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THE ENTHALPY OF INTERACTION OF MALONALDEHYDE WITH AMINO ACIDS AND MYOSIN: EFFECT OF pH

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ABSTRACT

The enthalpies of interaction between malonaldehyde with myosin and heavy meromyosin (HMM) in aqueous solution over a range of pH (3.6–9.0) at 25 °C have been measured as a function of malonaldehyde concentration by microcalorimetry. The enthalpies of interaction are strongly pH dependent and for myosin change sign, becoming exothermic in acid solution at pH 3.6. To aid interpretation of the data the enthalpies of interaction of malonaldehyde with the amino acids arginine, histidine, lysine and methionine have also been measured. It was found that the side chain guanidino and imidazolyl groups of arginine and histidine relative to the other amino acid side chains react very exothermically with malonaldehyde in acid solution (pH 3.6). However, the enthalpies of reaction with the amino acid side chains in the myosins cannot account for the observed enthalpies of interaction with malonaldehyde but the interaction initiates denaturation resulting in absorption of heat. As for thermal denaturation, malonaldehyde-induced denaturation is strongly pH dependent in acid solution.

INTRODUCTION

The reaction of malonaldehyde (propanedial) arising from the auto-oxidation of polyunsaturated fatty acids with proteins leads to adverse effects on food storage [1–5]. Malonaldehyde reacts preferentially with certain amino acid residues, specifically histidine, arginine, tyrosine, methionine and lysine residues in myosin [2]. However, we previously showed that the enthalpies of reaction of malonaldehyde with these amino acid residues in myosin could not account for the relatively large endothermic enthalpy of interaction between malonaldehyde and myosin, but that the reactions probably ini-

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tiated the denaturation of myosin resulting in the observed endothermic enthalpy [6]. It has also been shown that malonaldehyde can introduce intermolecular cross-links between proteins [7,8] although this process may not lead to thermal effects as large as myosin unfolding [9].

Microcalorimetry was found to be a convenient means of monitoring the initial interactions between malonaldehyde and myosin. Previous work, however, was confined to a single pH [6]. In the present study the effect of pH on the interactions has been investigated and it has been found that in acid solution (pH 3.6) the reactions between malonaldehyde and the histidyl and arginyl amino acid residues are sufficiently exothermic to dominate the enthalpy of interaction between malonaldehyde and myosin.

EXPERIMENTAL

Materials

Malonaldehyde was freshly prepared by acid-catalysed hydrolysis of 1,1,3,3-tetraethoxypropane as previously described [6]. Rabbit muscle myosin (product no. M1636) and heavy meromyosin (product no. M9014) were from Sigma Chemical Co. Ltd. They were dialysed for 24 h against the required buffer using Spectrapor membrane tubing (molecular weight cut-off 6000–8000). Three buffer systems were used; a phosphate buffer (pH 6.8, 0.45 M KCl) as previously described [6]; a glycine–HCl buffer (0.05 M glycine, 0.45 M KCl, pH 3.6) and a glycine–NaOH buffer (0.05 M glycine, 0.45 M KCl, pH 9.0). Although it has been reported that glycine may react with malonaldehyde the reaction is reversible and no evidence was found that this reaction occured to any significant extent under the conditions used here [2,10]. The amino acids L-arginine (A-5006), L-histidine (H-8000) and L-methionine (M-9625) were from Sigma. L-lysine monohydrochloride was from British Drug Houses.

Microcalorimetry

Enthalpy measurements were made at 25 °C with an LKB 10700 batch microcalorimeter calibrated electrically and operated as previously described [6].

RESULTS

Figures 1 and 2 show the enthalpies of interaction of myosin and heavy meromyosin (HMM) at 25 °C as a function of malonaldehyde concentration at pH 3.6, 6.8 and 9.0. It should be noted that these data were collected

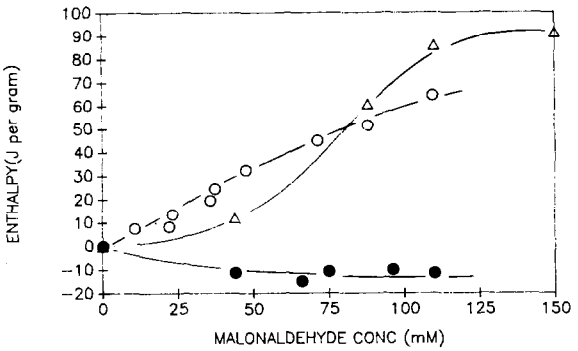

Fig. 1. Enthalpy of interaction between myosin and malonaldehyde in aqueous solution at 25 °C. The pH and myosin concentrations were: ●, pH 3.6 (0.26 μM); ○, pH 6.8 (0.26 μM); Δ, pH 9.0 (0.68 μM). The data at pH 6.8 were taken from ref. 6.

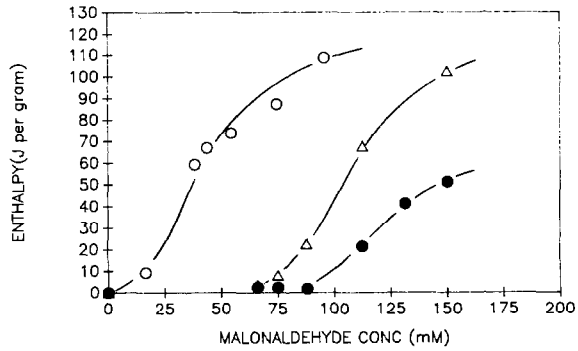

Fig. 2. Enthalpy of interaction between heavy meromyosin and malonaldehyde in aqueous solution at 25 °C. The pH and heavy meromyosin concentrations were: ●, pH 3.6 (2.66 μM); ○, pH 6.8 (0.83 μM); Δ, pH 9.0 (2.66 μM). The data at pH 6.8 were taken from ref. 6.

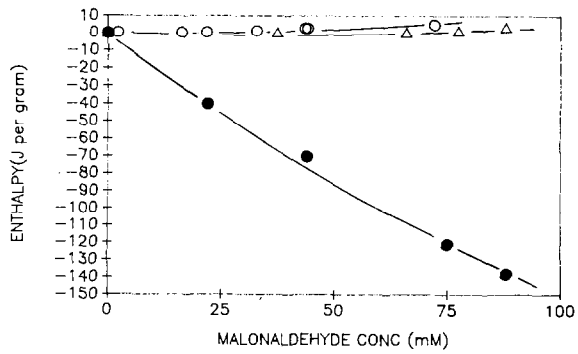

Fig. 3. Enthalpy of interaction between arginine and malonaldehyde in aqueous solution at 25 °C. The pH and arginine concentrations were: ●, pH 3.6 (6.72 mM); ○, pH 6.8 (7.20 mM); Δ, pH 9.0 (18.9 mM). The data at pH 6.8 were taken from ref. 6.

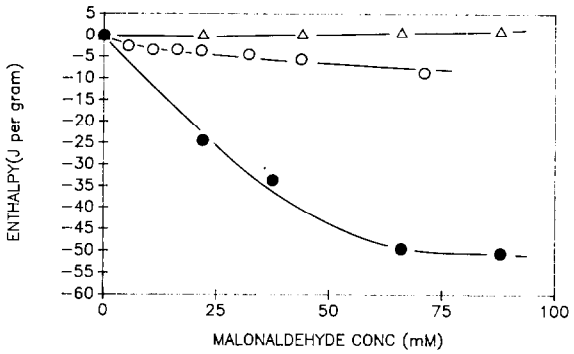

Fig. 4. Enthalpy of interaction between histidine and malonaldehyde in aqueous solution at 25 °C. The pH and histidine concentrations were: ●, pH 3.6 (11.0 mM); ○ pH 6.8 (10.5 mM); △, pH 9.0 (13.2 mM). The data at pH 6.8 were taken from ref. 6.

within a period of 30–40 min after mixing and thus relate to the initial interaction. The thermograms were of a conventional form, came back to the baseline within this time period and gave no indication of a continuing reaction. Although further reaction may occur it could only be proceeding at a very slow rate and was not detectable within the time scale of the experiments. For both myosin and HMM the enthalpy curves approach limiting values at high malonaldehyde concentration suggesting that at least the initial reactions (processes) had gone to completion.

Figures 3–6 show the enthalpies of interaction between malonaldehyde and the amino acids arginine, histidine, lysine and methionine as a function of malonaldehyde concentration at pH 3.6, 6.8 and 9.0. In very marked contrast to all the other systems the enthalpies of interaction of arginine and histidine with malonaldehyde in acid solution (pH 3.6) are large and exothermic (Figs. 3 and 4). The reaction with histidine is also exothermic at

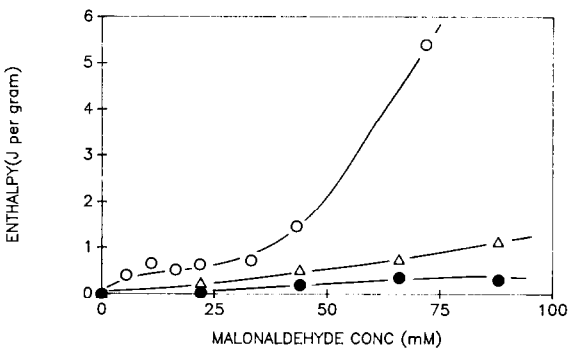

Fig. 5. Enthalpy of interaction between lysine and malonaldehyde in aqueous solution at 25 °C. The pH and lysine concentrations were: ●, pH 3.6 (27.9 mM); ○, pH 6.8 (34.2 mM); △, pH 9.0 (25.5 mM). The data at pH 6.8 were taken from ref. 6.

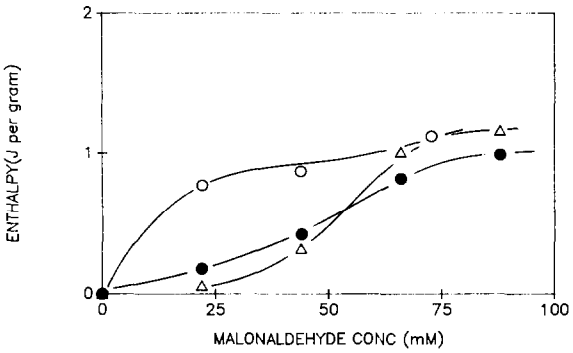

Fig. 6. Enthalpy of interaction between methionine and malonaldehyde in aqueous solution at 25 °C. The pH and methionine concentrations were: ●, pH 3.6 (33.6 mM); ○, pH 6.8 (33.5 mM); △, pH 9.0 (33.2 mM).

pH 6.8 but the enthalpy is not as large as at pH 3.6. Both lysine and methionine interact endothermically with malonaldehyde in the pH range 3.6–9.0 but the enthalpies are relatively small.

DISCUSSION

The enthalpies of interaction of malonaldehyde with both myosin and heavy meromyosin (HMM) are pH dependent. For myosin the interaction becomes exothermic in acid solution (pH 3.6) and for HMM considerably less endothermic (Figs. 1 and 2). It is clear from the enthalpies of interaction of malonaldehyde with arginine and histidine that reaction with these amino acids will contribute to the increasing exothermicity (or decreasing endothermicity in the case of HMM) of the malonaldehyde interaction with myosin and HMM. A comparison between the enthalpies of malonaldehyde interaction of, for example, methionine with those of arginine and histidine shows that the α NH_2 and carboxyl groups of the amino acids must make an almost negligible contribution to the enthalpy of interaction in the pH range 3.6–9.0 so that it is the reactions with the side chain guanidino and imidazolyl groups in arginine and histidine respectively which are the sites of the observed exothermic reactions.

The enthalpies of reaction of malonaldehyde with the amino acids can be used to estimate the contributions made by the reactive side chains of the residues to the overall enthalpy of interaction of malonaldehyde with myosin. The data of Fig. 1 show that the enthalpies approach limiting values at high malonaldehyde concentration. These limiting values can be compared with estimates of the sum of the contributions made by each amino acid residue on reaction with malonaldehyde calculated from the amino acid

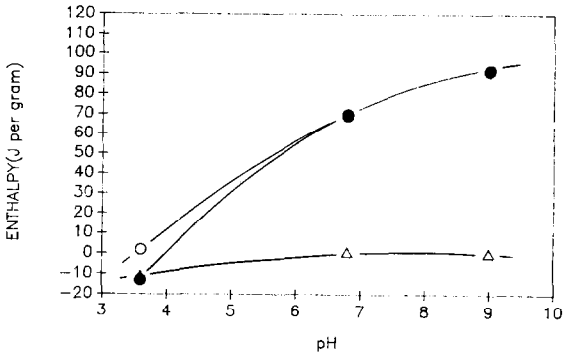

Fig. 7. Contributions to the enthalpies of interaction of malonaldehyde at limiting concentration with myosin in aqueous solution at 25 °C as a function of pH: ●, total enthalpy; △, enthalpy contribution of amino acid side chains; ○, enthalpy of denaturation.

composition of myosin [11] according to the equation

$$\Delta H_{aa} = \sum_i w_i \, \Delta H_i \tag{1}$$

where w_i and ΔH_i are the weight of amino acid i per gram of myosin and the limiting enthalpy of interaction (J g^{-1}) respectively. For arginine at pH 3.6 and lysine at pH 6.8, the curves in Figs. 3 and 5 do not reach limiting values at high malonaldehyde concentration so the values at the highest concentration were used. This procedure could lead to underestimates for the contributions although the highest malonaldehyde concentrations are comparable to the concentrations at which the interactions saturate in myosin (Fig. 1). The results of this approach are shown for myosin and HMM in Figs. 7 and 8 respectively. It should be noted that the possible contributions from the interaction of malonaldehyde with tyrosyl residues

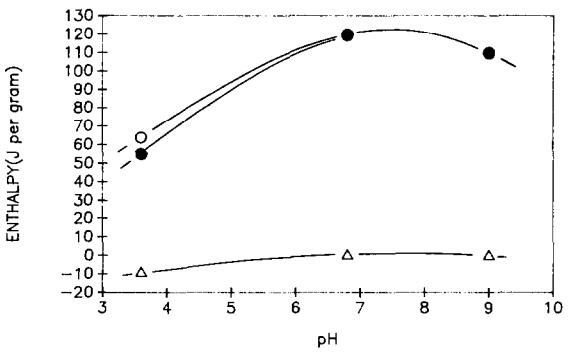

Fig. 8. Contributions of the enthalpies of interactions of malonaldehyde at limiting concentrations with heavy meromyosin in aqueous solution at 25 °C as a function of pH: ●, total enthalpy: △, enthalpy contribution of amino acid side chains; ○, enthalpy of denaturation.

could not be included because no data are available for the tyrosine reaction owing to its limited solubility. However, the tyrosyl content of myosin and HMM are only small, 3.3% and 3.4% by weight respectively, and it is unlikely that contributions from this source would change the results significantly.

At pH 6.8 and 9.0, the enthalpy contributions from the interactions between malonaldehyde and the amino acid side chains make almost negligible contributions to the overall enthalpy of the interaction with myosin. As previously discussed [6] the endothermic enthalpies of malonaldehyde–myosin/HMM interaction most likely arise from unfolding (denaturation) and/or aggregation initiated by reaction of malonaldehyde. The enthalpy of thermal denaturation of myosin is pH dependent and at 7.0 has a value of approximately 18 J g^{-1} at high ionic strength [9]. If the overall enthalpy of interaction of the myosins with malonaldehyde is written in terms of ΔH_{aa} and the enthalpy of denaturation (ΔH_d)

$$\Delta H = \Delta H_{aa} + \Delta H_d \tag{2}$$

the effect of pH on ΔH_d can be obtained from the measured value of ΔH and the estimated ΔH_{aa} value. The curves for ΔH_d are shown in Figs. 7 and 8 for myosin and HMM respectively. It follows that for both myosins the pH dependence of the enthalpies of the malonaldehyde reactions with the amino acid side chains make only a small contribution to the decreasing endothermicity of the overall enthalpy with decreasing pH. Hence it follows that ΔH_d is strongly pH dependent. This result is qualitatively consistent with the enthalpy of thermal denaturation of myosin which decreases from ~ 18 J g^{-1} at pH 7.0 to ~ 12 J g^{-1} at pH 5.5 (i.e. 33%). From Fig. 7, it can be seen that ΔH_d for myosin decreases from 75 to 45 J g^{-1} (i.e. 40%) over the same pH range.

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