used as the substrate, there was no stimulation in the presence of PKM.

Discussion. During the preparation of M-PK_I in this study, the step of using exceedingly high concentration of cGMP or histone was not employed as in the preparations of catalytic subunit of G-PK by Shoji et al.[11-13]. In our most recent studies on some other mammalian tissues, it has been demonstrated that M-PK_I is separated from the catalytic subunit of G-PK[14]. The mechanism of the PKM augmented phosphorylation of the protein substrates of M-PK_I is not clear; however, there are at least 3 different possibilities: a) the interaction between PKM and the protein substrates occurred first for the subsequent stimulation of M-PK_I; b) the interaction between PKM and M-PK_I was prior to the action of the enzyme on its substrates; c) the interaction among PKM, M-PK_I and substrates took place simultaneously. We have investigated the declined M-PK_I activities in testes during the course of sexual maturation[15]. Nevertheless, more effort is required to define the physiological role of this enzyme.

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Adenosine triphosphatase of *Aspergillus nidulans:* Stimulation by aminoacids

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Summary. Ca^{2+}-dependent ATPase of *Aspergillus nidulans* was found to be stimulated by aminoacids in vitro. Both histidine and arginine stimulated the enzyme more effectively than the aromatic aminoacids. Supplementation of the growth medium with basic or aromatic aminoacids increased the enzyme activity in vivo 2–6-fold. The enhanced activity observed in these cultures in vivo was not mediated through the synthesis of new isoenzyme, as observed in protein-enriched cultures, but appeared to be through the activation of enzyme activity.

Adenosine triphosphatase activity with different cations such as Na^+, K^+, Mg^{2+} and Ca^{2+} has been demonstrated in a large variety of bacteria and animal tissues[1-4]. In addition to cations, bicarbonate and imidazole have been reported as activators of this enzyme[5,6]. Further, in vitro addition of aminoacids stimulated a Mg^{2+}-dependent ATPase preparation from embryonic chick heart nuclei[7], and Na^+, K^+ dependent enzymes of renal medulla[8], chick brain[9], Ehrlich cell plasma membrane[10,11], and rat liver plasma membrane[12].

In *Aspergillus nidulans,* it has been shown in my laboratory that Ca^{2+} stimulates ATPase activity maximally while Mg^{2+} inhibits this stimulatory effect non-competitively[13]. Further it has been shown that this Ca^{2+}-dependent ATPase activity decreased when the cultures were subjected to heat stress during growth[14]. The decrease in the Ca^{2+}-ATPase activity is attributed to a change in the lipid composition of this enzyme[15]. Further, a 5–6-fold increase in the activity of this enzyme has been observed when cultures are grown in protein hydrolysate supplemented media at the optimal temperature, 37 °C[16]. This increase in enzyme activity is found to be mediated by the synthesis of another isoenzyme[16]. It is therefore of interest to investigate further whether the increased activity observed in cells grown in protein hydrolysate media is mediated through aminoacid activation. This communication reports the stimulatory action of aminoacids both in vitro and in vivo, and the enhanced activity in vivo occurs without the synthesis of another isoenzyme.

Materials and methods. A. nidulans, a green wild strain, was grown in Pontecorvo minimal medium[17] for 120 h in static culture at 37 °C. The mycelium was harvested and washed twice with distilled water and then with 0.01 M Tris-HCl buffer (pH 7.5). The mycelium was dried and stored at −10 °C for further use. To study the effect of protein hydrolysates and aminoacids, they were added separately to minimal medium in the following concentrations: yeast extract (Difco Lab, USA), peptone (BDH), meat extract and casein hydrolysate (Centron Res. Lab., Bombay) 0.5% each; L-aminoacids, group I (aliphatic neutral aminoacids), group II (Sulphur aminoacids), group III (Basic aminoacids), group IV (aromatic aminoacids) and group V (acidic aminoacids) 0.05% each. All the L-amino acids were obtained either from BDH, E. Merck, Fluka AG, Koch-Light or Sigma.

The extraction of enzyme from cells grown in different media and the assay of the enzyme were carried out as described earlier[16]. Disc electrophoresis in polyacrylamide gel was performed using the procedure of Davis[18], slightly modified, using a 7.5% separating gel prepared in 0.1 M Tris-Glycine buffer (pH 8.6). The enzyme activity was discerned on the gel by incubating the gel with ATP

(50 mM) in 0.05 M Tris-buffer (pH 7.5) containing $CaCl_2$ at 37 °C for 20 min. The white band appeared in the corresponding region of the enzyme protein.

Protein was estimated by Lowry's method[19] using bovine serum albumin as the standard.

Results and discussion. ATP hydrolysis was measured from the amount of Pi released and the method employed was not affected by the presence of aminoacids. ATPase of *A. nidulans* was stimulated by aminoacids only in the presence of Ca^{2+}-ions. Of the 12 aminoacids tested only histidine exerted a significant stimulation (60%). The other most effective aminoacids were arginine, lysine, tryptophan and phenylalanine (25–35%) (table). Imidazole also stimulated the enzyme effectively at a level comparable with the histidine effect. The effect of histidine concentration on the stimulation of Ca^{2+}-ATPase of *A. nidulans* is given in figure 1. The activation of ATPase shows saturation kinetics with an apparent Ka of about 15 mM.

To study further whether the activation by these aminoacids exhibited in vitro is effective in vivo also, the growth medium was supplemented with aminoacids. Table 2 summarises the Ca^{2+}-ATPase activities of cells grown in different aminoacid media. The cells grown in basic aminoacid media showed maximal activity (51.5 ± 3.8 units) while the cells grown in aromatic aminoacids showed 50% of that. The other aminoacid media had no effect on the enzyme activity. But in all protein hydrolysate media the cells exhibited 4–6-fold activity compared with cells grown in minimal medium.

Earlier studies[16] revealed that the cells grown in protein hydrolysate media showed 2 major isoenzymes (fast and slow), whereas cells grown in minimal medium showed 1 major isoenzyme (fast) and a barely visible minor isoenzyme (slow). To see whether the increased activity seen in basic aminoacid media was mediated through the activation of the 'slow' isoenzyme as observed in protein hydrolysate media, an isoenzyme distribution study was undertaken. It is interesting to note that the cells grown in basic aminoacid medium showed a pattern similar to that observed in cells grown in minimal medium even though the former exhibited 5–6-fold greater activity (figure 2).

Ca^{2+}-ATPase of *A. nidulans* was stimulated in vitro most effectively by basic aminoacids, histidine and arginine, and slightly by aromatic aminoacids; this effect is similar to that reported for other cation-dependent ATPases[7–12]. Further, the presence of these aminoacids in the growth medium

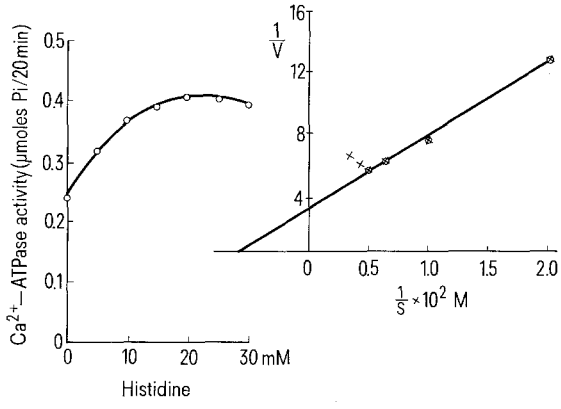

Fig. 1. Concentration effect of L-histidine in stimulation of Ca^{2+}-ATPase of *A. nidulans*. The points are means of 3 experiments carried out on different preparations. The insert represents a Lineweaver-Burk plot of the his-stimulated curve; the reciprocal of Ca^{2+}-ATPase activity (from which the basal Ca^{2+}-ATPase activity in the absence of histidine has been substracted) is plotted against the reciprocal of histidine concentration.

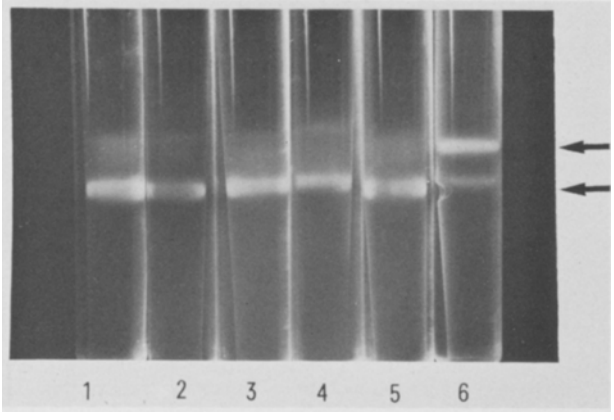

Fig. 2. Isoenzyme pattern of Ca^{2+}-ATPase of *A. nidulans* when grown in different growth media at 37 °C. Cells grown in *1* MM; *2* MM + group I*; *3* MM + group II; *4* MM + group III; *5* MM + group IV; *6* MM + yeast extract.
* Aminoacid groups are described in "Materials and methods".

Table 1. Effect of different aminoacid on Ca^{2+}-dependent ATPase of *A. nidulans* grown at 37 °C

Treatment	Ca^{2+}-ATPase activity (μmoles of Pi liberated/ 20 min)	p
None	0.80 ± 0.12 (8)	–
Glycine	0.75 ± 0.15 (7)	NS
L-Alanine	0.78 ± 0.11 (7)	NS
L-Methionine	0.80 ± 0.12 (7)	NS
L-Phenylalanine	1.08 ± 0.13 (9)	0.05
L-Tryptophan	1.05 ± 0.12 (10)	0.05
L-Proline	0.85 ± 0.10 (7)	NS
L-Histidine	1.32 ± 0.10 (12)	0.001
L-Arginine	1.20 ± 0.11 (12)	0.001
L-Lysine	1.00 ± 0.10 (10)	0.05
L-Aspartic acid	0.80 ± 0.12 (8)	NS
L-Isoleucine	0.78 ± 0.15 (7)	NS
L-Leucine	0.75 ± 0.12 (7)	NS
Imidazole	1.30 ± 0.11 (10)	0.001
Ouabain* + L-histidine	1.28 ± 0.11 (10)	0.001

Final concentration of amino acid was 10 mM; Results are mean ± SD; in parentheses, the number of experiments; p has been evaluated by Student's t-test; NS not significant. * 5 mM concentration.

Table 2. The effect of different growth media on Ca^{2+}-dependent ATPase of *A. nidulans* when grown at 37 °C

Medium	Ca^{2+}-ATPase
Minimal medium (MM)	7.7 ± 0.41
MM + yeast extract	41.5 ± 3.8*
MM + mean extract	52.5 ± 5.1*
MM + casein hydrolysate	41.0 ± 2.8*
MM + peptone extract	35.5 ± 3.1*
MM + group I**	8.2 ± 0.25
MM + group II	9.5 ± 0.50
MM + group III	51.5 ± 3.8*
MM + group IV	25.8 ± 2.1*
MM + group V	7.2 ± 0.40

Enzyme activities are expressed in units/mg protein/20 min. Results are mean ± SD. * Compared to cells grown in MM at 37 °C, $p < 0.01$; ** Aminoacid groups are described in 'Materials and methods'.

greatly influenced in vivo enzyme activity, the basic aminoacids being the most effective. The isoenzyme study revealed that in protein-enriched media, the slow moving component, normally present but barely visible in minimal medium, appeared predominantly. If the synthesis of this enzyme is stimulated by aminoacids, then one could expect the same in the aminoacid supplemented media also. However, this enzyme was not predominant in cultures enriched with aminoacids, so it could be small peptides rather than aminoacids that play a role in the synthesis of this enzyme. Hence, the increased activity observed in cultures grown in media supplemented with basic or aromatic aminoacids could be either through the protection of the existing major isoenzyme from degradation, or by activation of the activity of the enzyme species as suggested by in vitro studies.

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Reaction of pyridoxal-5′-phosphate with γ-carboxyglutamic acid

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Summary. Pyridoxal-5′-phosphate (PLP) reacts with γ-carboxyglutamic acid (Gla) to form a stable complex absorbing at 325 nm. It is suggested that a condensation occurs in which the formyl group of PLP reacts with the α-amino group and the γ carbon atom of Gla to give a pyrrolidine derivative.

The recently discovered amino acid γ-carboxyglutamic acid (Gla) has been found in proteins involved in blood clotting[1-4], in plasma proteins[5,6] and in bone proteins[7-9]. It has been found free in the urine of healthy humans[10]. Studies have been undertaken to see if Gla is used by enzymes involved in glutamate breakdown. In this context Federici et al.[11] have recently shown that Gla is a competitive inhibitor of glutamate dehydrogenase with NAD^+ and glutamate, and an activator, additive to ADP, of the reverse reaction. We have found however that Gla is not used by bacterial glutamate decarboxylase (type V from *E. coli,* Sigma Chem. Co., 25 units/mg protein) at pH 4.7, even with enzyme concentrations 5 times higher than those able to decarboxylate glutamate in standard conditions; furthermore Gla does not inhibit the enzyme, also at concentrations 10 times higher than the substrate concentration. The spectrum of holo glutamate decarboxylase, characterized between pH 3 and 5 by an absorption maximum at 415 nm, attributed to a hydrogen-bonded aldimine, is also not affected by the addition of 10^{-2} M Gla (synthesized according to Fernlund et al.[12]) at pH 5.0.

On the other hand, in the course of the experiments with glutamate decarboxylase, we noticed a rapid disappearance of the characteristic yellow colour of the pyridoxal-5′-phosphate (PLP, Fluka AG) added to the assay mixture, upon addition of Gla. This suggests the formation of a colorless adduct between the 2 reagents. The present communication reports results concerning this interaction.

When PLP and Gla are added in aqueous solution over a wide range of pH, disappearance of the maximum at

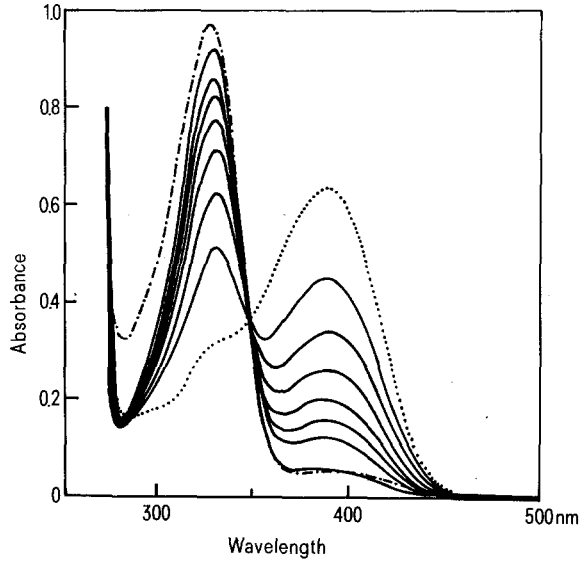

Fig. 1. Absorption spectra of a reaction mixture containing Gla and PLP at pH 4.75 as a function of time. The reaction mixture contains 1.38 mM PLP and 8.3 mM Gla in 0.3 M pyridine-acetate buffer, pH 4.75, 25 °C. Light path 0.1 cm. Spectra recorded with an ACTA 5260 Beckman spectrophotometer. · · · · ·, 1.38 mM PLP; ——, absorption spectra taken 10, 20, 30, 40, 50, 60, and 90 min after the addition of Gla; — · — ·, spectrum of the reacted mixture after addition of 0.3 M ethanolamine at final pH 7.9.