Studies on Proteolytic Digestion of Myosin from Red and White Skeletal Muscles of the Rabbit

Proteolytic enzymes are widely used as structural probes in studying molecular properties of myosin. In this study we have investigated tryptic and chymotryptic digestion of myosin isolated from red and white muscles of the rabbit in an attempt to obtain more information on the differences in the structure of myosins studied. Differences between these 2 kinds of myosin, reflected for example by different enzymatic activity, are important from the point of view of the relation between myosin structure and function.

Myosin was prepared by the dilution and precipitation procedure[1]. Red muscles used for the preparation of myosin were the soleus, semitendinosus, crureus and intertransversarius, white muscles were the adductor magnus and vastus lateralis.

Figure 1 shows that myosin from white muscles is digested by trypsin more rapidly than myosin from red muscles. This difference probably reflects differences in the molecular structure of these 2 myosins, studied for example by viscosity measurements after tryptic digestion[3]. Both viscosity measurements and the determination of NPN formed during proteolysis correspond to and reflect different processes. In our experiments, the different rate of tryptic digestion was observed in both earlier and later stages of proteolysis, suggesting that structural differences between red and white myosin concern the proteolytic sensitive area of myosin and other parts of the myosin molecule. The amount of peptides released by chymotrypsin was practically the same, when red and white myosins were compared. This result may possibly be due to the wider specificity of chymotrypsin.

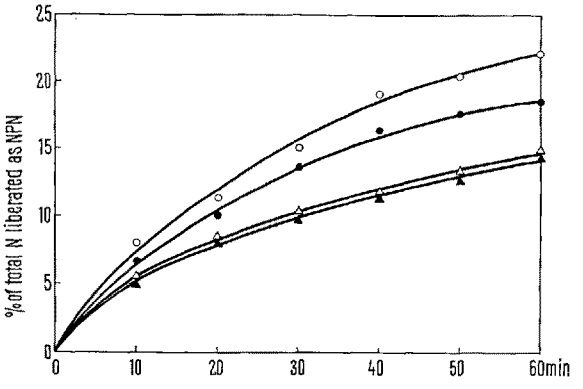

Fig. 1. Tryptic and chymotryptic digestion of myosins. To a solution of myosin containing 0.5 *M* KCl, 17 m*M* *Tris*, pH 7.5, and 7 mg of protein/ml, trypsin or chymotrypsin (twice respectively, 3 times crystallized) were added at 25 °C (ratio of enzyme to myosin of 1:250, w/w). Digestion was stopped by adding TCA (final concentration 5%). NPN was determined in the filtrate after digestion by the Conway micro diffusion technique[2]. Circles: tryptic digestion, triangles: chymotryptic digestion, open symbols: NPN from white myosin, closed symbols: NPN from red myosin.

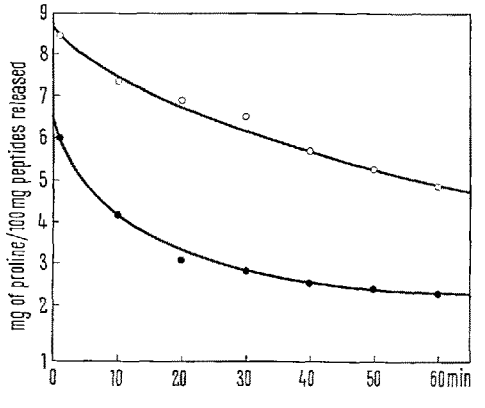

Fig. 2. The release of proline during tryptic digestion of red and white myosin. For details see Figure 1. Proline was determined by the method of TROLL and LINDSLEY[6]. o——o Peptides from red myosin, ●——● peptides from white myosin.

The content of proline in the protein molecule or in its part is characteristic and corresponds to the amount of α-helix in the molecule. It was shown that parts of the myosin molecule differ in the amount of proline. Proline is almost absent in LMM, higher amount of proline was found in HMM[4]. The proteolytic sensitive area of myosin contains an unusually high amount of proline[5]. We measured the content of proline in peptides released during tryptic digestion of red and white myosin. When the content of proline was determined in the peptides released as a function of the extent of proteolysis, essentially different results were obtained by both myosins (Figure 2). In the early stages of digestion high concentrations of proline were released, both by red and white myosin, but peptides from red myosin contained 30–40% more proline than peptides from white myosin. Most of the peptides released during early stages of tryptic digestion arise from the fast reaction process, presumably from the protease-labile belt. The content of proline was also determined in native myosins and it was found that red and white myosin contains the same amount of proline (3.05%). From these results it can be calculated that non-digested fragments of red myosin contain less proline than those of white myosin. Assuming that the model HMM and LMM with proteolytic sensitive area in the middle part of myosin molecule is valid also for red myosin, we can conclude that red myosin contains more proline in the proteolytic sensitive area and less proline probably in HMM than white myosin. Not very much is known about the differences between molecular properties of white and red myosin. Nevertheless, the different content of proline in peptides from red and white myosin appears to be related to the conformational state of native myosin molecules.

Zusammenfassung. Der Vergleich der tryptischen und chymotryptischen Myosinspaltungen aus weissem und rotem Kaninchenmuskel ergibt: Nichteiweißstickstoffbildung zeigt durch Trypsin eine schnellere Myosinspaltung im weissen Muskel; Chymotrypsin spaltet beide Myosine mit gleicher Geschwindigkeit.

I. SYROVÝ

Institute of Physiology, Czechoslovak Academy of Sciences, Praha (Czechoslovakia), 18 June 1968.

[1] S. V. PERRY, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. V. KAPLAN; Academic Press, New York 1955), vol. 2, p. 582.
[2] E. J. CONWAY, *Microdiffusion Analysis and Volumetric Error*, 4th edn (Crosby Lockwood, London 1957).
[3] F. A. SRETER, J. C. SEIDEL and J. GERGELY, J. biol. Chem. *241*, 5772 (1966).
[4] A. G. SZENT-GYORGYI and C. COHEN, Science *126*, 697 (1957).
[5] D. M. SEGAL, S. HIMMELFARB and W. F. HARRINGTON, J. biol. Chem. *242*, 1241 (1967).
[6] W. TROLL and J. LINDSLEY, J. biol. Chem. *215*, 655 (1955).