Regulation of skeletal-muscle AMP deaminase

Evidence for a highly pH-dependent inhibition by ATP of the homogeneous derivative of the rabbit enzyme yielded by limited proteolysis

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Limited proteolysis of rabbit skeletal-muscle AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) with trypsin results in conversion of the enzyme into a species which over the pH range 6.5–7.1 exhibits hyperbolic kinetics at low K^+ concentration even in the absence of ADP, but shows a 20% decrease in activity at saturating substrate concentration. Analysis by sedimentation-equilibrium techniques reveals the proteolysed enzyme to be homogeneous and to have a molecular mass of 222 000 Da, indicative of a trimeric structure with a subunit molecular mass of 72 000 Da, in contrast with the tetrameric structure of the native enzyme, composed of four 79 000-Da subunits. These observations suggest a role of the 7000-Da fragment which is removed by proteolysis in the maintenance of the three-dimensional structure of the subunit that causes the enzyme at low K^+ concentration to show homotropic positive co-operativity. Study of the influence of pH, isolated from that of K^+ , on the kinetics of AMP deaminase reveals a highly pH-dependent inhibitory effect by ATP which is completely absent at acid pH values and abruptly manifests itself just above neutrality. This phenomenon may have significance in the metabolism of exercising muscle, in connection with the pH-dependent interaction of AMP deaminase with the thick filament.

INTRODUCTION

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) is an oligomeric enzyme which is thought to play an important role in stabilizing the adenylate energy charge in many tissues (Chapman & Atkinson, 1973). The activity of skeletal-muscle AMP deaminase, which is very high in comparison with that of all other tissues, is sensitive to a number of physiologically relevant metabolites: nucleoside di- and tri-phosphates, inorganic and organic phosphate compounds (Ronca-Testoni *et al.*, 1970a; Zielke & Suelter, 1971a). Analysis of the kinetic parameters of the enzyme in the presence of its effectors is very complex because of the dependence of the enzymic activity on KCl concentration and pH of the assay medium. In the absence of K^+ the enzyme follows sigmoidal kinetics, which revert to hyperbolic kinetics on addition of 50–100 mM-KCl (Smiley & Suelter, 1967; Ronca *et al.*, 1972). This behaviour is observed at the optimal pH of 6.5, but not at pH 7.1, where the substrate-velocity curve is always sigmoidal, regardless of the KCl concentration (Ranieri-Raggi *et al.*, 1980). At alkaline pH the stimulation by nucleoside diphosphates, as well as the inhibition by nucleoside triphosphates, is strongly increased (Ronca-Testoni *et al.*, 1970a; Wheeler & Lowenstein, 1979). Furthermore, at acidic and basic pH two qualitatively distinct actions, namely induction of negative co-operativity and enhancement of positive co-operativity respectively, are observed in the modulation by ATP, which presumably is the most important physiological effector of the enzyme (Ranieri-Raggi *et al.*, 1980).

Structural studies on skeletal-muscle AMP deaminase have suggested that a subunit dissociation phenomenon is at the basis of the positive co-operativity shown by the enzyme at low K^+ concentration (Ashman & Atwell, 1972; Ranieri-Raggi & Raggi, 1984), whereas allosteric kinetics at basic pH are probably produced by a deprotonation-induced isomerization process that enhances the reactivity of thiol groups and the rate of sedimentation of the associated form of the enzyme (Ranieri-Raggi & Raggi, 1984) and also causes its cold-lability (Ranieri-Raggi & Raggi, 1983). It has also been shown that the extent of interaction of AMP deaminase with heavy meromyosin is decreased by

alkalinization (Barshop & Frieden, 1984). In a previous paper (Ranieri-Raggi & Raggi, 1979) we have shown that limited proteolysis with trypsin removes from rabbit skeletal-muscle AMP deaminase a 7000-Da fragment that is not required for catalytic activity but has a regulatory function, since in its absence the enzyme at optimal pH is no longer sensitive to adenine nucleotides and exhibits hyperbolic kinetics even at low K^+ concentration.

In the present paper we give evidence that at alkaline pH the proteolysed enzyme retains the desensitization towards stimulation by K^+ , thus allowing us to separate effects of K^+ and pH on the regulatory properties of the enzyme. We show that the pH transition 6.5 to 7.1 gives the enzyme the sensitivity to ATP inhibition. The significance that this pH-dependence may have in the metabolism of exercising muscle is discussed.

MATERIALS AND METHODS

Trypsin and nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); other chemicals were of analytical grade.

AMP deaminase was prepared as described previously (Ranieri-Raggi & Raggi, 1979) from fresh muscle dissected from the back and hind leg of rabbits. The specific activity of the enzyme was 1200 μ mol of AMP deaminated/min per mg of protein in 50 mM-imidazole/HCl (pH 6.5)/60 mM-KCl/2 mM-AMP at 20 °C. Since rabbit muscle AMP deaminase undergoes progressive fragmentation with storage, changing the kinetic properties of the enzyme (Ranieri-Raggi & Raggi, 1980), the kinetic studies with the native enzyme were performed in the first few days after the preparation. Quite similar results were obtained with different fresh preparations. Limited proteolysis of AMP deaminase with trypsin and separation of the proteolysed protein from trypsin and by-products were carried out as described by Ranieri-Raggi *et al.* (1985). The specific activity of the digested enzyme was 1000 μ mol of AMP deaminated/min per mg of protein when assayed in 50 mM-imidazole/HCl (pH 6.5)/60 mM-KCl/2 mM-AMP at 20 °C. The trypsin-treated enzyme from

several different preparations of AMP deaminase showed quite reproducible kinetic properties even when the analyses were performed after several weeks of storage at 4 °C. The AMP deaminase activity was determined spectrophotometrically at 265 or 285 nm; $\Delta\epsilon_{\text{mm}}$ values of 8.86 and 0.23 were used at 265 and 285 nm respectively for the calculation of the μmol of substrate deaminated (Ronca *et al.*, 1968). Spectrophotometric determinations were carried out in a Zeiss PMQ II spectrophotometer equipped with a Zeiss TE-converter and a Varian G-2000 recorder.

Electrophoresis in the presence of 0.1 % SDS at pH 8.3 was carried out in 8%-polyacrylamide slab gels as previously described (Ranieri-Raggi & Raggi, 1979).

Analytical ultracentrifugation was performed in a Beckman-Spinco model E ultracentrifuge equipped with an electronic speed-control unit and a temperature-control unit. Sedimentation-equilibrium experiments were performed at 20 °C in 10 mm cells equipped with Yphantis-style centrepieces. Equilibrium was achieved by centrifugation at 9341 rev./min for 24 h. Molecular masses were determined by the meniscus-depletion method of Yphantis (1964). Rayleigh interference fringes were analysed on a Nikon model 6 Shadowgraph micro-comparator. The enzyme solutions were dialysed for 72 h at 4 °C against 1 M-KCl adjusted to pH 7.0 with 1 M- K_2HPO_4 before ultracentrifugation. A density of 1.045 g/ml for 1 M-KCl was used. The partial specific volume of AMP deaminase was taken as 0.731 (Wolfenden *et al.*, 1968).

RESULTS

Dependence of native and trypsin-treated AMP deaminases on pH and KCl concentration

As shown for rat skeletal-muscle AMP deaminase (Ranieri-Raggi *et al.*, 1980), the activity of the native rabbit enzyme at substrate concentration lower than K_m (0.4 mM-AMP at pH 6.5; Zielke & Suelter, 1971a) is strongly influenced by the H^+ concentration. This is shown in Fig. 1, where the AMP deaminase activity at 0.1 mM-AMP is plotted against KCl concentration at pH 6.5 and 7.1. The increase in activity of native rabbit skeletal-

Fig. 1. Stimulation of native and trypsin-treated AMP deaminases by increasing KCl concentration at pH 6.5 and 7.1

Activity was measured in the presence of 50 mM-imidazole/HCl, 0.1 mM-AMP and the indicated KCl concentrations at pH 6.5 (○, ●) or 7.1 (△, ▲); ●, ▲, native enzyme; ○, △, trypsin-treated enzyme.

muscle AMP deaminase with increasing KCl concentrations shows a different nature at the two pH values, being hyperbolic at pH 6.5 and sigmoid at pH 7.1. When the dependence on KCl concentration was examined in the enzyme that had been treated with trypsin as described in the Materials and methods section, hyperbolic increases in activity were obtained at both pH values, although a significantly higher concentration (50 mM) was required for maximal stimulation at pH 7.1 than at pH 6.5 (10 mM). However, the K_A for K^+ stimulation, calculated from plots of $[\text{K}^+]/v$ against $[\text{K}^+]$, was shown to be 1 mM and 2 mM at pH 6.5 and pH 7.1 respectively for the proteolysed enzyme, compared with that of 12 mM calculated for the native enzyme at pH 6.5. It should be noted that, as reported for rat skeletal-muscle AMP deaminase (Ranieri-Raggi *et al.*, 1980), high salt concentrations inhibit the trypsin-treated as well as the native rabbit enzyme at pH 6.5, but considerably less at alkaline pH. It has been shown that the inhibition of AMP deaminase by high salt concentrations is due to the anionic moiety of the salt (Ronca *et al.*, 1972).

Effect of pH on the modulation of native and trypsin-treated AMP deaminases by nucleoside triphosphates

On the basis of the results reported above, our comparative study of the influence of pH on the regulatory properties of native and trypsin-treated AMP deaminases has been carried out in the presence of either low or high KCl concentrations in order to ascertain the role of the stimulating ion K^+ in the pH-dependence of the enzyme activity. The effect of pH on the modulation by 1 μM nucleoside triphosphates of the enzyme activity at 3 mM-KCl is shown in Fig. 2. In a previous paper (Ranieri-Raggi & Raggi, 1979) we reported that, after limited proteolysis with trypsin, rabbit skeletal-muscle AMP deaminase at optimal pH 6.5 and 3 mM-KCl showed a loss of sensitivity towards ATP, whereas the inhibitory effect of GTP was unmodified. Fig. 2 shows that the effect of GTP at the various pH values on the digested enzyme is very similar to that shown with the native enzyme. A clear pH-dependence of the inhibitory effect of ATP on the native enzyme is apparent from Fig. 2, in agreement with the observations reported previously (Ronca-Testoni *et al.*, 1970a; Wheeler & Lowenstein, 1979; Ranieri-

Fig. 2. Sensitivity of native and trypsin-treated AMP deaminases to nucleoside triphosphates at different pH values

Assays were performed in the presence of 50 mM-imidazole/HCl at the indicated pH values, 0.1 mM-AMP and 3 mM-KCl with the addition of 1 μM -ATP (○, ●) or -GTP (△, ▲). The ordinate shows the activity in the presence of the effector relative to that in its absence at each pH value considered. ●, ▲, Native enzyme; ○, △, trypsin-treated enzyme.

Fig. 3. Influence of pH and KCl concentration on the modulation of trypsin-treated AMP deaminase by ATP

Assays were performed at pH 6.5 (○, □) or pH 7.1 (●, ■) in the presence of 50 mM-imidazole/HCl, 0.1 mM-AMP and either 3 mM- (○, ●) or 100 mM- (□, ■) KCl.

Fig. 4. Substrate-versus-velocity curves of native (●) and trypsin-treated (○) AMP deaminases

The reaction mixture contained 50 mM-imidazole/HCl (pH 7.1), the reported AMP concentrations and 100 mM-KCl.

Raggi *et al.*, 1980). With the trypsin-treated enzyme, the effect of ATP is completely absent when the activity is assayed at acid pH values, but it manifests itself abruptly just above neutrality, with a degree of inhibition similar to that shown with the native enzyme.

The striking influence of pH on the modulation of the trypsin-treated enzyme by ATP is further clarified by Fig. 3, which presents the effect of ATP on the enzyme at pH 6.5 and 7.1 in the presence of either low or high KCl concentrations. The results concerning the dependence on ATP at pH 6.5 show an almost complete desensitization of the proteolysed enzyme towards the nucleotide. Under the same conditions, ATP exerts a biphasic effect on native skeletal-muscle AMP deaminase; $< 5 \mu\text{M}$ -ATP acts as an inhibitor, whereas $> 5 \mu\text{M}$ -ATP stimulates the enzyme, the extent of the stimulation being strongly decreased at optimal KCl concentrations (Ronca *et al.*, 1972; Raggi & Ranieri-Raggi, 1979; Ranieri-Raggi & Raggi, 1979). The data in Fig. 3 demonstrate that, in contrast with the observation made at pH 6.5, ATP exerts on proteolysed AMP deaminase at pH 7.1 a pronounced influence which is similar to that observed with the native enzyme (results not shown). At 3 mM-KCl the enzyme shows a biphasic modulatory response that is not observed at 100 mM-KCl, where increasing ATP concentrations provoke only an increase in enzyme inhibition up to a limited extent.

Effect of pH on the kinetics of native and trypsin-treated AMP deaminases in the presence of ATP

As previously reported, in the presence of 100 mM-KCl native and proteolysed AMP deaminases display hyperbolic saturation kinetics at pH 6.5 (Ranieri-Raggi & Raggi, 1979). Nevertheless, the two forms show distinct behaviours at pH 7.1 (Fig. 4). As shown for rat skeletal-muscle AMP deaminase (Ranieri-Raggi *et al.*, 1980) the native rabbit enzyme follows sigmoid kinetics (Hill coefficient, $h = 1.9$) at the alkaline pH even in the presence of high KCl concentration. In the same conditions, the sigmoidicity of the substrate-saturation curve of the trypsin-treated enzyme is clearly decreased ($h = 1.3$). The substrate-saturation plot of the proteolysed enzyme at pH 7.1 and at KCl concentration as low as 3 mM showed $h = 1.4$ (results not shown).

The above results indicate that, in contrast with the observation made with the native enzyme, alkalization has no effect on the

Fig. 5. Effect of ATP on the kinetics of trypsin-treated AMP deaminase at pH 6.5 and 7.1

Substrate saturation plots were determined in a reaction mixture containing 50 mM-imidazole/HCl, 100 mM-KCl and the reported AMP concentrations either in the absence (○, ●) or in the presence of $50 \mu\text{M}$ -ATP (△, ▲), with assays at pH 6.5 (○, △) and 7.1 (●, ▲).

kinetics of the trypsin-treated enzyme. However, study of the influence of ATP on the kinetics of the proteolysed enzyme (Fig. 5) reveals that at pH 7.1 the inhibition caused by the nucleotide at low substrate concentration induces sigmoid kinetics in the enzyme ($h = 2.1$), whereas at pH 6.5 the hyperbolic substrate-saturation plots obtained in the presence and absence of ATP are almost superimposable. Different from what was observed with ATP, the effect of GTP was to induce sigmoid kinetics in the proteolysed enzyme at pH 6.5 as well as at pH 7.1 (results not shown). As far as native rabbit skeletal-muscle AMP deaminase is concerned, the effect of both nucleoside triphosphates was to cause the hyperbolic substrate-velocity curve obtained at pH 6.5 in the absence of effectors to revert to sigmoidal curves, whereas

at pH 7.1 they increased the sigmoidicity of the substrate-saturation curve (results not shown).

Effect of limited proteolysis on sedimentation of rabbit skeletal-muscle AMP deaminase

Fig. 4 shows that higher AMP concentrations have a different effect on the activity at pH 7.1 of native and trypsin-treated enzymes, since the $V_{\max}$ of the proteolysed enzyme is 20% lower than that of the native enzyme. A quite similar phenomenon was observed by comparing the substrate-saturation plots of the two enzymes at pH 6.5 in the presence of either low or optimal KCl concentrations (Ranieri-Raggi & Raggi, 1979). This observation can be explained by an effect of limited proteolysis on the aggregation properties that lead to the formation of the fully active tetrameric form of the enzyme. This suggestion is confirmed by the determination of the molecular masses of native and proteolysed AMP deaminase by sedimentation-equilibrium analysis using the meniscus-depletion method of Yphantis (1964). The results of typical experiment are shown in Fig. 6. For the native enzyme the plot of the natural logarithm of concentration, represented by the displacement of Rayleigh interference fringes, versus the square of the radial distance showed a significant curvature, indicating the presence of at least two species. Taking the limiting slopes as representative of the molecular masses of each of the two species, values of 173 000 and 312 000 Da were obtained, which are consistent with the existence of a dimer-tetramer equilibrium, in accordance with a molecular mass of 79 000 Da for the enzyme subunit (Ranieri-Raggi & Raggi, 1979). It must be considered, however, that in all fresh preparations of rabbit skeletal-muscle AMP deaminase a minor contaminant of 73 000 Da is always present, owing to the liability of the enzyme to undergo fragmentation during the purification process (Ranieri-Raggi & Raggi, 1980). Gel electrophoresis in the presence of SDS of the enzyme used for analytical ultracentrifugation revealed that the intensity of the lower-molecular-mass band was approx. 20% of that of the 79 000-Da band. On this basis, the heterogeneity observed with the native enzyme should be interpreted as being due to the presence, in addition to the native tetrameric component, of the proteolysed species at a lower degree of aggregation.

Fig. 6. Effect of limited proteolysis on sedimentation equilibrium of rabbit skeletal-muscle AMP deaminase

Sedimentation was at 20 °C and 9341 rev./min for 24 h. The protein concentration was 0.4 mg/ml in 1 M-KCl (pH 7.0); ●, native enzyme; ○, trypsin-treated enzyme.

Fig. 6 shows that the plot corresponding to the proteolysed enzyme conforms to a straight line, indicative of homogeneity. Calculation of the molecular mass from the data gave a value of 217 000 Da, which is suggestive of limited proteolysis 'freezing' the enzyme in the trimeric conformation state, consistent with a molecular mass of 72 000 Da for the trypsin-truncated subunit (Ranieri-Raggi & Raggi, 1979). The average molecular mass of the trypsin-treated enzyme was found to be $222\,000 \pm 10\,000$ Da, whereas that for the heavier component of fresh preparations of AMP deaminase was $309\,000 \pm 17\,000$ Da.

DISCUSSION

The tetrameric nature of skeletal-muscle AMP deaminase is well documented in the literature. However, the molecular mass of the enzyme from various species ranges from 238 000 to 326 000 Da (Lee, 1957; Currie & Webster, 1962; Wolfenden *et al.*, 1968; Ronca-Testoni *et al.*, 1970b; Zielke & Suelter, 1971b; Ashman & Atwell, 1972; Coffee & Kofke, 1975; Boosman & Chilson, 1976; Ogasawara *et al.*, 1977; Barshop & Frieden, 1984). In the light of the observation that the rabbit enzyme undergoes fragmentation on storage, the 79 000 Da subunit being cleaved to a product of approx. 70 000 Da (Ranieri-Raggi & Raggi, 1980), the different reports on the molecular mass of skeletal-muscle AMP deaminase have been explained by the inherent instability of the enzyme, especially during the prolonged extraction step at room temperature. This view has been confirmed recently by the conversion *in vitro* of the 80 000 Da subunit into a 66 000 Da form of rat skeletal-muscle AMP deaminase observed during incubation of muscle extract at 37 °C (Marquetant *et al.*, 1989).

The observation in the present paper that, after limited proteolysis with trypsin, which degrades the enzyme subunit to a product of 72 000 Da (Ranieri-Raggi & Raggi, 1979), rabbit skeletal-muscle AMP deaminase sediments as a trimer of 222 000 Da, deserves consideration for the useful deductions that can be obtained in an attempt to correlate the kinetic parameters of AMP deaminase with the state of aggregation of the enzyme. In fact, in addition to the loss of the positive homotropic co-operativity effects at low K^+ concentration, the proteolysed enzyme shows a 20% decrease in $V_{\max}$ compared with that of the native enzyme. Interestingly, consequences on the kinetics of AMP deaminase qualitatively similar to those provoked by limited proteolysis were obtained by substitution of 12 non-essential thiol groups of rat skeletal-muscle AMP deaminase with 5,5'-dithiobis-(2-nitrobenzoic acid) (Raggi, 1971; Raggi *et al.*, 1972; Ranieri-Raggi & Raggi, 1984). The observation that at alkaline pH the proteolysed enzyme exhibits a desensitization towards the activating effect of K^+ similar to that observed at optimal pH supports the hypothesis that removal of a regulatory fragment by limited proteolysis causes a conformational change which completely abolishes the subunit dissociation phenomenon which is thought to cause AMP deaminase at low K^+ concentration to show positive co-operativity for substrate (Ashman & Atwell, 1972; Ranieri-Raggi & Raggi, 1984). On this basis, the proteolysed enzyme can be regarded as a simpler species in which the influence of pH is isolated from that of K^+ . This permits explanation of the more clear-cut consequences that the 6.5-to-7.1 pH transition causes in the regulation by ATP of the proteolysed enzyme with respect to that observed with the native enzyme.

A previous paper (Ranieri-Raggi *et al.*, 1985) showed that the sensitivity to ATP at pH 6.5 is restored in trypsin-treated AMP deaminase by interaction with stoichiometric amounts of native skeletal-muscle troponin T or phosphorylated 42-residue N-terminal peptide of troponin T. The suggestion that the formation

of the complex with troponin T caused in AMP deaminase effects diametrically opposite to those exerted by limited proteolysis is strengthened by the observations that, as a consequence of the interaction, the enzyme showed values of $V_{\max}$ and K_A for K^+ stimulation similar to those of the native enzyme. It was concluded that phosphorylated troponin T mimics the regulatory action exerted in the native enzyme by a region particularly susceptible to proteolysis at which adenine nucleotides, but not GTP, bind and influence the K_A for K^+ .

The observation in the present paper that the inhibitory effect of ATP is restored in the proteolysed enzyme also by a pH transition clearly indicates that the fragment which is removed by limited proteolysis is not essential for the binding of ATP, which may occur in another region of the enzyme, but it certainly has a regulatory function giving the enzyme sensitivity towards ATP inhibition even at optimal pH. Altogether, these observations give evidence of the complexity of the modulation of skeletal-muscle AMP deaminase by ATP and permit further distinction of this phenomenon from the inhibitory effect exerted by GTP, which appears to be dependent neither on pH nor on the inclusion in the AMP deaminase structure of the fragment removable by trypsin. This is in agreement with the marked quantitative differences observed in the binding of the two nucleotides to the enzyme. In fact 3.8 binding sites for ATP, but only 2.0 for GTP, were demonstrated in rabbit skeletal-muscle AMP deaminase (Tomozawa & Wolfenden, 1970). Using periodate-oxidized ATP and GTP, we found that 3.2 and 1.9 mol of these reagents respectively bind per mol of the rat skeletal-muscle enzyme (Ranieri-Raggi & Raggi, 1976; Raggi & Ranieri-Raggi, 1979).

The present data do not establish whether ATP binds to one site in each of four identical subunits of rabbit skeletal-muscle AMP deaminase, or to two sites shared by two regulatory subunits also possessing one site for GTP. However, the hypothesis of two binding sites for ATP in the native 80000 Da subunit of rat skeletal-muscle AMP deaminase was suggested by the observation that proteolytic removal of 14000 Da does not eliminate inhibition by nucleoside triphosphates, although this removal does decrease the binding of the enzyme to myosin in the presence of ATP (Marquetant *et al.*, 1989).

The observation reported in the present paper that the inhibition of the enzyme by ATP is clearly regulated by a pH transition over the range measured in exercising muscle (Dawson *et al.*, 1978) deserves consideration in the light of reports that AMP deaminase is a dynamic component of the myofibril of vertebrate skeletal muscle (Ashby & Frieden, 1977; Barshop & Frieden, 1984). AMP deaminase binds to myosin heavy chain, the binding site being localized to the α -helical hinge region of myosin. Formation of the ternary complex between two molecules of AMP deaminase and one molecule of heavy meromyosin is highly pH-dependent, with less complex formed at pH above neutrality. The fraction of AMP deaminase bound to myofibrils increases on electric stimulation of rat skeletal muscle, with concomitant decrease in ATP concentration and increase in ammonia liberation (Shiraki *et al.*, 1981).

Although the physiological role of the association of AMP deaminase with the thick filament remains unclear at present, the regulation of this phenomenon appears to be in close relationship with the modulation of AMP deaminase, acidification of the tissue and falling ATP concentration being factors that may

cause association as well as stimulation of the enzyme during sustained contractile activity. In this context also, the presence in the AMP deaminase structure of a regulatory domain that causes inhibition by ATP at acidic pH deserves attention. This phenomenon can play a role in protecting the muscle against the danger of unrestrained activity of AMP deaminase, which would ultimately lead to depletion of adenine nucleotide stores.

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REFERENCES

- Ashby, B. & Frieden, C. (1977) *J. Biol. Chem.* **252**, 1869–1872
- Ashman, L. K. & Atwell, J. L. (1972) *Biochim. Biophys. Acta* **258**, 618–625
- Barshop, B. A. & Frieden, C. (1984) *J. Biol. Chem.* **259**, 60–66
- Boosman, A. & Chilson, O. P. (1976) *J. Biol. Chem.* **251**, 1847–1852
- Chapman, A. G. & Atkinson, D. E. (1973) *J. Biol. Chem.* **248**, 8309–8312
- Coffee, C. J. & Kofke, W. A. (1975) *J. Biol. Chem.* **250**, 6653–6658
- Currie, R. D. & Webster, H. L. (1962) *Biochim. Biophys. Acta* **64**, 30–40
- Dawson, M. J., Gadian, D. G. & Wilkie, D. R. (1978) *Nature (London)* **274**, 861–866
- Lee, Y. P. (1957) *J. Biol. Chem.* **227**, 993–998
- Marquetant, R., Sabina, R. L. & Holmes, E. W. (1989) *Biochemistry* **28**, 8744–8749
- Ogasawara, N., Goto, H., Yamada, Y. & Yoshino, M. (1977) *Biochem. Biophys. Res. Commun.* **79**, 671–676
- Raggi, A. (1971) AMP deaminasi del muscolo scheletrico, pp. 73–76, Scuola Normale Superiore ed., Pisa
- Raggi, A. & Ranieri-Raggi, M. (1979) *Biochim. Biophys. Acta* **566**, 353–361
- Raggi, A., Ranieri, M., Ronca, G. & Rossi, C. A. (1972) *Biochim. Biophys. Acta* **271**, 102–113
- Ranieri-Raggi, M. & Raggi, A. (1976) *Biochim. Biophys. Acta* **445**, 223–233
- Ranieri-Raggi, M. & Raggi, A. (1979) *FEBS Lett.* **102**, 59–63
- Ranieri-Raggi, M. & Raggi, A. (1980) *Biochem. J.* **189**, 367–368
- Ranieri-Raggi, M. & Raggi, A. (1983) *Biochim. Biophys. Acta* **742**, 623–629
- Ranieri-Raggi, M. & Raggi, A. (1984) *Ital. J. Biochem.* **33**, 155–176
- Ranieri-Raggi, M., Bergamini, C. & Raggi, A. (1980) *Ital. J. Biochem.* **29**, 238–250
- Ranieri-Raggi, M., Moir, A. J. G. & Raggi, A. (1985) *Biochim. Biophys. Acta* **827**, 93–100
- Ronca, G., Raggi, A. & Ronca-Testoni, S. (1968) *Biochim. Biophys. Acta* **167**, 626–629
- Ronca, G., Raggi, A. & Ronca-Testoni, S. (1972) *Ital. J. Biochem.* **21**, 305–321
- Ronca-Testoni, S., Raggi, A. & Ronca, G. (1970a) *Biochim. Biophys. Acta* **198**, 101–112
- Ronca-Testoni, S., Ranieri, M., Raggi, A. & Ronca, G. (1970b) *Ital. J. Biochem.* **19**, 262–276
- Shiraki, H., Miyamoto, S., Matsuda, Y., Momose, E. & Nakagawa, H. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1099–1103
- Smiley, K. L., Jr. & Suelter, C. H. (1967) *J. Biol. Chem.* **242**, 1980–1981
- Tomozawa, Y. & Wolfenden, R. (1970) *Biochemistry* **9**, 3400–3404
- Wheeler, T. J. & Lowenstein, J. M. (1979) *J. Biol. Chem.* **254**, 8994–8999
- Wolfenden, R., Tomozawa, Y. & Bamman, B. (1968) *Biochemistry* **7**, 3965–3970
- Yphantis, D. A. (1964) *Biochemistry* **3**, 279–317
- Zielke, C. L. & Suelter, C. H. (1971a) *Enzymes* 3rd Ed. **4**, 47–78
- Zielke, C. L. & Suelter, C. H. (1971b) *J. Biol. Chem.* **246**, 2179–2186