The extracellular matrix proteins laminin and fibronectin modify the AMPase activity of 5′-nucleotidase from chicken gizzard smooth muscle

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Laminin and fibronectin, but not collagen, affect the AMPase activity of the purified transmembrane protein 5′-nucleotidase. Laminin stimulates whereas fibronectin inhibits the AMPase activity of this ectoenzyme. The AMPase-modulating effects by these components of the extracellular matrix require a preincubation period of several hours when detergent-solubilized 5′-nucleotidase is employed, they can, however, instantaneously be elicited with liposome-incorporated 5′-nucleotidase.

5′-Nucleotidase *Laminin* *Fibronectin* *Actin* *Laminin receptor*

1. INTRODUCTION

The mechanism by which extracellular matrix molecules affect cell functions such as migration, spreading or differentiation is not yet clear. Experimental evidence is available that cells bind to matrix proteins via specific receptors in the plasma membrane. Membrane proteins able to bind extracellular matrix proteins such as laminin [1,2], fibronectin [3] and particular collagen types [4,5] have been isolated from different sources. However, nothing is known about the molecular mechanism by which the extracellular matrix influences cell function.

Here we show that the AMPase activity of 5′-nucleotidase (EC 3.1.3.5) purified from chicken gizzard smooth muscle [6] can be modulated by the extracellular matrix proteins laminin and fibronectin. The enzymic activity of this integral plasma membrane protein [7] is stimulated by laminin by 200–400% and inhibited by fibronectin to more than 50%.

Although the biological function of 5′-nucleotidase is still not fully explained, this ectoenzyme [8] is assumed to be the main producer of adenosine which acts as a local hormone influencing a number of cellular activities [9]. Furthermore, we have recently presented evidence that 5′-nucleotidase is also able to interact with filamentous actin [10,24,25], a component of the intracellular cytoskeleton.

Since both laminin and fibronectin were shown to effect the organisation of the cytoskeleton [11], we tested the ability of 5′-nucleotidase to interact with various components of the extracellular matrix. Here, we report initial results indicating a specific interaction of laminin and fibronectin with purified 5′-nucleotidase. To our knowledge this is the first experimental indication for the regulation of an enzymic activity normally localized on the cell surface by components of the extracellular matrix.

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Abbreviations: AMP, adenosine 5′-monophosphate; DEAE, diethylaminoethyl

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2. MATERIALS AND METHODS

Chickens were obtained from a local farm. Adenosine deaminase was from Boehringer, Mannheim. 5′-AMP-Sepharose was from Pharmacia, München.

2.1. *Protein preparations*

5′-Nucleotidase from chicken gizzard smooth muscle was prepared essentially as described by Dornand et al. [12] with modifications [6]. In principle, after tissue homogenisation the enzyme was solubilized using the zwitterionic detergent 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfate (Chaps) and further purified by 2 consecutive affinity chroamtographic columns using immobilized lens culinaris lectin and 5′-AMP-Sepharose 4B. Final purification was achieved by absorbing impurities to DEAE-cellulose [6]. Purified 5′-nucleotidase was stored in buffer A (5 mM Hepes-NaOH, 0.5 mM NaN_3, 0.1 mM $CaCl_2$) supplemented with 0.1% Triton X-100.

The preparation of laminin and collagen type IV from a transplantable mouse tumor (EHS tumor, sarcoma) has been described [13]. Fibronectin was purified from human plasma following the procedure of [14] with the modifications in [15].

Collagen type I and III were prepared from lathyritic rats by neutral salt extraction followed by fractionated salt precipitation [16–18].

2.2. *Protein determination*

Protein concentration was determined as described [19].

2.3. *Enzyme tests*

AMPase activity was determined spectrophotometrically [20] or by a radioisotopic assay for 5′-nucleotidase [21]. The matrix proteins were dialyzed against buffer A containing 0.15 M NaCl. The proteins were preincubated with 5′-nucleotidase from chicken gizzard overnight at 4°C and the AMPase activity determined. When using the optical assay system the final detergent concentration was set at 0.01% Triton X-100 in a 1 ml test cuvette. Using the radio-assay procedure [^{3}H]AMP was added to the preincubation mixtures giving a final concentration of 0.1 mM. The detergent concentration was then set at 0.05% Triton X-100. The rate of hydrolysis of [^{3}H]AMP was measured after precipitation of non-hydrolyzed [^{3}H]AMP with $Ba(OH)_2$ and $ZnSO_4$ [21].

3. RESULTS

5′-Nucleotidase from chicken gizzard smooth muscle was purified from detergent extracts as in [6]. A homogeneous enzyme preparation was obtained with an apparent M_r of 79000 as determined by polyacrylamide gel electrophoresis in the presence of SDS [6].

The effect of the high-M_r glycoproteins laminin, fibronectin and of collagen type I, III and IV on the AMPase activity of 5′-nucleotidase was investigated. To this end purified 5′-nucleotidase was preincubated overnight with the extracellular matrix proteins in question and aliquots of the incubation mixtures were used to determine its AMPase activity spectrophotometrically [20]. Alternatively, the conversion of AMP into adenosine was followed by a radio-assay procedure using [^{3}H]AMP as substrate [21].

As shown in fig.1 and table 1, laminin stimulated the AMPase activity of the enzyme in a concentration-dependent manner. A maximal stimulation between 200 and 400% was achieved at a 3–10-fold molar excess of laminin over 5′-nucleotidase. No AMPase activity could be detected in our laminin preparations. Therefore, we can exclude that the stimulation by laminin might be due to a contamination by traces of AMP-hydrolysing enzymes.

The complex formation between 5′-nucleotidase and the extracellular matrix proteins leading to an elevated or reduced AMPase activity was found to be time-dependent and required a preincubation of 5′-nucleotidase with laminin or fibronectin for at least several hours (not shown). The integrity of the enzyme after the long incubation steps could be verified by staining immunoblots of the incubation mixtures after SDS-polyacrylamide gel electrophoresis with polyclonal antibodies against 5′-nucleotidase [6,22] (not shown).

In contrast to the isolated detergent-solubilized enzyme, 5′-nucleotidase incorporated into artificial liposomes (B. Niggemeier, J. Dieckhoff and H.G. Mannherz, to be published) was instantaneously stimulated by 200% after addition of laminin. Prolonged incubation (14 h) resulted in an increase of 300% of the AMPase activity of

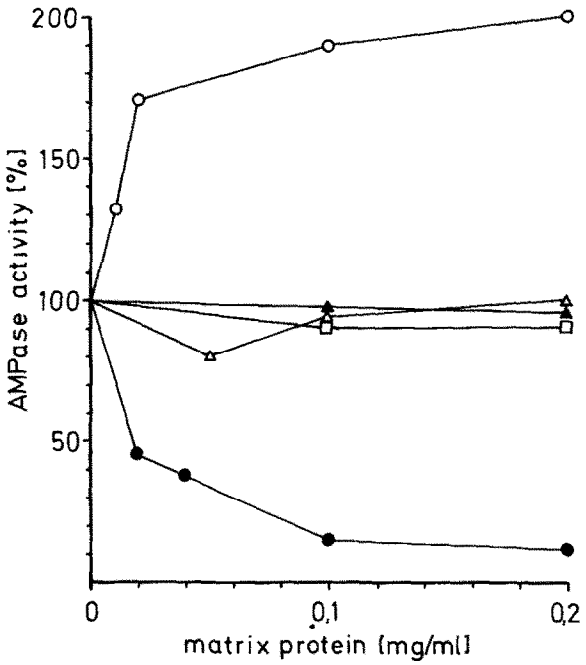

Fig.1. Influence of matrix proteins on the AMPase activity of purified 5′-nucleotidase from chicken gizzard. (●) Fibronectin, (○) laminin, (△) collagen type I, (□) collagen type III and (▲) collagen type IV. Ordinate gives relative AMPase activity of 2.5 μg 5′-nucleotidase as measured by the optical assay system [20]. Abscissa gives final concentration of matrix proteins in test cuvette.

Table 1

Comparison of the effect of four different laminin and fibronectin preparations (I–IV) on the AMPase activity of 5′-nucleotidase from chicken gizzard

Laminin preparation	5′-Nucleotidase activity (%)	Fibronectin preparation	5′-Nucleotidase activity (%)
I	325	I	20
II	200	II	50
III	360	III	25
IV	400	IV	80

Influence of different laminin and fibronectin preparations on the AMPase activity of purified 5′-nucleotidase from chicken gizzard. 2.5 μg 5′-nucleotidase was incubated with 0.2 mg laminin or fibronectin overnight at 4°C and the relative AMPase activity measured by a radioisotopic assay as described in section 2

liposome incorporated 5′-nucleotidase (table 2). In contrast to laminin, fibronectin was found to inhibit the enzymic activity of 5′-nucleotidase in a concentration-dependent manner (fig.1, table 1). Interestingly 5′-nucleotidase activity was only inhibited by fibronectin batches prepared by elution from gelatin-Sepharose with 1 M NaBr [15], but not by fibronectin eluted with 4 or 8 M urea [23]. Furthermore, it was noted that fibronectin batches completely lose their ability to inhibit the AMPase activity of the enzyme after 1–4 weeks of storage at 0°C (not shown).

The effect of laminin and fibronectin on the AMPase activity of 5′-nucleotidase could not be suppressed by the addition of other proteins, e.g. bovine serum albumin or ovalbumin, indicating

Table 2

Comparison of the effect of laminin on the AMPase activity of 5′-nucleotidase before and after incorporation into liposomes

	Laminin added (μg)	5′-Nucleotidase activity (%)
5′-Nucleotidase incorporated into liposomes, incubated with laminin overnight at 4°C	0	100
	150	230
	300	380
5′-Nucleotidase incorporated into liposomes, incubated with laminin 5 min at 4°C	0	100
	150	190
	300	300
Incubation of isolated solubilized 5′-nucleotidase with laminin overnight at 4°C	0	100
	150	180
	300	200
Incubation of isolated solubilized 5′-nucleotidase with laminin 5 min at 4°C	0	100
	150	110
	300	110

Liposomes with incorporated 5′-nucleotidase (Niggemeyer et al., to be published) were prepared as described [5] and incubated with laminin in a total volume of 500 μl buffer A. 5′-Nucleotidase activity was determined spectrophotometrically [20]. The effect of laminin on the AMPase activity of detergent solubilized 5′-nucleotidase was measured under identical conditions in the presence of 0.01% Triton X-100

that the measured effects are not due to unspecific interaction of these sticky matrix proteins with 5′-nucleotidase. Collagen type I, III and IV (fig.1) and many other proteins (bovine serum albumin, vinculin, α-actinin, DNase I) had no effect on the AMP breakdown under identical conditions (not shown).

4. DISCUSSION

Our results clearly show that the AMPase activity of the ectoenzyme 5′-nucleotidase can be altered differently by laminin and fibronectin. The extent of either stimulation or inhibition of the AMPase activity was shown to be concentration-dependent and saturated at a 3–10-fold molar excess of laminin over 5′-nucleotidase and a 6–20-fold molar excess of fibronectin over the enzyme. In addition, preliminary results also demonstrate the formation of stable complexes of laminin and fibronectin with 5′-nucleotidase, namely the retention of the enzyme on Sepharose 4B columns containing immobilized laminin or fibronectin and the cosedimentation of laminin and 5′-nucleotidase using sucrose gradient centrifugation (to be published). From the affinity gels the enzyme can subsequently be eluted in an enzymatically active form by increasing the NaCl concentration to 0.1 and 1 M using laminin- and fibronectin-Sepharose, respectively (to be published). These results indicate that the interaction of 5′-nucleotidase and these extracellular matrix molecules is reversible. A large difference in the time dependence of the stimulation of the AMPase activity was observed after addition of laminin to detergent-solubilized and liposome-incorporated 5′-nucleotidase (table 2). We attribute the time lag of the rise in AMPase activity of the solubilized enzyme to an unfavourable environment in the detergent micelles or the absence of natural phospholipids. The instantaneous effect observed with the liposome-incorporated enzyme might therefore resemble more closely the natural situation.

Our observation that 5′-nucleotidase binds to fibronectin and laminin is not necessarily in conflict with reports on the identification of specific receptor proteins for these molecules [1–3], since the binding of cells to the extracellular matrix has been shown to be a multistep process involving recognition, followed by alterations of cell morphology and the reorganization of the intracellular cytoskeleton [11]. It remains to be shown that the modulation of the AMPase activity of 5′-nucleotidase by laminin and fibronectin can also be elicited on intact culture cells and that this process is one of the possible mechanisms by which extracellular components influence cell function.

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