Effects of storage on activity and subunit structure of rabbit skeletal-muscle AMP deaminase

Maria RANIERI-RAGGI and Antonio RAGGI
Istituto di Chimica Biologica dell' Università di Pisa, 56100 Pisa, Italy

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On storage, AMP deaminase is converted into a form exhibiting hyperbolic kinetics even at low KCl concentration. This effect results from cleavage of the enzyme subunit (mol.wt. 79 000) to a product of similar size to the component of approx. mol.wt. 70 000 present in trace amounts in AMP deaminase just prepared from fresh muscle.

In a previous paper (Ranieri-Raggi & Raggi, 1979) we have shown that gel electrophoresis of rabbit muscle AMP deaminase in the presence of sodium dodecyl sulphate gave rise to a major protein band of mol.wt. 79 000, termed α , and a faint band of mol.wt. 73 000, termed α' . On limited proteolysis of AMP deaminase with trypsin the α -band disappeared and a single band was found, which migrated with a similar mobility to that of the minor contaminant of the native enzyme. After incubation with trypsin, the enzyme exhibited hyperbolic kinetics even at low KCl concentration and in the absence of ADP.

Here we report that an activation of the enzyme similar to that brought about by trypsin and resulting from a limited cleavage of the α -subunit occurs on storage of both the muscle and the purified enzyme.

Materials and methods

AMP deaminase was prepared as previously described (Ranieri-Raggi & Raggi, 1979) from fresh muscle dissected from the back and from the hind leg of the rabbit. Preparations were also made from muscle dissected from the eviscerated skinned carcass stored at 4°C. The specific activity of different preparations of purified enzyme was in the range 1000–1200 μ mol of AMP deaminated/min per mg of protein when assayed in 2 mM-AMP/50 mM-imidazole/HCl (pH 6.5)/60 mM-KCl, at 20°C. The enzyme at a concentration of about 1 mg/ml was stored at 4°C in 1 M-KCl adjusted to pH 7.0 with 1 M-K₂HPO₄. AMP deaminase activity was routinely determined spectrophotometrically at 20°C as described by Ronca *et al.* (1968).

Molecular-weight determination by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was carried out as described by

Weber & Osborn (1969), except that 0.1 M-Tris/0.1 M-Bicine [*NN*-bis-(2-hydroxyethyl)glycine] (pH 8.3) was used as buffer system. The following proteins were used as standards: phosphorylase (mol.wt. 94 000), bovine serum albumin (66 500) and glutamate dehydrogenase (53 000).

Results and discussion

In the presence of 3 mM-KCl and 0.1 mM-substrate, the activity of different preparations of AMP deaminase isolated from fresh skeletal muscle varied little around the value of 20 μ mol of AMP deaminated/min per mg of protein, i.e. about 7% of the activity at 60 mM-KCl (see Table 1 for a typical case). At the low salt concentration the enzyme always showed a sigmoidal substrate-concentration/velocity curve, which reverted to a hyperbolic one on addition of optimal KCl concentrations (50–100 mM).

By contrast with AMP deaminase prepared from fresh muscle, the enzymes isolated from muscle that had been stored at 4°C for some days showed a much higher specific activity at 0.1 mM-AMP in the presence of 3 mM-KCl, whereas that in the presence of 60 mM-KCl was only slightly increased. The effect of storage was thus a decrease in the ratio of the activity in 60 mM-KCl to that in 3 mM-KCl, which was approx. 1 in AMP deaminase prepared from muscle after 7 days storage (Table 1). This enzyme migrated on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate as a single species of approximate mol.wt. 70 000, its mobility being very similar to that of the minor component (α' -band) of AMP deaminase prepared from fresh muscle (Ranieri-Raggi & Raggi, 1979). This observation suggests that on storage AMP deaminase is susceptible to proteolytic attack and that limited cleavage of α -subunit, of mol.wt.

Table 1. *Alteration of kinetic properties of AMP deaminase with time of storage of the muscle and aging of the purified enzyme*

Assays were performed on a 1000-fold dilution of the enzyme in a reaction mixture containing 0.1 mM-AMP, 50 mM-imidazole/HCl (pH 6.5) and the indicated KCl concentration. Preparations were AMP deaminase prepared from fresh muscle (I), or AMP deaminase from muscle stored at 4°C for 3 days (II) or 7 days (III).

Preparation	Specific activity of freshly purified enzyme ($\mu\text{mol/min per mg}$)		Activity ratio (A/B) at the indicated times of storage of the enzyme at 4°C (days)					
	60 mM-KCl	3 mM-KCl						
	(A)	(B)	0	7	11	17	22	28
I	298	22	13.6	5.4	3.8	2.6	1.4	1.0
II	391	200	2.0	1.3	1.4	1.1	1.1	0.9
III	430	451	1.0	0.9	0.8	0.9	0.9	0.9

79 000 (Ranieri-Raggi & Raggi, 1979), was responsible for the activation observed.

This is confirmed by the changes occurring in the kinetic behaviour of AMP deaminase with aging of the purified enzyme at 4°C (Table 1). The enzyme preparations characterized by an activity ratio higher than 1 showed on storage an increase in the activity at 3 mM-KCl until it reached the activity at 60 mM-KCl. The intensity of the α -band simultaneously decreased and that of the α' -band increased. In every case, when the α -to- α' transition was complete, AMP deaminase followed hyperbolic kinetics even at 3 mM-KCl. The electrophoretic and kinetic properties of the enzyme did not vary on further storage, indicating that the α' -subunit was resistant to proteolysis. The observation that the α -to- α' conversion does not cease once the enzyme has been isolated from muscle indicates that the system responsible for the process, possibly a proteinase(s), still acts at the different stages of enzyme purification. This gives a probable explanation of the different reports in the literature on the kinetic properties (Zielke & Suelter, 1971) and the subunit molecular weight of muscle AMP deaminase, ranging from 60 000 to 79 000 (Ashman & Atwell, 1971; Boosman *et al.*, 1971; Scopes & Penny, 1971; Coffee & Kofke, 1975; Ogasawara *et al.*, 1977). In the light of the observations in the present paper, conflicting results have probably arisen because of the failure of the investigators to appreciate the inherent instability of AMP deaminase, especially during the prolonged extraction step at room temperature.

A limited cleavage of the α -subunit was also observed to be a consequence of incubation of AMP deaminase with trypsin, enhancing the activity at low salt concentration (Ranieri-Raggi & Raggi, 1979). This suggests that trypsin, as well as a proteinase(s) present in muscle, removes from AMP deaminase a fragment acting at least partially to hold the enzyme in an inactive conformation.

This could not be considered as a physiological mechanism, since it is irreversible. However, this activation process can be envisaged to be at the basis of the large ammonia accumulation occurring in skeletal muscle subjected to strong tetanic contraction or passing into rigor mortis (Parnas & Mozolowski, 1927; Parnas, 1932; Bendall & Davey, 1957). This suggestion is supported by the recent observation of an irreversible increase of AMP deaminase activity during skeletal-muscle contraction (Rahim *et al.*, 1979).

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