Acta Biochimica Polonica

Vol. 51 No. 1/2004

213–218

QUARTERLY

This paper is dedicated to the memory of late Professor Jerzy Popinigis (1936–2003)

AMP-deaminase from hen stomach smooth muscle — physico-chemical properties of the enzyme

Anna Swieca[1]✉, Iwona Rybakowska[1], Anna Koryziak[2], Jerzy Klimek[2] and Krystian Kaletha[1]

[1]*Department of Biochemistry and* [2]*Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdańsk, Poland*

Received: 23 July, 2003; revised: 20 January, 2004; accepted: 06 February, 2004

Key words: AMP-deaminase, smooth muscle

AMP-deaminase from hen stomach smooth muscle was isolated and physico-chemical properties of the purified enzyme were investigated. The enzyme had an activity optimum at pH 6.5, and poorly deaminated the substrate analogues tested.

At optimum pH (6.5), in the absence of regulatory ligands (control conditions), the enzyme manifested hyperbolic substrate–saturation kinetics with half-saturation constant ($S_{0.5}$) of about 4.5 mM. Additions of adenine nucleotide effectors (ATP, ADP) activated the enzyme strongly at all the concentrations tested, diminishing significantly the value of $S_{0.5}$ constant. In contrast, the regulatory effect of orthophosphate was variable, and depended on the orthophosphate concentration used.

The molecular mass of the enzyme subunit determined in SDS/PAG electrophoresis was about of 37 kDa.

The obtained results suggest that in different types of hen muscle, similarly as in humans and rats, expression of AMP-deaminase is under the control of independent genes.

AMP-deaminase (EC 3.5.4.6; AMP aminohydrolase) is an enzyme catalyzing irreversible deamination of AMP to IMP. The physiological role of the enzyme is not completely eluci-

✪This study was supported financially by the Medical University of Gdansk (grants ST-40, ST-41).

✉Corresponding author: Anna Swieca, Department of Biochemistry, Medical University of Gdansk, Dębinki 1; 80-211 Gdańsk, Poland; tel./fax (48 58) 349 1465; e-mail: aswiec@amg.gda.pl

dated, but it seems to be connected with stabilization of the adenylate energy charge (Chapman & Atkinson, 1973) in the cell.

In human and rat three main isoforms (Ogasawara *et al.,* 1975; 1982) of AMP-deaminase, encoded by separate genes of a multigene family (Sabina *et al.,* 1990) have been identified. In skeletal muscle of these species intensive expression of the highly active isoform M takes place (Sabina *et al.,* 1990), causing the activity of AMP-deaminase in this tissue to be 1–2 order higher (Ogasawara *et al.,* 1975; Makarewicz & Stankiewicz, 1974) than in other tissues, including those of heart (Kaletha & Składanowski, 1979; Kaletha *et al.,* 1979) and uterus (Nagel-Starczynowska *et al.,* 1991).

No data concerning the presence and distribution of AMP-deaminase isoforms in the tissues of birds are available. In skeletal muscle of hen and pigeon (Stankiewicz *et al.,* 1979; Kaletha, 1983; 1988), similarly as in skeletal muscle of human and rat, activity of AMP-deaminase is high, and surpasses manifold that observed in the heart (Kaletha & Składanowski, 1984; Kaletha *et al.,* 1987).

In this paper the physico-chemical properties of AMP-deaminase isolated from hen stomach smooth muscle are presented.

MATERIALS AND METHODS

Preparation of the enzyme. Freshly obtained hen stomachs were washed, mucose layer was removed, weighed and homogenized in 3 vol. (v/w) of extraction buffer (0.089 M phosphate buffer, pH 6.5, containing 0.18 M KCl, 1 mM thioethanol, 1 mM phenylmethulsylfonyl fluoride (PMSF) and 1 μg/ml of trypsin inhibitor), in a Warring blender-type homogenizer. The homogenate was centrifuged twice (first 30 min at 3000 × ***g***, and then 30 min at 18 000 × ***g***), and the supernatant obtained was applied on a phosphocellulose column (2.6 cm × 20 cm), according to the procedure of Smiley *et al.* (1967). The phosphocellulose column was subsequently washed (first with 0.5 l of extraction buffer, then with 0.2 l of 0.4 M KCl, and finally with 0.1 l of 0.75 M KCl), and the enzyme adsorbed on the resin was eluted with linear gradient of 0.75–2.0 M KCl. The most active fractions eluted were pooled, applied on another phosphocellulose column (2.6 cm × 20 cm), washed (first with 0.1 l of 0.4 M KCl, and then with 0.1 l of 0.75 M KCl), and subsequently eluted (at a flow rate of 7 ml/h) with 2.0 M KCl. The most active fractions collected were used for electrophoresis and kinetic experiments.

Enzyme assay. The activity of AMP-deaminase was determined colorimetrically, by estimating production of ammonia, according to the phenol-hypochlorite method of Chaney & Marbach (1961). Incubation medium, in a final volume of 0.5 ml, contained 0.1 M potassium succinate buffer (pH 6.5), 100 mM KCl and different concentrations of the substrate (AMP). After equilibration of the incubation medium at 32°C, 50 μl of enzyme solution was added into the medium to start the reaction. All incubations were carried out for 15 min, and the average amount of ammonia liberated from three parallel incubations was determined. No symptoms of enzyme denaturation were observed as judged from the proportionality of ammonia liberation *versus* time for the period of 15 min.

Protein concentration was determined according to the method of Bradford (1976).

Electrophoresis. Electrophoresis in the presence of 0.4% SDS was carried out on 12% (w/v) polyacrylamide slab gel in a 0.025 M Tris/0.192 M glycine/0.1% SDS, pH 8.3, buffer. Low molecular weight standards (LMW) were used to calibrate the gel. Samples were run in duplicate for Coomassie Brilliant Blue staining.

Measurement of kinetic parameters. The kinetic parameters of the reaction (maximum velocity – V_{max}, half-saturation constant – $S_{0.5}$, and cooperativity coefficient – n_H) were calculated with the aid of a computer pro-

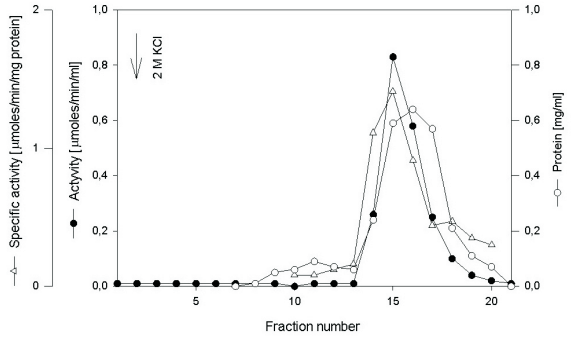

Figure 1. Purification of AMP-deaminase from hen stomach muscle – second chromatography on a phosphocellulose column.

Fractions of 1 ml were collected.

gram, as described previously (Nagel-Starczynowska *et al.*, 1991).

Reagents. 5′-AMP (free acid) and trypsin inhibitor were supplied by Sigma (St. Louis, MO, U.S.A.). PMSF (phenylmethylsulfonylfluoride) was from Boehringer (Mannheim, Germany). Cellulose phosphate was from Whatman (Maidstone, Kent, U.K.). Electrophoretic reagents were supplied by BioRad (Richmond, CA, U.S.A.); LMW standards by Pharmacia (Uppsala, Sweden). All other chemicals were from Polskie Odczynniki Chemiczne (Gliwice, Poland).

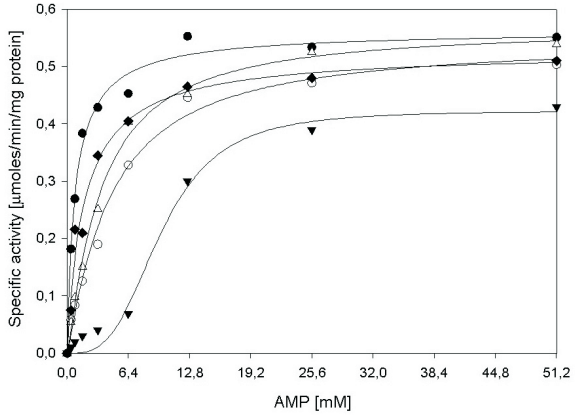

Figure 2. Regulatory effect of ATP, ADP and P_i on AMP-deaminase from hen stomach muscle.

Reaction was measured in a medium containing 0.1 M potassium succinate buffer (pH 6.5) with 100 mM KCl, in the absence (○), or in the presence of 1 mM ATP (●), 1 mM ADP (◆), 0.5 mM P_i (Δ) and 2.5 mM P_i (▼).

RESULTS

Figure 1 illustrates the result of chromatography of AMP-deaminase. The purification procedure used was effective and single symmetrical activity peak, well correlated with the protein released appeared in the eluate after the second phosphocellulose chromatography.

Figure 2 illustrates the regulatory properties of the AMP-deaminase. As can be seen from the figure, all the kinetic curves generated manifested a similar hyperbolic substrate-saturation profile, with the half-saturation constant ($S_{0.5}$) value being the highest (about 5 mM – see Table 1) in the absence of allosteric effectors. One mM ATP and 1 mM ADP activated potently the AMP-deaminase, decreasing the value of $S_{0.5}$ much below the value observed at control conditions (Table 1). In contrast, the regulatory effect of ortophosphate was not uniform and depended on its concentration used. Orthophosphate at 0.5 mM slightly activated the enzyme, but an inhibitory effect was visible when higher concentrations were present. At 2.5 mM orthophosphate the reaction catalysed by the studied AMP-deaminase manifested a clearly sigmoidal substrate–saturation profile, and a significant increase of $S_{0.5}$, correlated with a decrease of V_{max} could be observed in these conditions (Table 1).

Figure 3 illustrates the results of SDS/PAG electrophoresis of AMP-deaminase. A single sharp protein band of approximate molecular mass of 35 kDa was revealed in the result of electrophoresis performed.

Data presented in Table 2 illustrate the substrate specificity of hen stomach muscle AMP-deaminase studied.

DISCUSSION

The specific activity of AMP-deaminase in a homogenate from hen stomach smooth muscle (0.022 μmoles/min per mg of protein) was distinctly lower than that measured in hen

Table 1. Effect of some important ligands on the kinetic parameters of the reaction catalyzed by the AMP-deaminase from hen stomach muscle

Effectors added	$S_{0.5}$ (mM)	V_{max}	n_H
None	4.52	0.55	1.12
1 mM ADP	1.81	0.53	0.96
1 mM ATP	0.85	0.56	0.72
0.5 mM P_i	3.45	0.57	1.16
2.5 mM P_i	9.87	0.42	3.21

Data represent mean of three independent determinations; ±S.D. was within ±10% of the calculated values. Assay conditions: 0.1 M succinate buffer (pH 6.5) with 0.1 M KCl.

skeletal muscle homogenate (Kaletha, 1983). This suggested that different molecular forms of AMP-deaminase in skeletal and stomach muscle of hen may operate. The experimental results presented in this paper seem to support such a suggestion. In fact, the AMP-deaminases isolated from the two types of hen muscle manifested distinctly different kinetic and regulatory properties. In control conditions, when no allosteric effectors were added, the $S_{0.5}$ constant value calculated for AMP-deaminase isolated from hen stomach muscle was relatively high (4.5 mM), and surpassed nearly 30-fold that (0.3 mM) calculated for the enzyme isolated from hen skeletal muscle (Kaletha, 1983). The presumably reason of this difference could be the presence of potassium. However, potassium ions – the most potent activator of skeletal muscle AMP-deaminase (Smiley *et al.*, 1967) does not affect significantly the enzyme isolated from non-skeletal muscle tissues.

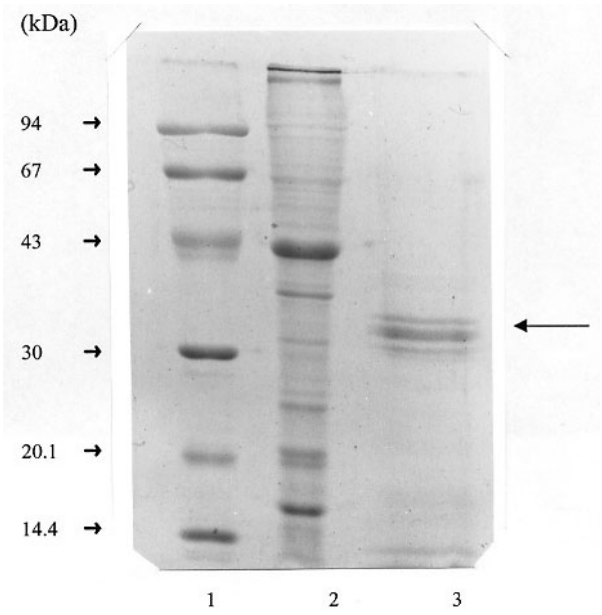

Figure 3. SDS/polyacrylamide gel electrophoresis of AMP-deaminase from hen stomach muscle.

Electrophoresis of muscle homogenate (lane 2) and purified enzyme (lane 3) are presented. Phosphorylase *b* (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsine inhibitor (20.1 kDa) and lactalbumin (14.4 kDa) were used as standards (lane 1).

SDS/PAG electrophoresis (Fig. 3) led to an estimate of the subunit molecular mass of AMP-deaminase studied as close to 35 kDa. This is only half of that assumed for AMP-deaminase subunit isolated from hen skeletal muscle (69 kDa) (Sammons & Chilson, 1978), but is very similar to that 37 kDa determined for AMP-deaminase isolated from human uterine smooth muscle (Nagel-Starczynowska *et al.,* 1991).

Developmental changes in the isoenzyme pattern of AMP-deaminase, mimicking those reported for rat and human enzyme, were observed also in hen skeletal muscle (Sammons & Chilson, 1978), but no data concerning distribution of AMP-deaminase isoenzymes in hen tissues were published. Studies of Ogasawara (1975) demonstrated that multiple molecular forms of AMP-deaminase, expressed by independent genes (Sabina *et al.,*

Table 2. Deamination of some structural analogues of 5′-AMP by AMP-deaminase isolated from adult hen stomach muscle and % of control (values in brackets)

Substrate (10 mM)	Relative velocity of the reaction (% of control)	
5' -AMP (control)	0.9	(100)
cAMP[a]	0.03	(3.6)
dAMP[b]	0.04	(4.5)
2' AMP	0.04	(4.5)
3' AMP	0.04	(4.5)
AMN[c]	0.04	(4.5)
AMPN[d]	0.05	(5.1)
Adenosine	0.05	(5.1)

[a]cAMP, adenosine 3′:5′-cyclic-monophosphoric acid; [b]dAMP, 2′-deoxyadenosine 5′-monophosphate; [c]AMN, adenosine 5′-mononicotinate; [d]AMPN, adenosine 5′-monophosphoramidate. Experimental data represent mean of three independent determinations. ±S.D. was within ±10% of the calculated values. Assays were carried out in 0.1 M succinate buffer, pH 6.5, with 0.1 M KCl.

1990) exist in various tissues of human and rat. The same seems to be true in the case of birds. The experimental data presented here suggest that different genes are responsible also for expression of AMP-deaminase in different types of muscle in hen.

From the evolutionary point of view it is interesting that the properties of AMP-deaminase from smooth hen muscle are so close to those of smooth muscles of mammals (Stankiewicz *et al.*, 1979) including human (Nagel-Starczynowska *et al.*, 1991). This holds also for the molecular mass of the enzyme subunits.

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