E14

DETERMINATION OF HYDROPHOBICITY OF FAST AND SLOW MYOSINS FROM RABBIT SKELETAL MUSCLES: IMPLICATION IN HEAT-INDUCED GELATION

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OBJECTIVES

Heat-gelling ability is probably the most important functional property of myosin in meat products, and occurs in all the technological processes involving a heat-treatment. Besides the great influence of physico-chemical factors (pH, ionic strength), the muscle type also determines the heat-gelation properties of myosin. Indeed, the myosin from white muscles exhibits a greater gelling ability than that from red muscles. Recent investigations (Culioli *et al.*, 1993) suggested that these differences could be related to the surface hydrophobicity of both types of myosin which determines the solubility and the heat-stability of the molecules. Furthermore, protein surface hydrophobicity can affect the type of molecular interactions occuring during gelation and, thus, the viscoelastic properties of the resulting gel. The aim of this work was (i) to investigate both aliphatic and aromatic surface hydrophobicities of fast and slow myosins by fluorescence methods, (ii) to follow the changes in the myosin hydrophobicity properties during heating and finally (iii) to determine, in relation with the muscle type, the involvement of myosin hydrophobic binding sites in the heat-induced gelation process.

METHODS

Myosin was prepared from rabbit *Psoas major* (PM) (type IIB) and *Semimembranosus proprius* (SMp) (type I) muscles according to Culioli *et al.* (1993), and then equilibrated in a 40mM potassium phosphate buffer (pH 6; 0.6M KCl).

Cis-Parinaric Acid (cPA) (0.5mM) and 8-Anilino-1-Naphtalene-Sulfonic Acid (ANSA) (5mM) were used to determine aliphatic and aromatic hydrophobicities of myosin, respectively. Each probe was added to the myosin solutions (0.01 to 0.04g/l) at a final concentration of 2µM and 16µM for cPA and ANSA, respectively. The cPA-protein conjugates were excited at 325nm ($\lambda_{exc.}$) and the relative fluorescence intensity was measured at 410nm ($\lambda_{em.}$). For ANSA-protein conjugates the values of $\lambda_{exc.}$ and $\lambda_{em.}$ were 380 and 475nm, respectively. According to Sklar et *al.* (1977), the initial slope (S0) of the fluorescence intensity versus protein concentration was taken as an index of protein hydrophobicity for both fluorescent probes. The hydrophobic properties have been determined on myosin either native (20°C) or gradually-heated (0.5°C/min) from 20 up to 80°C. All fluorescence measurements were performed after having cooled the samples at room temperature (1h).

The heat-induced gelation of PM and SMp myosins, without or with ANSA (0.1 to 0.4mM), was studied using a Carri-Med CSL 100 rheometer in oscillatory conditions. Myosin samples (12g/l) were heated between the 2 parallel plates of the rheometer (Φ = 4cm, gap = 0.1cm) from 30°C to 80°C at a rate of 0.75°C/min. Rheological measurements were carried out with a maximal deformation of 2%. Storage modulus (G') which indicates the elasticity of the gels was determined after every 0.5°C increment.

RESULTS & DISCUSSION

Surface hydrophobicities of PM and SMp pure myosins: As shown in table 1, the aliphatic surface hydrophobicity of SMp native myosin (S0) was about 1.4 times greater than that of PM native myosin. This result is in total accordance with the previous investigations of Ouali *et al.* (1988), carried out on the same rabbit muscles, in which the ratio value of S0 for SMp myosin versus S0 for PM myosin was 1.37. Similarly, the aromatic surface hydrophobicity of SMp native myosin (S0) appeared to be 1.5 times higher than that of PM myosin (table 1). It was therefore clear that the global surface hydrophobicity of slow-twitch red native myosin is higher than that of the fast-twitch white myosin.

Furthermore, the higher aliphatic and aromatic hydrophobicities of myosin from red muscle appeared very well correlated with its lower solubility properties revealed by either turbidity measurements or ammonium sulphate precipitation (results not shown). Penzer (1972) reported that the fluorescence enhancement following the binding of ANSA to the molecules is not necessarily specific of their hydrophobic binding sites. However, the use of ANSA still remained interesting because Nakai *et al.* (1986) presented a general hypothesis in which the aromatic hydrophobicity might play a more important role in protein solubility than the aliphatic hydrophobicity. Our investigations partly corroborated these results. Nevertheless, in our study, it seemed that aromatic and aliphatic hydrophobicities were both closely related to the solubility of myosin molecules.

Changes in PM and SMp myosin hydrophobicity during heating: The hydrophobicity of heat-denaturated or unfolded proteins was called Se (Nakai *et al.*, 1986). Whatever the muscle type and the fluorescent probes used, the hydrophobicity of myosin increased during heating. As shown in figure 1, the enhancement of aliphatic Se was insignificant up to 30°C, for both myosin types. Then, the hydrophobicity steeply increased between 30 and 45°C, remained constant up to 50°C and finally increased again from 50 to 80°C. However, the total increase in the hydrophobicity of SMp myosin (x 1.9) was less pronounced than that of PM myosin (x 3). The higher enhancement of PM myosin aliphatic hydrophobicity during heating led to the crossing of curves at about 55°C. As a consequence, beyond this temperature, the aliphatic hydrophobicity of the PM myosin became greater than that of SMp myosin (figure 1).

	20°C		80°C	
	PM	SMp	PM	SMp
Aromatic hydrophobicity (ANSA)	16 (±0.5)	22 (±1)	79 (±7)	56 (±6)
Aliphatic hydrophobicity (cPA)	55 (±11)	79 (±14)	173 (±7)	150 (±3)

Table 1: Hydrophobicities (S0 and Se) of native and heated PM and SMp myosins. The values were given by µM of myosin and by µM of probe (number of experiments > 7).

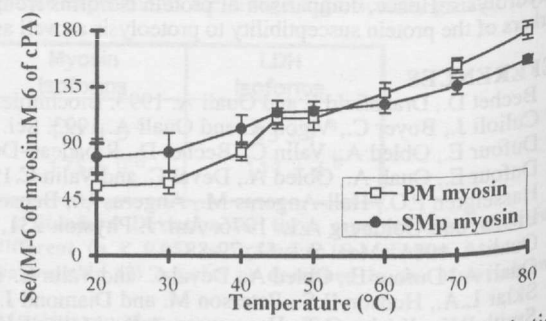

Figure 1: Effect of a gradual heating (0,5°C/min) on the aliphatic hydrophobicity of PM and SMp myosins (number of experiments > 7).

The maximal rise in aromatic hydrophobicity of both PM and SMp myosins occurred between 30 and 45°C (figure 2). Beyond this temperature, the hydrophobicity increased more slowly. Like the changes in aliphatic hydrophobicity, the aromatic hydrophobicity enhancement of PM myosin during heating was higher than that of SMp myosin. The PM and SMp myosins heated at 80°C exhibited aromatic Se values respectively 5 and 2.5 times higher than those of their corresponding native myosins. These increases are larger than those observed for aliphatic hydrophobicity and the curves crossed at 40°C, a lower temperature than in the case of aliphatic hydrophobicity.

Blocking of hydrophobic binding sites - Effect on heat-induced gelation: Blocking of hydrophobic binding sites by ANSA have been performed before heating on myosin. As shown in figure 3, the final strength of white fast-twitch myosin gels was always greater than that of red slow-twitch myosin gels. The blocking of hydrophobic binding sites affected differently the gelation of PM and SMp myosins. The ANSA induced a slight decrease in the rigidity of SMp myosin gels but a strong increase in the rigidity of PM myosin gels.

As described by Borejdo (1983), most of surface hydrophobic sites are located on the myosin heads. In addition, several studies (Yasui *et al.*, 1990; Sharp and Offer, 1992) reported that the interactions between myosin heads initiate the heat-gelation of myosin monomers. So, for the SMp myosin hydrophobic interactions seem to be necessary to initiate the gelation process. For PM myosin, the blocking of the hydrophobic binding sites did not prevent the heat-induced gelation process, and, on the contrary, might allow interactions between myosin molecules which lead to the development of stronger gels. This confirms that the molecular mechanisms involved in the gelation process, especially during the first steps of gel formation, are muscle type dependent. These different molecular mechanisms could explain the great differences in viscoelastic properties and structure of red and white myosin heat-induced gels described in the literature (Boyer *et al.*, 1992; Culioli *et al.*, 1993; Xiong, 1994).

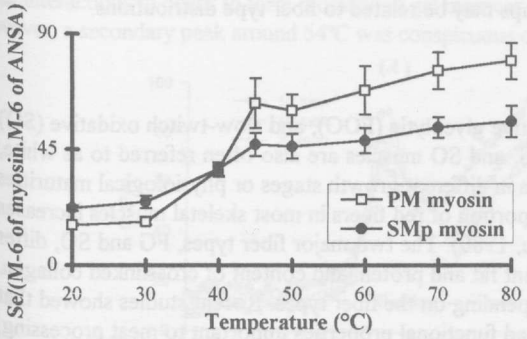

Figure 2: Effect of heating on the aromatic hydrophobicity of PM and SMp myosins (number of experiments > 7).

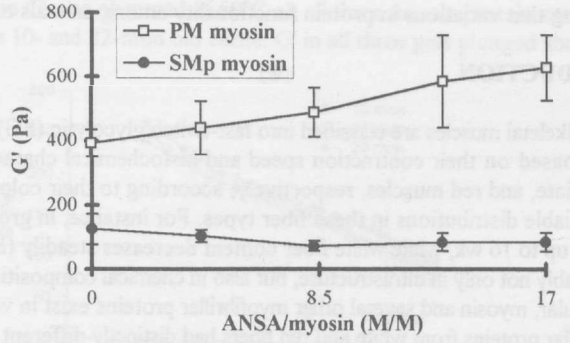

Figure 3: Effect of blocking of hydrophobic sites by ANSA on G' of PM and SMp myosin (12g/l) gels obtained at 80°C after a gradual heating (0.75°C/min) (number of experiments > 3).

CONCLUSIONS

This study emphasized the great incidence of the muscle type on the hydrophobic properties of myosin molecules. Indeed, the myosin from red slow-twitch muscle exhibits at their surface more aliphatic and aromatic amino acid residues than the myosin from white fast-twitch muscle. The hydrophobic characteristics of both myosin types were in total accordance with their solubility and heat-stability properties. As already described (Ouali *et al.*, 1988; Culioli *et al.*, 1993; Xiong, 1994), the solubility of myofibrillar proteins from red muscles was lower and the heat-stability higher than those of proteins from white muscles.

The changes in hydrophobicity during the gradual heating of myosin are also muscle type dependent. During heating, the fast-twitch myosins display more hydrophobicity than the slow-twitch one. This could be due either to a greater unfolding during heating or to a higher amount of internal hydrophobic residues in the native fast-twitch myosin molecule than in the slow-twitch isoform.

The hydrophobicity properties of both fast- and slow-twitch myosins could explain the differences in their heat-gelation properties. The higher hydrophobicity of red slow-twitch myosins could prevent molecular interactions which induce the gelation process in the first step of heating. The better the solubilization of myosin before heating the more easily the interactions between molecules during heating can occur.

Considering that the hydrophobic bonds are stabilized by heating, the hydrophobic binding sites could greatly contribute to the development of myosin heat-gels. Indeed, the higher hydrophobicity enhancement of fast-twitch myosin isoform during heating is in accordance with the higher increase in gel rigidity of this myosin isoform.

Finally, the blocking of hydrophobic binding sites on myosin molecule confirms that the molecular interactions occuring during gelation were strongly related to the muscle type. However, these assays did not allow determination of the specific mechanisms involved in the heat-gelation processes of both myosin types, except that the native hydrophobicity of myosin is essential in the inducing of slow myosin gelation while it is unfavourable to the gelation of fast myosin isoforms.

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