GRZEGORZ NOWAK and KRYSTIAN KALETHA

ISOLATION AND REGULATORY MECHANISMS OF SMOOTH MUSCLE AMP-DEAMINASE

Department of Biochemistry, Medical School of Gdańsk, Dębniki 1; 80-211 Gdańsk, Poland

AMP-deaminase from cow uterine smooth muscle has been purified. The enzyme activity is regulated by the two cooperating mechanisms: allosteric and dissociation-association.

AMP-deaminases (EC 3.5.4.6) from skeletal and heart muscle of various species have been isolated and their physico-chemical properties were characterized. That is not the case for the enzyme from smooth muscle. So far, contradictory reports concerning the occurrence of AMP-deaminase in smooth muscle have been published. Purzycka found the activity of this enzyme in stomach smooth muscle [1], whereas Hayashi & Olmsted [2] did not detect its presence in human uterus.

In this report isolation and regulatory properties of AMP-deaminase from cow uterine smooth muscle are presented.

MATERIALS AND METHODS

Isolation of the enzyme. AMP-deaminase from cow uterine smooth muscle was isolated by chromatography on phosphocellulose, essentially according to the procedure of Smiley et al. [3]. The enzyme adsorbed on phosphocellulose was eluted first by 0.75 M KCl, and then by a linear 0.75-2.0 M KCl gradient. The most active fractions of the second activity peak were rechromatographed on phosphocellulose, concentrated and then applied onto Sepharose CL-6B column. The enzyme was eluted with 0.05 M succinate/KOH buffer, pH = 6.5, containing 0.1 M KCl and 1 mM thioethanol.

Enzyme assay. AMP-deaminase activity was determined by measuring ammonia liberated with the use of the phenol-hypochlorite method of Chaney & Marbach [4].
RESULTS AND DISCUSSION

Chromatography on phosphocellulose revealed the existence of two forms of uterine smooth muscle AMP-deaminase differing in kinetic and regulatory properties. The main part (about 95%) of activity (form B) was released from the phosphocellulose column at 1.1 M KCl concentration. Form A of AMP-deaminase was eluted from the column in the 0.75 M KCl fraction. The two enzyme activities displayed different pH-dependence profiles, as well as different substrate-saturation curves; form A exhibited a nearly hyperbolic profile ($S_{0.5} = 3.4$ mM, $h = 1.1$), whereas form B a distinctly sigmoid-shaped one ($S_{0.5} = 6.4$ mM, $h = 1.9$). Apart from this, the two enzyme forms distinctly differed from each other in the magnitude of modulatory effects exerted by ATP, ADP or GTP and orthophosphate.

Gel filtration of the rechromatographed enzyme on Sepharose CL-6B allowed to obtain a highly purified and electrophoretically homogeneous solution of the main enzyme form (form B). The experiments performed on this form of the enzyme revealed that it was an oligomeric protein with the native molecular mass of about 140 kDa, and of subunit close to 37 kDa.

The detailed kinetic experiments interpreted in terms of the concerted transition theory of Monod et al. [5] revealed that the regulation of smooth muscle AMP-deaminase activity consisted of two coexistent and cooperating regulatory mechanisms: allosteric regulation and dissociation-association. The basis of this first one are homo- and heterotropic effects exerted by substrate and allosteric effectors. The strongest allosteric effectors of smooth muscle AMP-deaminase are ATP (activator) and orthophosphate (inhibitor). Both ligands affect the equilibrium existing between the two predicted conformational states of the enzyme, and their effect may be expressed in terms of the change of the allosteric equilibrium constant value ($L$) (Fig. 1). Orthophosphate, independently of its influence on the equilibrium of the

---

![Graph](image-url)

Fig. 1. Linearization of the simplified saturation function [6]. $x$, control curve ($n = 2.8$, $L = 58$) ○, in the presence of 0.5 mM H$_3$PO$_4$ ($n = 4.8$, $L = 151000$) ▽, in the presence of 0.025 mM ATP ($n = 2.8$, $L = 5$)
allosteric transition, induced also a change in enzyme aggregation. This was revealed by the increase of the n parameter from the original value $n \approx 2$ (which corresponds to the functionally dimeric structure of the enzyme) to $n \approx 4$ (corresponding to the functionally tetrameric structure of the enzyme). Apart from this, the degree of enzyme aggregation was influenced also by pH as well as the ionic strength of the medium.

The experiments with enzyme filtration on various gels (Sephadex G-200, Sepharose CL-6B, Sepharose CL-2B) demonstrated the existence (apart from monomeric, inactive form of the enzyme) of at least three active forms of smooth muscle AMP-deaminase, differing both in the aggregation degree and kinetic characteristics. These forms correspond to tetrameric, octameric and polymeric aggregation structures of this protein.

REFERENCES