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THE INTERACTION OF LINOLEIC ACID WITH MYOSIN AND ITS SUBFRAGMENTS: A MICROCALORIMETRIC STUDY

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ABSTRACT

The interaction of rabbit muscle myosin (M), heavy meromyosin (HMM) and light meromyosin (LMM) with the amphiphiles sodium linoleate (L) and sodium n-dodecyl-sulphate (SDS) in aqueous solutions has been investigated by microcalorimetry. At pH 6.4, M and LMM interact endothermically with L whereas for HMM at pH 6.4, and M, HMM and LMM at pH 8.0, the interaction is exothermic. A significant exothermic thermal transition is found at pH 6.4 for the interaction of M with L at a ratio of approximately 0.1 mmol L per mg M which can be associated with the HMM subfragment. The interaction enthalpy for the linoleate–myosin and SDS–myosin systems are not additively related to those for linoleate and SDS interaction with the isolated subfragments, indicating that within the myosin molecule the subfragments do not interact with these amphiphiles independently, particularly at high amphiphile to protein ratios.

INTRODUCTION

Interactions between unsaturated fatty acids and their oxidation products with muscle tissue have been implicated in the deterioration of foodstuffs, particularly seafoods and fishery products, during prolonged storage: there have been numerous studies concerned with the nature of these interactions and their consequences [1–11] as well as studies on model systems [12–14]. The thermal stability of the native conformation of myosin (M), its component subfragments light meromyosin (LMM), heavy meromyosin (HMM), and subfragment S1, has been studied by differential scanning calorimetric (DSC) [15 and refs. cited therein], and the thermal stability of subfragment

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S1 has been studied by polyacrylamide gel electrophoresis and related methods [16,17]. The DSC studies have established that myosin undergoes multiple transitions related to discrete and separate regions of the molecule [15]. Recently [11] DSC studies on mixtures of fatty acids (octanoate, decanoate, dodecanoate) and sodium n-dodecyl sulphate (SDS) with bovine myosin have suggested that these amphiphiles increase the thermal stability and give rise to a more ordered aggregation (gelation) on heating.

It is possible to obtain data by DSC on the thermal properties of pre-mixed mixtures of myosin with an interacting amphiphile, but to study the energy of interaction between myosin and an amphiphile a mixing calorimetric measurement is required. Here we have used a microcalorimeter to obtain information on the heat changes associated with the mixing of myosin, HMM and LMM with linoleic acid and SDS over a range of concentrations and pH values.

EXPERIMENTAL

Materials

Linoleic acid (as the sodium salt, product No. L8134), rabbit muscle myosin (product No. M1636), light meromyosin (product No. M9139), and heavy meromyosin (product No. M9014) were obtained from the Sigma Chemical Co. Ltd. Sodium n-dodecyl sulphate (especially pure grade) was obtained from British Drug House Ltd. Three buffer systems were used, two phosphate buffers with a total phosphate concentration of 5 mM (Na_2HPO_4 plus NaH_2PO_4) pH 6.4 and pH 8.0, and a glycine (50 mM)–sodium hydroxide buffer pH 10.0. As well as the buffering ions, each buffer contained 0.6 M KCl and 0.02% (w/v) sodium azide to inhibit bacterial growth. The linoleic acid was dispersed in the required buffers at a concentration of 20 mM using a magnetic stirrer, and the pH was adjusted to the desired value by addition of NaOH. The myosins were dialysed against the required buffer for 24 h using Spectrapor membrane tubing (molecular weight cut-off 6000–8000).

Microcalorimetry

Enthalpy measurements were made with an LKB 10700 batch microcalorimeter which utilizes the twin-vessel principle, each vessel being divided into two compartments [18]. The instrument was operated at 25 °C, and was calibrated electrically. Aliquots of the required myosin and linoleate solutions were placed in the reaction vessel, and aliquots of the corresponding amounts of buffer and linoleate were placed in the reference vessel. The additions were made by weight using 2-ml syringes. On rotation of the

calorimeter the heats of dilution of the linoleate solutions in the reaction and reference vessels are identical. This was confirmed in separate experiments in which linoleate solutions were mixed with buffer in both vessels. The heats of dilution of the myosins under these conditions were negligible. The net heat measured was thus that of the interaction between the myosin and the linoleate. The amounts of myosin and linoleate solution (20 mM) were varied to cover the concentration range ~ 20–200 mmol linoleate per mg myosin

Some measurements were made of the heat of interaction of myosins with SDS at 30 °C using a Beckman 190B twin-cell microcalorimeter [19]. In these experiments 2 ml of myosin solution (initial concentration in the range 0.4–1.5 mg ml^{-1}) were mixed with SDS solution (initial concentration in the range 0–25 mM). As above, the heats of dilution of the SDS solution were balanced out in the reference vessel. The temperature of 30 °C was chosen because potassium n-dodecylsulphate precipitated from the solutions at 25 °C due to the high KCl concentration (0.6 M) in the buffer (pH 6.4).

Sedimentation analysis

Measurements of sedimentation velocity were made in cells with 12-mm Epon centrepieces with 4° sector angles, by using an AnD rotor in a Spinco model E analytical ultracentrifuge operating at approximately 55,000 rpm at 20 °C. Schlieren optics were used and photographs were taken at 8-min intervals. The myosin concentrations were 1 mg ml^{-1} (pH 6.4) and 2 mg ml^{-1} (pH 8.0).

RESULTS AND DISCUSSION

Myosin exhibits complex behaviour in aqueous solutions and under conditions of low ionic strength and low pH forms polymers and filaments [20,21]. The experiments reported here were all carried out under conditions of high ionic strength ($I > 0.6$) and pH (6.4–10.0) where myosin (M) and its subfragments (HMM and LMM) are monomeric. Sedimentation analysis of M, HMM and LMM gave single schlieren peaks with low S values characteristic of monomeric material [21] as shown in Table 1. Addition of linoleate (L) to HMM and LMM at pH 6.4 and 8.0 resulted in the formation of a precipitate: M plus L only precipitated at pH 6.4. At pH 8.0 the sedimentation coefficient of the M–L complex was measurable and larger than for uncomplexed M. These observations clearly demonstrate an interaction between M (HMM and LMM) and L at pH 6.4 and 8.0.

The enthalpies of interaction between L and the myosins are shown in Figs. 1 and 2 over a ratio of mmol L per mg myosin up to approximately 0.2 (for myosin (molecular weight 480,000 [22]) this ratio corresponds to a molar

TABLE 1

Sedimentation analysis of myosin and myosin subfragments

System	Sedimentation coefficient (S)	
	pH 6.4 [a]	pH 8.0 [b]
Myosin	4.035 ± 0.061	4.089 ± 0.028
HMM	3.680 ± 0.177	4.008 ± 0.030
LMM	1.808 ± 0.026	1.135 ± 0.021
M + linoleic acid (10 mM)	pptd	5.531 ± 0.032

[a] Concentration 1 mg ml^{-1}.
[b] Concentration 2 mg ml^{-1}.

ratio 94×10^3 mol L per mol myosin). There are a number of possible thermal effects which could arise in these systems which could include binding of L to the myosins to form complexes, unfolding (denaturation) of the myosin, aggregation of the complexes and subsequent precipitation and gelation. It has been demonstrated that myosin has hydrophobic surface properties and an affinity for long chain fatty acyl groups [23] and one or two very high affinity sites for the conjugated polyene fatty acid *cis*-parinaric acid [24]. For the range of molar ratios used in Figs. 1 and 2 we are concerned with the binding of fatty acid to large numbers of low affinity sites. In general terms the binding of amphiphiles to proteins is invariably exothermic [25], and this is so for SDS interaction with myosin (see below). It follows that the endothermic interaction enthalpies observed at pH 6.4 for M and LMM most likely arise from unfolding (denaturation) and/or

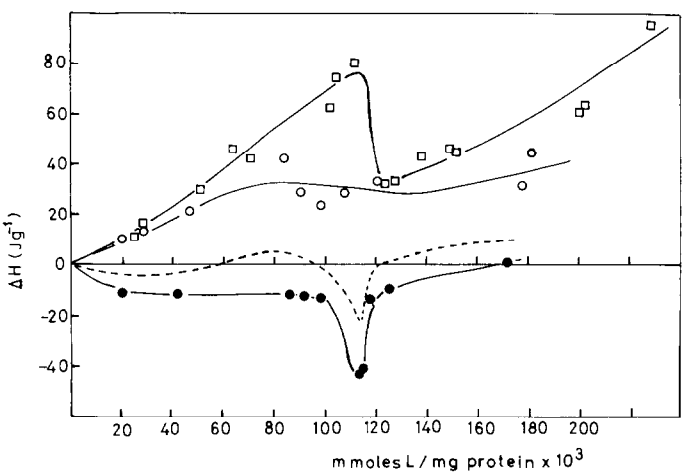

Fig. 1. Enthalpy of interaction of linoleate with myosins in an aqueous solution of pH 6.4 (ionic strength 0.6) at 25 °C. □, myosin; ●, heavy meromyosin; ○, light meromyosin; broken line, calculated from eqn. (1).

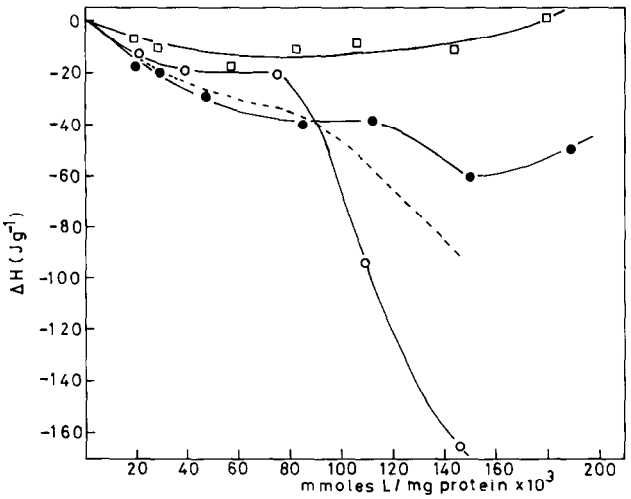

Fig. 2. Enthalpy of interaction of linoleate with myosins in an aqueous solution of pH 8.0 (ionic strength 0.6) at 25 °C. □, myosin; ●, heavy meromyosin; ○, light meromyosin; broken line, calculated from eqn. (1).

association of complexes in solution which precedes aggregation. It should be noted that the heat flux–time curves (thermograms) are obtained within 20–30 min of mixing myosins and L, so that the data refer to the enthalpy of initial interaction. Gel "strengthening" or ordered aggregation is a relatively slow process (~ hours) [11] and would not be detectable in the time scale of our experiments. Thermal denaturation of M and HMM is endothermic and is associated with enthalpy changes of 15–25 J g^{-1} at high ionic strength [15].

It can be seen from Fig. 1 that there is a very significant transition in the curve for M at a ratio of approximately 0.1 mmol L per mg M. This indicates the onset of an exothermic contribution to the interaction enthalpy which is clearly associated with the HMM fragment. It is possible that this exothermic effect arises from the binding of L to hydrophobic sites exposed during a conformational transition in the HMM fragment, if exothermic binding more than compensates for the endothermic unfolding process. The enthalpy of interaction of L with M ΔH_M can be estimated from the enthalpies of interaction of L with HMM ΔH_{HMM} and LMM ΔH_{LMM} according to the equation

$$\Delta H_M = 0.3\Delta H_{LMM} + 0.7\Delta H_{HMM} \qquad \text{J g}^{-1} \tag{1}$$

where the weight fractions were calculated from the molecular weights 480,000 (M), 340,000 (HMM), and 140,000 (LMM) [22]. Comparison of the experimental and calculated curves for M shows that simple additivity does not hold, implying that the HMM and LMM regions of M do not interact independently with L.

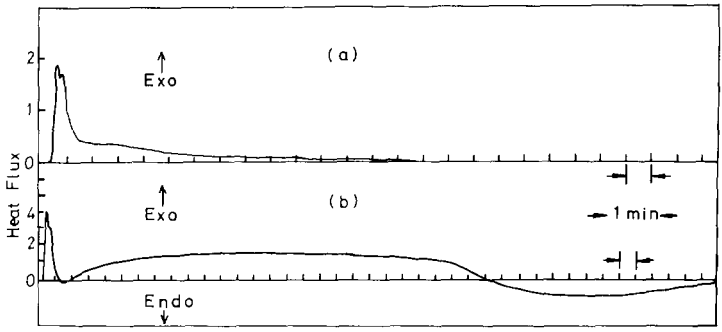

Fig. 3. Thermograms for the interaction of heavy meromyosin (a) and light meromyosin (b) with linoleate, pH 8.0 (ionic strength 0.6) at 25 ° C. (a) 0.112 mmol L per mg HMM; (b) 0.109 mmol L per mg LMM. Note the scale difference in heat flux (given in arbitrary units) between (a) and (b).

At pH 8.0 L interacts exothermically with M, HMM and LMM (Fig. 2), suggesting that the dominant contribution is binding. Here binding to HMM and LMM is more exothermic than binding to M and, as at pH 6.4, there is a marked deviation between the experimental results for M and those predicted from eqn. (1). Above a ratio of 0.08 mmol L per mg M the character of the thermograms changed significantly. Figure 3 shows a comparison between thermograms for HMM and LMM at a ratio of approximately 0.1 mmol L per mg. The thermograms for LMM + L showed a long duration (~ 25 min) exotherm followed by a relatively long duration (~ 15 min) endotherm. It is clear that the processes giving rise to the large net exothermic interaction with LMM must be largely inhibited in the whole myosin molecule by the adjoining HMM subfragment.

At pH 10 interaction between myosin and linoleate was found to be athermal, and this sytem was not studied further.

Figure 4 shows the enthalpy of interaction of SDS with myosins at pH 6.4 as a function of SDS concentration corresponding to ratios up to 0.02–0.03 mmol SDS per mg protein. In contrast to the interaction of L with the myosins at pH 6.4, SDS interacts exothermically with M and LMM. The initial stage in the interaction of SDS with proteins is often the binding of the monomeric anion to cationic residues [25]. At pH 6.4 these will be the lysyl and arginyl residues since the histidyl residues will be largely protonated. Aspartyl and glutamyl residues have an inhibitory effect on SDS binding [26]. The ratio of cationic to anionic residues in the myosins based on the amino acid analysis [22] are 0.62 (M), 0.62 (HMM), and 0.58 (LMM). Although the interaction enthalpy for LMM is lower than for M and HMM, the interaction enthalpy for HMM is larger than for M, suggesting contributions from other sources such as hydrophobic binding. The calculated interaction enthalpy for a M based on eqn. (1) is in agreement with the experimental curve at low SDS concentrations, but clearly deviates at higher

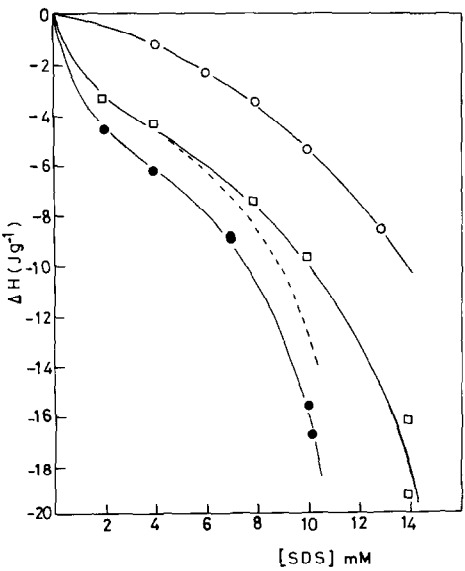

Fig. 4. Enthalpy of interaction of sodium n-dodecyl sulphate with myosins, pH 6.4 (ionic strength 0.6) at 30 °C. □, myosin; ●, heavy meromyosin; ○, light meromyosin; broken line, calculated from eqn. (1).

concentrations as was found for interactions with L. Interestingly, at an ionic strength of 0.6 the SDS will be predominantly in micellar form (the critical micelle concentration < 0.6 mM [27]) and the activity of monomeric SDS will be approximately constant. The increasing exothermicity with increasing SDS concentration thus indicates that micelles are interacting directly with the myosins, since if the interaction was only with monomer the enthalpy would be constant at a constant protein concentration.

CONCLUSIONS

Linoleate interacts with M, HMM and LMM to form complexes at pH 6.4 and 8.0. The enthalpies of interaction between linoleate and the myosins are dependent on pH and the ratio of linoleate to protein.

At pH 6.4 there is a significant transition for the M plus linoleate system which is related to interaction with the HMM fragment.

The interaction enthalpy for the linoleate–M system is not additively related to that for linoleate plus the subfragments suggesting that the subfragments do not interact independently with linoleate within the myosin molecule, particularly at high linoleate to protein ratios. This observation contrasts with thermal denaturation where subfragment unfolding within the whole molecule is discrete.

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REFERENCES

1 I.D. Desai and A.I. Tappel, J. Lipid Res., 4 (1963) 204.
2 K. Takama, K. Zamma and H. Igarashi, Nippon Suisan Gakkaishi, 38 (1972) 607.
3 R.J. Braddock and L.R. Dugan, J. Am. Oil. Chem. Soc., 50 (1973) 343.
4 S.Y.K. Shenouda and G.M. Pigott, J. Food Sci., 40 (1975) 520.
5 S.Y.K. Shenouda and G.M. Pigott, J. Food Sci., 40 (1975) 523.
6 L. Jarenback and A. Liljemark, J. Food Tech., 10 (1975) 437.
7 J. Pokorny, Riv. Ital. Sostanze Grasse, 54 (1977) 389.
8 H.W. Gardner, J. Agric. Food Chem., 27 (1979) 220.
9 L. Stodolnik and M. Knasiak, Int. J. Refrig., 7 (1984) 291.
10 S.D. Rao, Indian J. Biochem. Biophys., 21 (1984) 278.
11 B. Egelandsdal, K. Fretheim and O. Harbitz, J. Food Sci., 50 (1985) 1399.
12 S. Matsushita, J. Agric. Food Chem., 23 (1975) 150.
13 M. Karel, K. Schaich and R.B. Roy, J. Agric. Food Chem., 23 (1975) 159.
14 Z. Nakhost and M. Karel, J. Food Sci., 48 (1983) 1335.
15 D.J. Wright and P. Wilding, J. Sci. Food Agric., 35 (1984) 357.
16 A. Setton and Muhlrad, Arch. Biochem. Biophys., 235 (1984) 411.
17 M. Burke, S. Zaager and J. Bliss, Biochemistry, 26 (1987) 1492.
18 I. Wadso, Acta Chem. Scand., 22 (1968) 927.
19 T.H. Benzinger and R. Hems, Proc. Natl. Acad. Sci. U.S.A., 42 (1956) 896.
20 H.E. Huxley, J. Mol. Biol., 7 (1963) 281.
21 R. Josephs and W.F. Harrington. Biochemistry, 5 (1966) 3474.
22 S. Lowey, H.S. Slayter, A.G. Weeds and H. Baker, J. Mol. Biol., 42 (1967) 1.
23 G. Pineav, A. Tartakovsky, V.P. Shanbhay, G. Johansson and L. Backman, Mol. Cell. Biochem., 48 (1982) 65.
24 J. Borejdo, Biochemistry, 22 (1983) 1182.
25 M.I. Paz-Andrade, M.N. Jones and H.A. Skinner, J. Chem. Soc. Faraday Trans 1, 74 (1978) 2923.
26 M.N. Jones, P. Manley and A.E. Wilkinson, Biochem. J., 203 (1982) 285.
27 P. Mukerjee and K.J. Mysels, in Critical Micelle Concentrations of Aqueous Surfactant Systems, National Bureau of Standards, U.S. Dept. of Commerce, Washington DC, NSRDS-NBS 36, 1971.