Properties of Atrial and Ventricular Myosin in Mammals of Various Size

I. SYROVÝ

Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czechoslovakia

Abstract. Myosin was isolated from atria and ventricles of adult rats, rabbits and pigs, and characterized by ATPase activities, the effects of temperature on the latter, the influence of alkaline preincubation on enzymatic activity and by electrophoretic fractination of myosin peptides. It was shown that ventricular myosins are clearly distinguished by their ATPase activities and their response to pH and temperature, whereas atrial myosins were more similar to each other in this respect. However, the electrophoretic patterns of rat, rabbit and pig atrial myosin peptides produced by digestion with *S. aureus* V8 protease were different.

Key words: Atrial myosin — Ventricular myosin — ATPase activity — Myosin peptides

Introduction

Myosin isolated from atria has been shown to differ from ventricular myosin with respect to ATPase activity, electrophoretic mobility in non-dissociating gels and structure of the myosin light and heavy chains (Syrový et al. 1976; Long et al. 1977; Flink et al. 1978; Hoh et al. 1978; Yazaki et al. 1979; Dalla Libera and Sartore 1981; Price et al. 1980; Clark et al. 1982). The higher actin-activated ATPase activity of atrial myosin probably accounts for the higher speed of shortening of atrial muscle as compared to ventricular muscle (Korecky and Michael 1974; Urthaler et al. 1975; Wikman-Coffelt et al. 1982). A comparison of atrial and ventricular myosins from adult mammals of various size shows that ventricular myosin is much more differentiated than atrial myosin (Lompre et al. 1981). On the other hand, in all species atrial myosin is mostly of the α type, and it is not known how atrial myosins differ from each other. Measurements of

Ca^{2+}-ATPase activities of atrial and ventricular myosins in adult mammals of various size led to the conclusion that the larger the animal species size, the greater the divergence between atrial and ventricular myosin ATPase; this is mainly due to a decrease of ventricular myosin ATPase activity (Syrový 1985). Also, the effects of hemodynamic overload and hormonal imbalance is much more pronounced in ventricular than in atrial myocardium (Samuel et al. 1986; Syrový 1987).

Aimed at detecting whether various atrial myosins are more structurally related than the corresponding ventricular myosins, we examined ATPase activities of adult rat, rabbit and pig atrial and ventricular myosins, their temperature dependence, the effects of alkaline pH on ATPase activities and the electrophoretic pattern of peptides obtained after digestion of myosins with *S. aureus* V8 protease.

Materials and Methods

Adult rats (4-month-old, Wistar strain), adult rabbits (3 kg b.w., local supply) and adult pigs (cca 80 kg b.w., from slaughterhouse) were used for experiments. Myosin was prepared from the atria and ventricles and each myosin preparation consisted of 2—3 g of tissue. In the pig, a representative sample of about 4 g of tissue was cut from the outer wall of the myocardium. Myosin was prepared as described previously (Syrový and Gutmann 1977). Ca^{2+}-activated ATPase was measured in a medium containing 0.05 mol/l Tris-HCl, pH 7.5; 10 mmol/l $CaCl_2$; 0.025 mol/l KCl; 5 mmol/l ATP; and 0.3 mg of protein/ml, at 26 °C. Protein concentration was determined by the Conway microdiffusion technique after digestion with a catalyst mixture (Conway 1957).

ATPase activities were also measured after preincubation at pH 7.5 (control values) or pH 9.5. Preincubation at pH 7.5 was done by adding 0.4 ml myosin solution to 0.2 ml of 1 : 1 mixture (pH 7.5) of glycine and imidazole buffers, as used for preincubation at pH 9.5. Preincubation at pH 9.5: Myosin (0.4 ml, 5 mg per ml) was adjusted to pH 9.5 with 0.1 ml of 0.4 mol/l glycine-KOH buffer and incubated at 25 °C for 10 min. The pH was then adjusted back to 7.5 with 0.1 ml of 0.4 mol/l imidazole-HCl buffer and ATPase activity was determined in the presence of $CaCl_2$. For details see Syrový (1975).

Proteolytic digestion of myosin. Partial proteolytic cleavage of SDS-denaturated myosin with *S. aureus* V8 protease (Miles) was performed according to the method of Cleveland et al. (1977). Myosin (in 0.3 mol/l NaCl, 5 mmol/l sodium phosphate, pH 7.0 50 % glycerol) was diluted with 0.125 mol/l Tris-HCl, pH 6.8, to a final concentration of 0.5 mg/ml, supplemented with 0.5 % SDS and 10 % glycerol, and heated for 3 min to 100 °C. Proteolysis was performed using *S. aureus* protease (17 µg/ml for 30 min at 37 °C). The reaction was terminated by heating for 2 min in the presence of 2 % SDS and 6 % mercaptoethanol. Peptides released were analysed by the one-dimensional SDS-PAGE (Laemli 1970).

Results and Discussion

The ATPase activities of rat, rabbit and pig atrial and ventricular myosins and the dependence of ATPase activities on temperature are shown in Fig. 1. It can

be seen that Ca^{2+}-ATPase activities of different atrial myosins are similar, and that the temperature dependences of atrial myosins are also essentially the same. Fig. 1 also illustrates that, unlike atrial myosin, ventricular myosin of the rat, rabbit and pig differ in both their ATPase activities and temperature dependence. The ATPase activity of rat ventricular myosin is 1.8 times higher than that in the pig. On the other hand, rat atrial myosin ATPase activity is only 1.2 times higher than that in the pig. The ATPase activity of ventricular myosin of the pig is more dependent on temperature than that of the rat, the ATPase activity of rabbit ventricular myosin having intermediate temperature dependence.

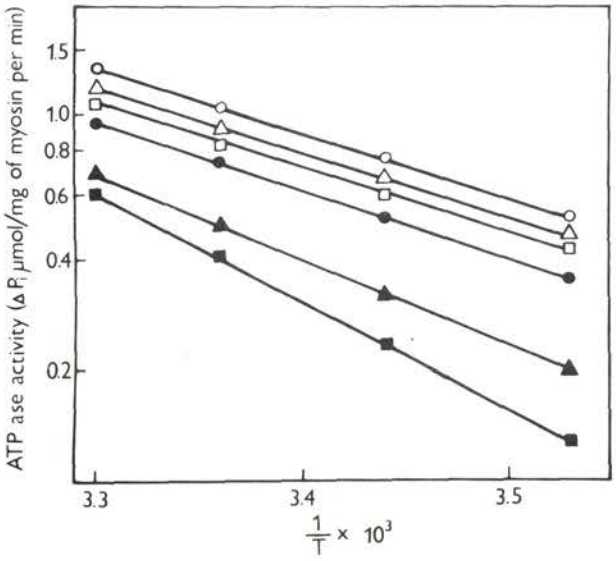

Fig. 1. The temperature dependence of myosin Ca^{2+}-ATPase activities. T = absolute temperature. The values represent means of three experiments. ● — rat atrial myosin; ○ — rat ventricular myosin; △ — rabbit atrial myosin; ▲ — rabbit ventricular myosin; □ — pig atrial myosin; ■ — pig ventricular myosin.

Table 1 illustrates the effect of alkaline preincubation on myosin Ca^{2+}--ATPase activity. Myosins from rat, rabbit and pig atria were not influenced by alkaline preincubation. Ventricular myosins showed different responses to alkaline preincubation: Ca^{2+}-ATPase activity in rat ventricular myosin was not altered by alkaline preincubation, while 21 % and 35 % of ATPase activity were lost in rabbit and pig ventricular myosin respectively. Also, the effects of alkaline pH on Ca^{2+}-myosin ATPase activity distinguished between atrial and ventricular myosins.

Polypeptide mapping of myosin digested with a proteolytic enzyme is a usefull tool for comparing myosin structures. We therefore compared polypep-

Table 1. The effects of alkaline preincubation on Ca^{2+}-ATPase activities of myosins from cardiac muscles. Activity is expressed in μmole P_i/mg protein/min. Each value represents the mean ± SD of three experiments.

Source of myosin	Ca^{2+}-ATPase activity Preincubation at pH		Activity ratio pH 9.5/pH 7.5
	7.5	9.5	
Rat atrial	1.03 ± 0.09	1.04 ± 0.1	1.01
Rat ventricular	0.75 ± 0.06	0.71 ± 0.05	0.94
Rabbit atrial	0.90 ± 0.07	0.90 ± 0.08	1.00
Rabbit ventricular	0.50 ± 0.03	0.38 ± 0.02	0.79
Pig atrial	0.85 ± 0.06	0.82 ± 0.05	0.96
Pig ventricular	0.42 ± 0.03	0.27 ± 0.03	0.65

tide fragments obtained by partial proteolytic digestion of myosin. One-dimensional analysis of the peptide fragments produced by digestion with *S. aureus* V8 protease is shown in Fig. 2. Slightly different peptide maps were obtained from rat, rabbit and pig atrial myosins. It can be concluded that subtle structural differences exist between the myosins studied. The ventricular myosins were not analyzed in this respect since they have been already shown to be strictly

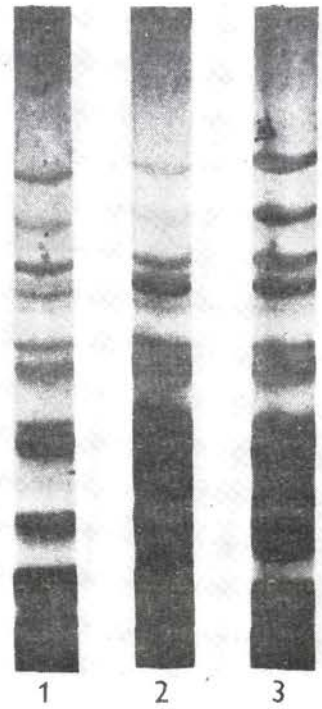

Fig. 2. Gel analysis of peptides produced by partial proteolysis of SDS-denaturated atrial myosins with *S. aureus* V8 protease. 1 — rat atrial myosin; 2 — rabbit atrial myosin and 3 — pig atrial myosin.

different in various species. Differences observed in the electrophoretic patterns of peptides of atrial myosin between the species studied are not surprising, because myosin cannot be expected to have the same structure in different species.

It can be concluded from our study that atrial myosins are structurally more related than various ventricular myosins. Lompre et al. (1981) showed that the isoenzymic composition of ventricular myosin depends on the size of the adult animal. In rat V_1 is predominant, and smaller amounts of V_2 and V_3 are present; in rabbit V_1 is predominant, and a small amount of V_3 is probably present, while in pig only V_3 is present. Ventricular isomyosins V_1, V_2 and V_3 differ in their peptide maps, suggesting that their structures are different. Much less information is available about atrial myosins. Our results are in agreement with results of Dechesne et al. (1985) indicating that in all species atrial myosin is essentially of the α (V_1) type and that the amino acid sequences of different atrial myosins are largely similar.

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