original cGMP-dependence after restoring 7 days at −20 °C, apparently having converted to a modulator-dependent enzyme with characteristics similar to M-PK_{II}. This suggests that diluted enzyme is less stable than the concentrated form. Arginine-rich histone was demonstrated to be an excellent substrate for all 3 enzymes (PK of A, B, C in table), while protamine chloride (grade V) was poor. When partially purified PKM_s from the Sephadex G-100 step[7,8] was used to replace PKM in the above tests, similar stimulation of the enzymes was observed (data not shown). However, when partially purified PKM_I was used, no stimulation or inhibition was detectable (data not shown).

Discussion. One of the difficulties of purifying mammalian GPK[4,12], after the discovery of athropod GPK[2,3], was that the dilution of the enzyme during the purification process induced instability of the enzyme, causing the lose of its cyclic GMP dependent characteristics. It has been shown that the crude preparation of PKM contained both PKM_s and PKM_I[8]. It has also been shown that PKM_I depresses both A-PK[8] and its catalytic subunit (C_A)[11]. In reapplied Sephadex G-200 chromatography in this study, I-PK (peak 4) had presumably derived from A-PK (peak 1) as suggested by its smaller molecular weight, cAMP-independent and PKM-depressing characteristics. Similarly, M-PK_{II} (peak 3) had presumably derived from and is the catalytic subunit of G-PK[9,10], as suggested by its smaller molecular weight, cGMP-independent and PKM (or PKM_s)-stimulating characteristics. All these findings suggest the possible conversion of A-PK and G-PK into their subunits after longterm storage at −20 °C. According to recent privileged communications with Dr M. Shoji and Dr J.F. Kuo, it seems our M-PK_{II} is the same as their stimulatory modulator-dependent protein kinase from Morris hepatoma 9618A, based on the similarity of substrate protein specificity. However, in several respects, either M-PK_{II} or stimulatory modulator-dependent protein kinase is different from our newly discovered modulator-dependent protein kinase (type I) from many mammalian tissues[13].

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Separation of modulator-dependent protein kinase (type I) from cyclic GMP-dependent protein kinase in mouse testes

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Summary. A new type of enzyme, modulator-dependent protein kinase (type I) (M-PK_I), was successfully isolated from the cytosol fraction of mouse testes. It was eluted slightly after the peak of cyclic GMP-dependent protein kinase (G-PK) by Sephadex G-200 gel filtration. Unlike either cyclic AMP-dependent protein Kinase (A-PK) or G-PK, its maximal activity depended exclusively on the presence of crude protein kinase modulators (PKM) or partially purified stimulatory modulator (PKM_s).

It has been shown that either crude PKM[3-8] or partially purified PKM_s[5-8] from mammalian tissue is able to stimulate G-PK[5-10] and its catalytic subunit[11-13], much smaller in molecular weight[8,12]. Unlike G-PK, this catalytic subunit is totally independent of cyclic GMP (cGMP)[11-13]. While investigating the separation of PKM_s from G-PK in lungs and some other tissues by gel filtration, we found that the active fractions of A-PK were contaminated not only with cGMP-independent but also with PKM-dependent activity. This led us to further studies of the contaminant enzyme, which might differ from the catalytic subunit of G-PK.

Materials and methods. [γ-^{32}P] ATP was purchased from New England Nuclear. Cyclic AMP (cAMP) and cGMP were obtained from Boehringer Mannheim (BRD); arginine-rich histone (HA) was obtained from Worthington; other histones (type II-S, VI-S, and V-II-S), protamine chloride (grade V), lipase (type VI) and phosphatase (alkaline, type XVII) were obtained from Sigma. Sephadex G-100 and G-200 were from Pharmacia.

Sexually premature male ICR mice (weighing 10.0 ± 2.6 g) were used exclusively. Crude PKM was prepared from liver extracts of ICR mice by boiling and trichloroacetic acid-precipitation[3-8]. PKM_s and inhibitory modulator (PKM_I) were partially purified by Sephadex G-100 gel filtration[5-8]. Preparation of M-PK_I actively were as follows. Fresh testes (6.7 g) from 30 ICR mice were homogenized in 3.5 ml of ice cold 50 mM potassium phosphate buffer, pH 7.0, using a glass-teflon homogenizer. The homogenate was centrifuged for 15 min at 30,000 × g. The supernatant fluid (crude extract) was filtered through 2 layers of glass wool to remove fat, and then applied to a Sephadex G-200 column (3.5 × 46 cm). Potassium phosphate (50 mM, pH 7.0) was used to equilibrate and elute each column. The fraction size in each case was 2.5 ml, and the flow was 0.4 ml/min.

The standard assay system[9] for protein kinase activity contained, in a final volume of 0.2 ml, potassium phosphate buffer, pH 7.0, 10 μmoles; theophylline, 0.5 μmoles; substrate protein, 40 μg; MgCl, 2 μmoles; [γ-^{32}P] ATP, 1 nmole

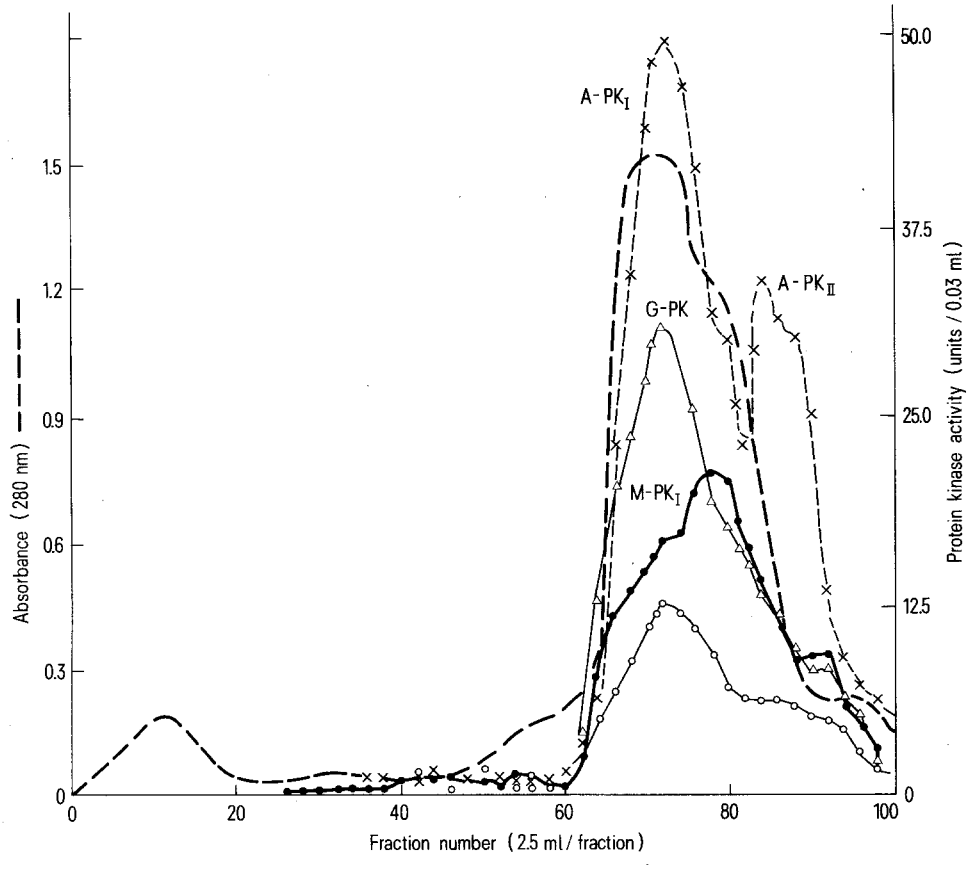

Fig. 1. Sephadex G-200 chromatography. ○—○, Basal; ●—●, PKM; △—△, PKM-cGMP; ×---×, cAMP.

Substrate protein specificity of modulator-dependent protein kinase (type I) from active fractions of Sephadex G-200 step

Substrate	None		+cGMP	
	−PKM (units)	+PKM	−PKM	+PKM
Histone, type II-S	6.9	9.4	7.1	9.5
Histone, type VI-S	4.4	8.4	3.0	8.0
Histone, type VII-S	5.1	7.2	4.7	7.0
Arginine-rich histone	6.8	11.8	7.0	12.0
Protamine chloride, grade V	6.6	17.7	5.4	17.4
Lipase, type VI	7.2	6.0	-	-
Phosphatase, alkaline	7.7	7.9	-	-

Assay conditions were as described in the text, except for the variation of substrates and 3.4 µg of M-PK$_I$ used. Each value shown represents the mean of triplicate samples.

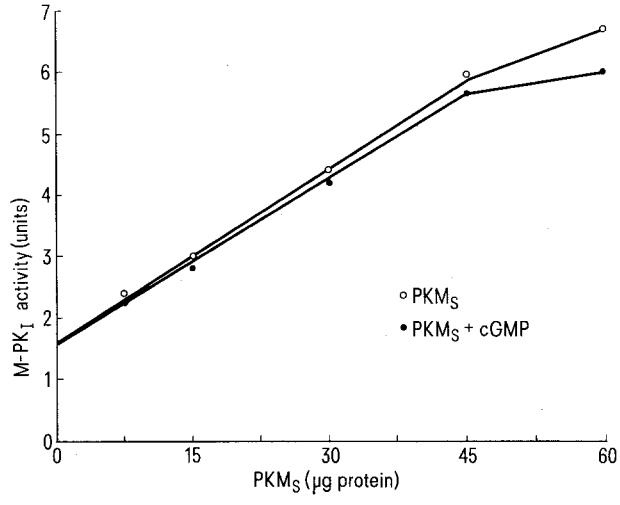

Fig. 2. Relationship between M-PK$_I$ activity and PKM$_s$ dose.

containing about 1.2×10^6 cpm; with or without cAMP or cGMP, 60 pmoles; with or without modulator; and appropriate amounts (3.4–15.5 µg) of protein kinase preparations. The reaction was carried out for 10 min at 30 °C. 1 unit of enzyme activity was defined as the amount of enzyme that transferred 1 pmole of ^{32}P from [γ-^{32}P] ATP in recovered substrate protein under the assay conditions.

Results. M-PK$_I$ activity was separated from the G-PK activity also present in the crude extract of premature testes by Sephadex G-200 filtration, when 40 µg of arginine-rich histone was used as the substrate under assay conditions (figure). The sequences of the eluted peak activity fractions were G-PK, M-PK$_I$, and then followed by A-PK$_{II}$. Maximal G-PK activity depended on the presence of both PKM and cGMP, while maximal M-PK$_I$ activity depended exclusively on PKM.

The dose dependence on PKM$_s$ of M-PK$_I$ isolated by Sephadex G-200 step was observed when 20 µg of arginine-rich histone was used as the substrate (figure). A linear relationship between them was noted up to 45 µg of PKM$_s$. Again, the stimulation of M-PK$_I$ depended on PKM$_s$ but not on cGMP.

Several proteins (table), 40 µg each, were examined for their substrate specificities to M-PK$_I$ from active fractions of the Sephadex G-200 step. Protamine chloride (grade V) was shown to be the best substrate in the presence of PKM. When either lipase (type VI) or phosphatase (alkaline) was

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