E13

TYPE I AND TYPE IIB MUSCLE PROTEIN SUSCEPTIBILITY TO PROTEOLYSIS AS RELATED TO THEIR HYDROPHOBICITY

G. FARIAS-MAFFET, C. TASSY, A. OUALI
Muscle Biochemistry Unit, SRV, INRA-Theix, 63122 ST Genès Champanelle, France

INTRODUCTION

Although not clearly understood, it has been stressed that, in normal physiological conditions, both myofibrillar and non-myofibrillar protein breakdown rates were higher in slow twitch muscles (Type I) than in fast twitch (type II) muscles (Li and Goldberg, 1976; Hasselgren *et al.*, 1990). To explain this difference, one major reason often emphasized was the higher level of endopeptidases in the former muscle type. However, although often neglected in such studies, the substrate, i.e. the highly polymorphic myofibrillar and sarcoplasmic muscle proteins, might be a limiting factor to the intracellular endopeptidases efficiency. Therefore, the present work intended to evaluate the potential contribution of the intracellular proteinaceous substrates to this muscle type difference by determining the relative susceptibility to proteolysis of different myofibrillar and sarcoplasmic protein fractions obtained from rabbit *Semimembranosus proprius* [SMp] and *Psoas major* [PM] rabbit muscles which were shown to be pure type I and IIB muscles, respectively (Dufour et al., 1989) . We further investigated the relationship between the rate of protein hydrolysis by various endopeptidases and their effective hydrophobicity which was suggested to greatly influence the susceptibility of proteins to proteolysis (Wiederanders *et al.*, 1981).

MATERIAL AND METHODS

Myofibrillar and sarcoplasmic protein fractions were prepared from SMp and PM muscles as described previously (Ouali, 1984). Rabbit lactate dehydrogenase (LDH) isoforms, bovine cathepsin D and papain were purchased from Sigma Chemicals (Grenoble, France). Cathepsin L was purified from chicken liver as reported by Dufour *et al.* (1987). SMp and PM mysosins were purified according to Culioli et al. (1993). Incubations of muscle protein substrates (4-5 mg) with proteases (at a W/W ratio varying between 1/500 and 1/1000) were performed at pH 6 and 30° C in the presence of 0.6 M NaCl with added 5 mM dithiothreitol when necessary. The rate of hydrolysis was estimated by measuring spectrophotometrically (A_{280}) the concentration of 5% TCA soluble material released per minute. The method of Sklar *et al.* (1977) using *cis*-parinaric acid (9,11,13,15-octadecatetraeneoic acid : PNA) as a probe (PNA commercially available from Molecular Probes, Interchim, Montluçon, France) was applied to determine the effective hydrophobicity of muscle protein fractions (So). Briefly, 10 µl of a 0.5 mM PNA ethanolic stock solution were added to 2 ml of protein solution in 5 mM potassium phosphate buffer, pH 7.0, containing 0.6 M KCl. Fluorescence measurements were performed using a Perkin-Elmer LS5 spectrofluorometer. Protein concentration was determined by the bicinchoninic method of using bovine serum albumin as a standard (Smith et al., 1985).

RESULTS

1 - Comparative Hydrophobicity of protein fractions from SMp and PM rabbit muscles

The effective hydrophobicity of proteins is believed to play a major role in the definition of their structure-function relationship. The usefulness of *cis*-parinaric to estimate the effective hydrophobicity of proteins has been emphasized by Kato and Nakai (1980) who clearly demonstrated that the hydrophobicity of a variety of proteins as determined by fluorescence enhancement following binding of this probe correlates very well with the classical parameters of hydrophobicity including the hydrophobic partition coefficient, the interfacial tension and the emulsifying index. With this technique, the initial slope (So) of fluorescence intensity *vs.* protein concentration was used as an index of the protein hydrophobicity. This method was used to determine the effective hydrophobicity of various protein fractions prepared from pure type IIB and type I rabbit muscles in which post-mortem proteolysis has been shown to be much extensive in the former type (Ouali et al., 1987).

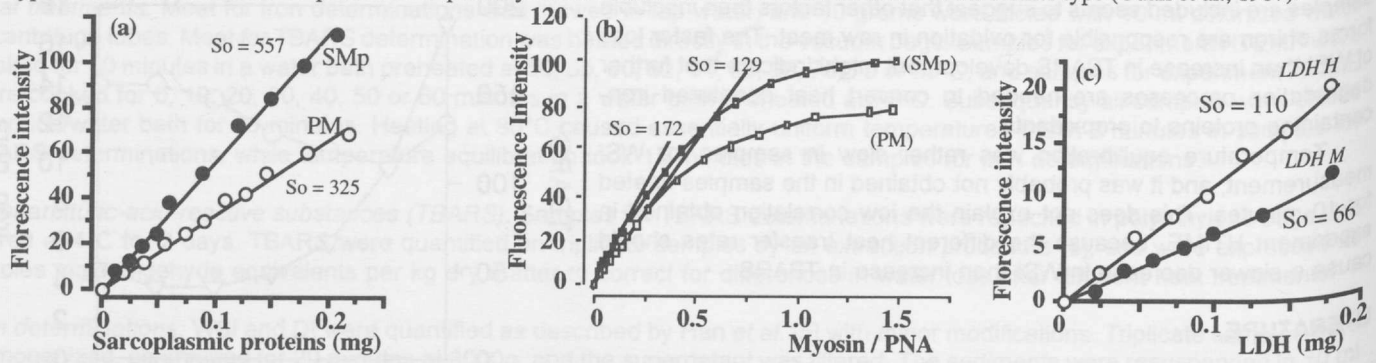


Figure 1: Plot of the *cis*-parinaic acid fluorescence versus protein concentration or protein/probe molecular ratio. These plots were obtained for the total sarcoplasmic extract (a), the purified myosin isoforms (b) and the purified LDH isoforms (c). The initial slope of the plots drawn for each protein fraction sample corresponded to the So value indicated for each plot.

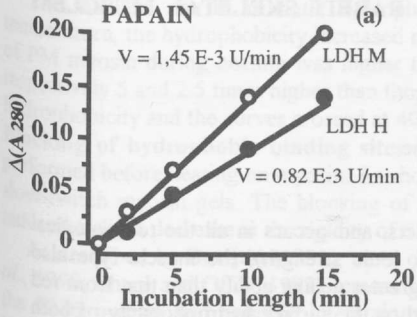
As reported in Fig. 1a, a total sarcoplasmic extract from rabbit SMp muscle exhibited a significantly greater hydrophobicity (So) than the same protein fraction from PM muscle. Similar conclusions were drawn for the purified type I and IIb myosin isoforms (Figure 1b) as well as for the Heart (LDH H) and skeletal muscle (LDH M) LDH isoforms (Figure 1c). As summarised in Table 1, for all protein fractions tested including the soluble myofibrillar protein fraction, the mean values obtained for So from three separate experiments are always significantly greater for the fractions prepared from the SMp muscle.

Protein Fractions →	Sarcoplasmic Extract	Myofibrillar Extract	Myosin Isoforms	LDH Isoforms
PM muscle [1]	338 ± 5^{a}	108.3 ± 1.7^{a}	127 ± 3^{a}	55 ± 7^{a}
SMp muscle [2]	555 ± 3^{b}	192.3 ± 0.3^{b}	175 ± 9^{b}	111 ± 4^{b}

Table 1: So values for the different muscle protein fractions. figures are the Mean ± Standard Deviation for three separate determinations. Within columns, values followed by different superscript letters are significantly different ($p < 0.05$) according to the student t-test. Proteins fractions were obtained from rabbit *Psoas major* (1) and *Semimembranosus proprius* (2) muscles as described in the Material and Methods section.

2 - Comparative susceptibility to proteolysis of protein fractions from SMp and PM rabbit muscles

The time course hydrolysis of sarcoplasmic proteins by Papain and cathepsin D reported in Figure 2 suggested that the PM muscle fraction is degraded approximately at twice lower rate than the same fraction from SMp muscle. This finding was confirmed by the data obtained for the other muscle protein fractions studied (Table 2) and, for all of them, the differences between PM and SMp muscles were of high significance ($p < 0.05$). According to the results presented in Table 2 and irrespective of the protease considered, all protein fractions from SMp muscle



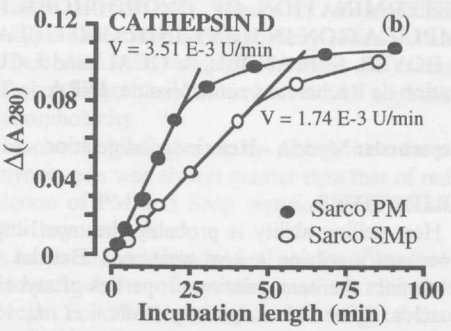


Figure 2: Time course hydrolysis of sarcoplasmic proteins by papain (a) and cathepsin D (b). The ordinate represents the absorbance difference at 280 nm between the assay sample and the control incubated in the same conditions in which TCA was added at time 0.

clearly showed a significantly lower susceptibility to proteolysis than the same fractions from PM muscle (Type IIB).

Proteinase tested	Sarcoplasmic Extract		Myofibrillar Extract		Pure Myosin isoforms		Pure LDH isoforms	
	PM muscle	SMp muscle	PM muscle	SMp muscle	PM muscle	SMp muscle	PM muscle	SMp muscle
Papain	6.94 ± 0.29	4.30 ± 0.21	5.04 ± 0.55	3.06 ± 0.26	ND	ND	1.41 ± 0.15	0.89 ± 0.05
Cathepsin D	3.61 ± 0.28	1.86 ± 0.23	ND	ND	ND	ND	ND	ND
Cathepsin L	ND	ND	ND	ND	0.41 ± 0.08	0.10 ± 0.02	ND	ND

Table 2: Rate of hydrolysis ($*10^{-3}$) of the different muscle protein fractions by papain, cathepsin D and cathepsin L. Figures are the Mean ± Standard Deviation for three separate determinations. The rate of hydrolysis was expressed as the increase in the ΔDO at 280 nm of the 5% TCA soluble material ($*10^{-3}$) per minute. ND: Not Determined

3 - Relationship between susceptibility to proteolysis and protein hydrophobicity

From the findings reported above, one might conclude that proteins with greater hydrophobicity were less sensitive to proteolysis. Attempts to correlate protein hydrolysis rates to their surface hydrophobicity values using the whole set of data were however unsuccessful. By contrast, highly significant correlations were obtained within muscle when papain hydrolysis rates obtained for the different protein fractions from either PM (r = 0.94) or SMp (r = 0.93) muscles were plotted versus Log So (Figure 3). Unexpectedly, these correlations were positive, a finding which did not reflect the conclusions drawn upon comparison of SMp v.s. PM muscle protein fractions. Therefore, within muscle, protein susceptibility to proteolysis increased with their effective hydrophobicity but whatever the fraction considered, highest hydrolysing rates were found for PM muscle proteins. Hence, it can be stressed that protein hydrophobicity can not be considered as general marker for the protein susceptibility to proteolysis suggesting that this information is coded differently as suggested by a series of previously published works (For review see Bechet et al., 1993).

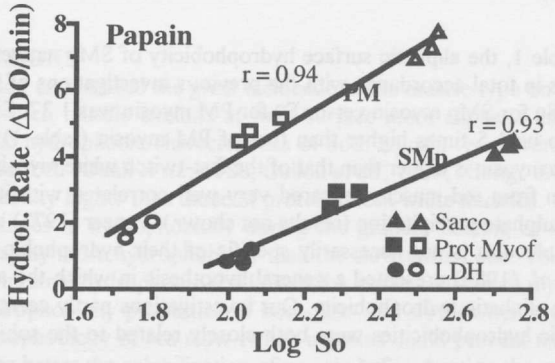


Figure 3 : Within muscle Relationship between the papain hydrolysing rates of the different protein fractions and their effective hydrophobicity value So.

CONCLUSIONS

The present results stressed forward that all protein fractions from SMp muscle were more hydrophobic and less susceptible to proteolysis than their counterparts from PM muscle, a finding which does not fit with the higher *in situ* rate of protein hydrolysis in SMp muscle (Hasselgren et al., 1990). If the substrate contribution is of no significance for the *in vivo* protein dagradation rate, this would probably not the case in postmortem muscle where extent of protein degradation is much lower in type I than in type IIB muscles (Ouali et al., 1987). Furthermore, the muscle type variation in the susceptibility to proteolysis of both sarcoplasmic and myofibrillar proteins can not be explain by a difference in their effective hydrophobicity a finding in contradiction with the positive correlation obtained for each muscle between protein So values and their rate of hydrolysis. Hence, comparison of protein isoforms from type I and type IIB muscles migth constitute a good model for the identification of markers of the protein susceptibility to proteolysis as well as of markers of the proteins half-life.

REFERENCES

Bechet D., Dransfield E and Ouali A. 1993. Biochimie. 75, 837-838.

Culioli J., Boyer C., Vigon X. and Ouali A. 1993. Sci. Aliments. 13, 249-260.

Dufour E., Obled A., Valin C., Bechet D., Ribadeau-Dumas B. and Huet J.C. 1987. Biochemistry, 26, 5689-5695.

Dufour E., Ouali A., Obled A., Deval C. and Valin C.1989. Biochimie, 71, 625-632.

Hasselgren P.O., Hall-Angeras M., Angeras U., Benson D., James J.H. and Fischer J.E. 1990. Biochem. J. 267, 37-44

Li J.B. and Goldberg A.L. 1976. Am. J. Physiol. 231, 441-448

Ouali A. 1984 . Meat Sci. 11, 79-88.

Ouali A., Dufour E., Obled A., Deval C. and Valin C. 1987. Reprod. Nutr. Develop. 28, 839-844.

Sklar L.A., Hudson B.S., Peterson M. and Diamond J. 1977. Biochemistry, 16, 813-818.

Smith P.K., Krohn G.T., Hermanson A.K., Mallia F.H., Gartner M.D., Provenzano E.K. Fujimoto N.M., Goeke B.J., Olson B.J. and Klenk D.C. 1985. Anal. Biochem. 150, 76-85.

Wiederanders B., Bohley P. and Kirschke H. 1981. In : Proteinases and their Inhibitors (Turk V. & Vitale L.J. Eds), Maldinska Knjiga-Pergamon Press, Ljubljana. pp 25-32.