1967). The increase in activity of some enzymes, however, is accompanied by decreases in the amounts of some other proteins most notably myofibrillar components (Josepovits et al. 1957). Thus, it seemed justified to investigate the possibility of proteolytic enzymes of striated muscle being responsible for breakdown of myofibrillar proteins. Enhanced activity of calcium activated protease has been detected in skeletal muscle of rabbits (Dayton et al. 1979) and rats, respectively (Otsuka et al. 1985). It has also been shown that the early elimination of Z-line proteins in this state of dystrophy is due to the same protease (Dayton et al. 1979). On the other hand, elevated cathepsin D and B activity were found in the muscles

of vitamin E-deficient guinea pigs (Spanier et al. 1977; Spanier and Bird 1982). This can be explained by a decreased amount of the inhibitor. In addition to the above endopeptidases the activities of certain dipeptidases were also reported to be increased in muscles of vitamin E-deficient rabbits (Weinstock et al. 1956). It may be of interest that in muscles of vitamin E-deficient rabbits the activity of other lysosomal enzymes increases as well (Zalkin et al. 1962).

However, there has so far been no comprehensive investigation of proteases in striated muscle in vitamin E-deficient dystrophy. A similar investigation has to be based on a precious characterization of the proteolytic enzymes with respect to their specific substrates and inhibitors. In the present study changes in the activities of different cathepsins and some neutral proteinases (MMP-7ase) were measured in fast (m. semimembranosus) and slow (m. soleus) muscles of vitamin E-deficient rabbits. Significantly differing patterns of changes were observed for the individual proteases.

Materials and Methods

Vitamin E-deficient rabbits were fed Goettsch-Pappenheimer diet (Goettsch and Pappenheimer 1931) until signs of dystrophy became manifest (approx. six weeks). The muscles were solubilized in 0.15 mol.l⁻¹ NaCl+0.1% Triton-X-100 in Ultra-Turrax (FRG), for 2x10 s. and centrifuged at 20,000 x *g* for 20 min. The supernatant was used for activity measurements.

The activities of cathepsin H and B were measured with the method of Kirschke et al. (1983), using Arg-AMC (AMC: 7-amino-4-methyl-coumarin) and Z-Arg-Arg-AMC (Z: benzyloxy-carbonyl) as substrates for cathepsin H and cathepsin B, respectively. The activity of MMP-7ase was measured with Suc-Ala-Ala-Pro-Phe-AMC as a substrate (Sohár et al. 1987). AMC substrates were supplied by Enzyme System Products (Livermore, CA., USA). The activity was expressed in terms of $\mu\text{mol.l}^{-1}$ AMC liberated per minute at 40°C. A Hitachi 650-105 apparatus was used for fluorimetric measurements (Ex:360 nm, Em.: 460). The activity of cathepsin D was measured with the method of Barrett (1972) using hemoglobin (Sigma, Saint Louis, USA) at pH 3.5. The activity of ATNase was determined photometrically with N-acetyl-*p*-nitroaniline as a substrate at 381 nm (Sohár et al. 1987). The protein content was determined with the microbiuret method of Goa (1953).

Myofibrils were prepared and the SDS-gelelectrophoretic characterization was carried out according to the method of Takács et al. (1977 and 1981). Student's *t*-test was used for statistical analysis.

Results

Figures 1-5 show the average values of enzyme activities measured in fast and slow muscles of 11 normal and 9 vitamin E-deficient rabbits. Of the enzymes investigated, the activity of cathepsin B showed the most pronounced change (Fig 1). This predominantly concerned the fast muscle, the activity of which was approx.

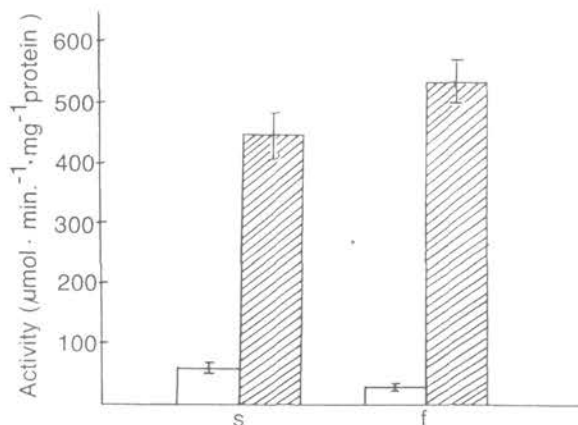


Figure 1. Cathepsin B activity in normal (empty columns) and dystrophic (hatched columns) slow (s) and fast (f) muscles.

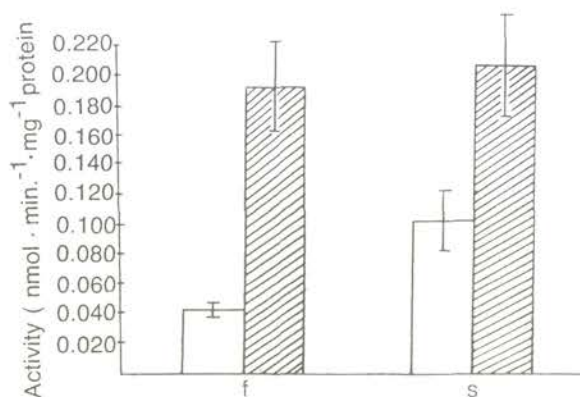


Figure 2. Cathepsin D activity in normal and dystrophic slow and fast muscles. For symbols see legend to Fig. 1.

twenty times higher than that of a normal muscle. In the case of the slow muscle, however, the increase was eightfold. The differences in the enzyme activities between normal and vitamin E-deficient rabbits were highly significant ($p < 0.01$) in both cases. It should be noted that in normal rabbits, the activity of the soleus muscle was considerably higher than that of the fast muscles. In dystrophic animals this difference could not be observed.

In Figure 2 the average activities of cathepsin D are shown. Though less than that of cathepsin B the increase in the activity of cathepsin D in dystrophy induced

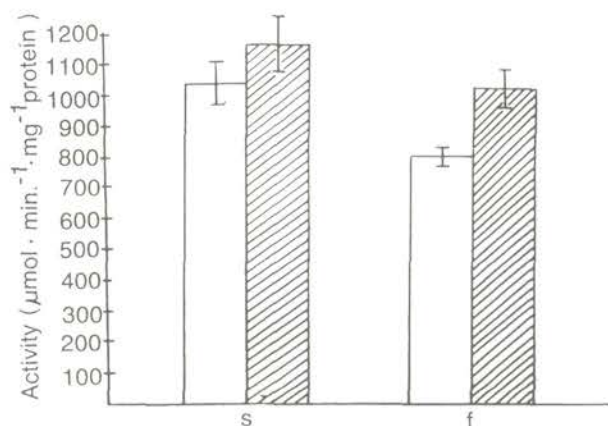


Figure 3. Cathepsin H activity in normal and dystrophic slow and fast muscles. For symbols see legend to Fig. 1.

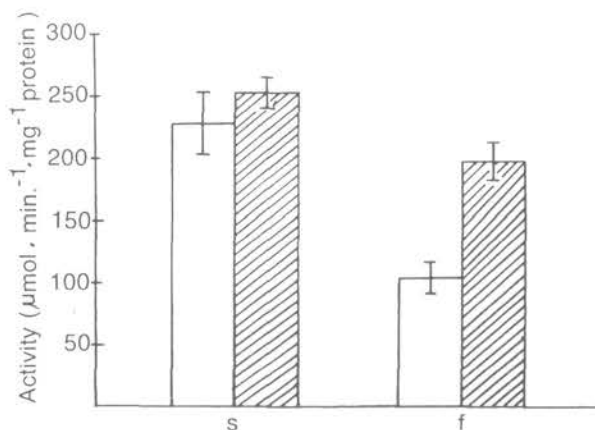


Figure 4. MMP-7ase activity in normal and dystrophic slow and fast muscles. For symbols see legend to Fig. 1.

by vitamin E-deficiency is highly significant ($p < 0.01$). While the activity in the dystrophic fast muscle was approx. four times higher than in a normal muscle, a roughly twofold increase in the activity was measured in the slow muscles. In normal rabbits the activity of the fast muscles was lower than that of the soleus, while in the vitamin E-deficient animals virtually no difference could be found.

Cathepsin H was the only enzyme the activity of which in the fast and slow muscles of vitamin E-deficient rabbits remained effectively unchanged (Fig. 3). No significant difference in cathepsin H activity of slow and fast muscles could be found

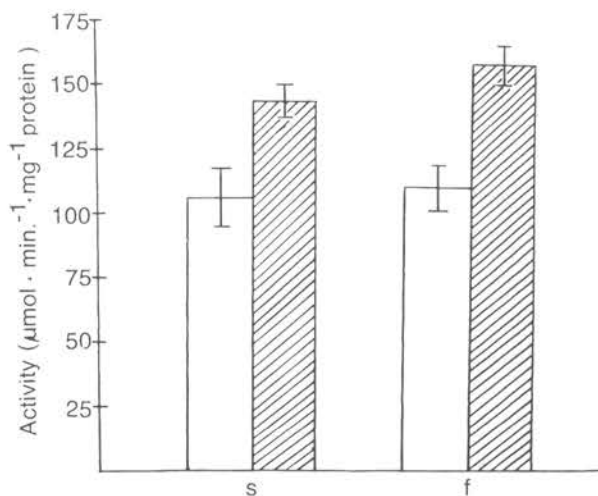


Figure 5. ATNase activity in normal and dystrophic slow and fast muscles. For symbols see legend to Fig. 1.

between normal and vitamin E-deficient animals.

A considerable difference could be observed, however, concerning the vitamin E-deficiency induced increase of MMP-7ase activity in the fast and slow muscles (Fig. 4). Opposed to the twofold increase in the activity measured in the fast muscles ($p < 0.01$), effectively no change could be detected in the slow muscles ($0.1 > p > 0.05$). In normal rabbits there was a considerable difference in the activities between the fast and slow muscles in favour of the latter, no similar observation could be made for dystrophic muscles.

A similar change was observed in vitamin E-deficiency concerning the activity of ATNase, another neutral proteinase (Fig. 5). The average increases in the activity were less than 50%, nevertheless, the increases were significant in both the fast ($p < 0.01$) and the slow ($0.02 > p > 0.01$) muscles. There was no difference in the activities between the two types of muscles, either in normal or the vitamin E-deficient rabbits.

In summary, the increases in the activities measured in the dystrophic muscles were more pronounced in the fast than in the slow muscles: this dystrophy preferably damages the fast muscle fibers. On SDS-gelelectrophoresis of the myofibrillar fraction, there were no detectable differences in the electrophoretic pattern of preparations between normal and vitamin E-deficient animals (data not shown). Although in dystrophy the proportion of myofibrillar proteins decreased, the relative amounts of the different components showed no considerable changes.

Discussion

In skeletal muscles proteolysis is catalyzed by different lysosomal and non-lysosomal proteases. Upon muscle damage of various ethiology the activities of these enzymes change to different extent (see below). Based on the mechanism of action of lysosomal proteases, aspartate (cathepsin D) and cysteine-proteinases (cathepsin B, H, L) can be distinguished. The cathepsins can split myofibrillar proteins (for a review see Bird et al. 1977; 1980; Sohár et al. 1979).

Among the non-lysosomal proteases, MMP-7ase has a characteristic substrate: Suc-Ala-Ala-Pro-Phe-AMC pH 7 (Sohár et al. 1987). ATNase with an optimum pH in slightly alkaline region has a specific substrate N-acetyl-tyrosinyl-nitroanilide (the enzyme obtained its name from the substrate) (Sohár et al. 1987).

Changes in protease activities observed in dystrophies such as Duchenne muscular dystrophy, are of special interest. Increase of activities of cathepsin B and Ca^{2+} -activated protease, a muscle dipeptidase (Kar and Pearson 1978; Pearson and Kar 1979), and a significant enhancement of the activity of cathepsin H in the serum of patients (Sohár et al. 1988) have been reported. Increase of activities of cathepsin B and H were found in the sera of homozygous children with cystic fibrosis (László et al. 1988). Increased cathepsin B activity in the muscles of genetically dystrophic chickens has been regarded as a determining factor (Iodice et al. 1972). In this context, no difference could be observed in the enzyme activities between hypertrophic and atrophic muscles of chickens (Peterson et al. 1972). Cathepsin C and D activities have been reported to be considerably increased in the same dystrophy (Lee et al. 1984). In myoblast cultures of dystrophic chickens, increased cathepsin B, H, and L activities were measured (Sohár et al. 1985). An enhanced breakdown of myofibrillar proteins was indicated by the increased excretion of 3-methylhistidine (Hillgartner et al. 1981).

The changes observed in vitamin E-deficiency can be attributed to a considerable growth in the amount of free oxygen radicals (due to the absence of a free radical scavenger), resulting in the activation of cysteineproteinase (such as cathepsin B and MMP-7ase). The presumed mechanism is that SH groups in the active center of the enzyme are liberated from disulfide bridge, thereby allowing the activated enzyme to catalyze the proteolytic reaction (Davies et al. 1987). The posttranslational synthesis of lysosomal cysteine-proteases from preenzymes is brought about by a not yet known metalloprotease (or perhaps MMP-7ase) (Hara et al. 1988). It is also known that an active enzyme can be produced from the preenzyme (e.g. from procathepsin B) by cathepsin D (Nishimura et al. 1988). In addition, the activity of cathepsin B can be enhanced due to the reduction of cystatins which are the specific inhibitors of cathepsin B in muscles (Schwartz and Bird 1977). Presumably, this reduction can be a result of an increased activity of cathepsin D (Lenarcic et al. 1988). Another possibility is that procathepsin D is

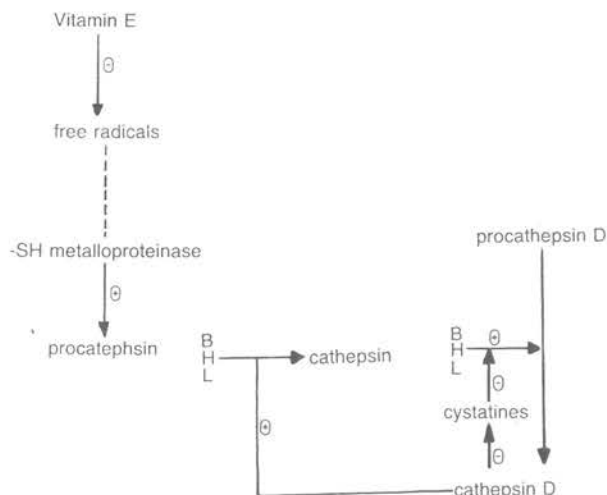


Figure 6. Schematic representation of the post-translational activation of cellular proteases (a hypothesis).

+ : activation, - : inhibition

activated by cathepsin B as a result of a limited proteolysis (Samarel et al. 1983). This cycle (Fig. 6) remains operative until the amount of cathepsin preenzymes starts decreasing as a result of a decrease in protein synthesis. Our measurements of protease activities were carried out at a stage of muscle damage, when the effect of the decreased synthesis had not yet been manifest.

From the results presented it may be concluded that the increased activity of skeletal muscle proteases induced by the accumulation of free radicals as a result of vitamin E-deficiency is much higher than that due to immobilization, treatment with glucocorticoids, castration, hypoxia or lack of ATP. The sensitivity of the enzymes decreases in the following order: cathepsin B, cathepsin D, MMP-7ase and ATNase. The response to vitamin E-deficiency was especially strong in cathepsin B: its activity increased four to five times higher than in conditions as mentioned above. The pronounced enhancement in cathepsin B activity as a result of vitamin E-deficiency is likely due to the activation of proteinases secondary to the triggering of the covalent modification of cathepsin B (see above). This is followed by activation of cathepsin D via limited proteolysis catalyzed by cathepsin B. Cathepsin B is further activated through limited proteolysis and breakdown of cystatins.

In view of all the above, we suppose that the striking increase in cathepsin B activity is a result of three different processes playing a role in the activation of the enzyme; moreover, the activation is a self-catalyzed chain reaction.

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