Concentrations of substrates in the assay mixture were: 60 mM L-glutamine, 15 mM hydroxylamine (NH_2OH), 0.4 mM Na_2ADP, 3 mM $MnCl_2$, 20 mM KH_2AsO_4, and 40 mM imidazole. Incubations were maintained at 25 °C and pH 6.7. Activity was measured by formation of a relatively stable (5–30 min) complex of $FeCl_3$ and γ-glutamyl hydroxamate in acid solution[15] compared with a γ-glutamyl hydroxamate standard at 500 nm. A unit of glutamine synthetase activity is defined as 1 µmole γ-glutamyl hydroxamate produced per min at 25 °C. Protein was determined by the biuret method of Zamenhof[16] with bovine serum albumin as a standard.

The specific activity of each *Squalus acanthias* brain region shows little variation as shown by the standard deviations in column 4 of the table. As observed by others[17–19], brain regions have different, but not a highly regionalized, specific activity of glutamine synthetase. The region of highest activity differs among these reports and may relate to the relative need of the brain regions to detoxify ammonia[8,19]. The relationship of glutamine synthetase to glial content[8,19] of each brain region could also reflect the relative glial content of these regions. As discussed by Martinez-Hernandez et al.[8], this could indicate control by glutamine synthetase of the glutamate pool and regulation of brain excitability. If this is the case, the different excitability of brain regions as expressed by glutamine synthetase activity in *Squalus acanthias* and other animals may be associated with the prominence of each region's function relative to each species' central nervous system adaptations.

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Conversion of mammalian cyclic GMP-dependent protein kinase into modulator-dependent protein kinase (type II) in vitro

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Summary. The spontaneous conversion of mammalian cyclic GMP-dependent protein kinase (G-PK) into modulator-dependent protein kinase (type II) ($M\text{-}PK_{II}$) in the absence of cGMP or histone was observed in vitro. The findings, together with similarity in substrate protein specificity, suggest that $M\text{-}PK_{II}$ is the catalytic subunit of mammalian G-PK.

Following the initial discovery of G-PK in arthropod tissues[2,3], the cGMP target enzyme was subsequently found in some mammalian tissues[4]. Recently, substantial progress concerning mammalian G-PK has been made, for example: the discovery that crude protein kinase modulator (PKM)[5–8] or purified stimulatory protein kinase modulator (PKM_s)[7,8] is required for maximal G-PK activity under the incubation condition of lower Mg++ concentration; the dissociation of G-PK holoenzyme into its catalytic subunit in the presence of exceedingly high concentration of cGMP or histone[9,10]; and the stimulation of its catalytic subunit in the presence of stimulatory modulator alone[10]. In spite of the above progress, some tasks yet remain. Among them are the conversion of G-PK holoenzyme into its subunits and the reversal of the process in vitro under low concentrations of histone, cGMP, or Mg++; and the stabilization of the subunits in vitro. We now report on the spontaneous conversion of G-PK into $M\text{-}PK_{II}$ in vitro in the absence of cGMP or histone.

Materials and methods. [γ-^{32}P] ATP was purchased from New England Nuclear. cGMP and cAMP were obtained from Boehringer Mannheim (FRG); arginine-rich histone (HA) was obtained from Worthington; another histone (type II-S) and protamine chloride (grade V) were obtained from Sigma. Sephadex G-100 and G-200 were from Pharmacia.

Crude PKM was prepared from liver extracts of 8-month-old ICR mice by boiling and trichloracetic acid-precipitation[5–8]. PKM_s and inhibitory modulator (PKM_I) were partially purified by Sephadex G-100 gel filtration[7,8]. G-PK from guinea-pig fetal lung was purified by Sephadex G-200[12], and the active fractions were pooled, concentrated to about 5 ml (Amicon concentrator using UM-10 membrane). This concentrated G-PK was stored at −20 °C for 8 months

and reapplied to a Sephadex G-200 column (1×46 cm). Potassium phosphate (50 mM, pH 7.0) was used to equilibrate and elute the column. The fraction size was 0.5 ml, and the flow was 0.1 ml/min.

The standard assay system[4] for protein kinase activity contained, in a final volume of 0.2 ml, potassium phosphate buffer, pH 7.0, 10.0 µmoles; theophylline, 0.5 µmoles; substrate protein, 40.0 µg; $MgCl_2$, 2.0 µmoles; [γ-^{32}P] ATP, 1 nmole containing about 1.2×10^6 cpm; with or without crude protein kinase modulator (containing 56.2 µg of protein); with or without cAMP or cGMP, 60 pmoles; and appropriate amounts (1.1–25.0 µg) of protein kinase preparations. The reaction was carried out for 10 min at 30 °C. 1 unit of enzyme activity was defined as that amount of enzyme that transferred 1 pmole of ^{32}P form [γ-^{32}P] ATP in recovered substrate protein under the assay conditions.

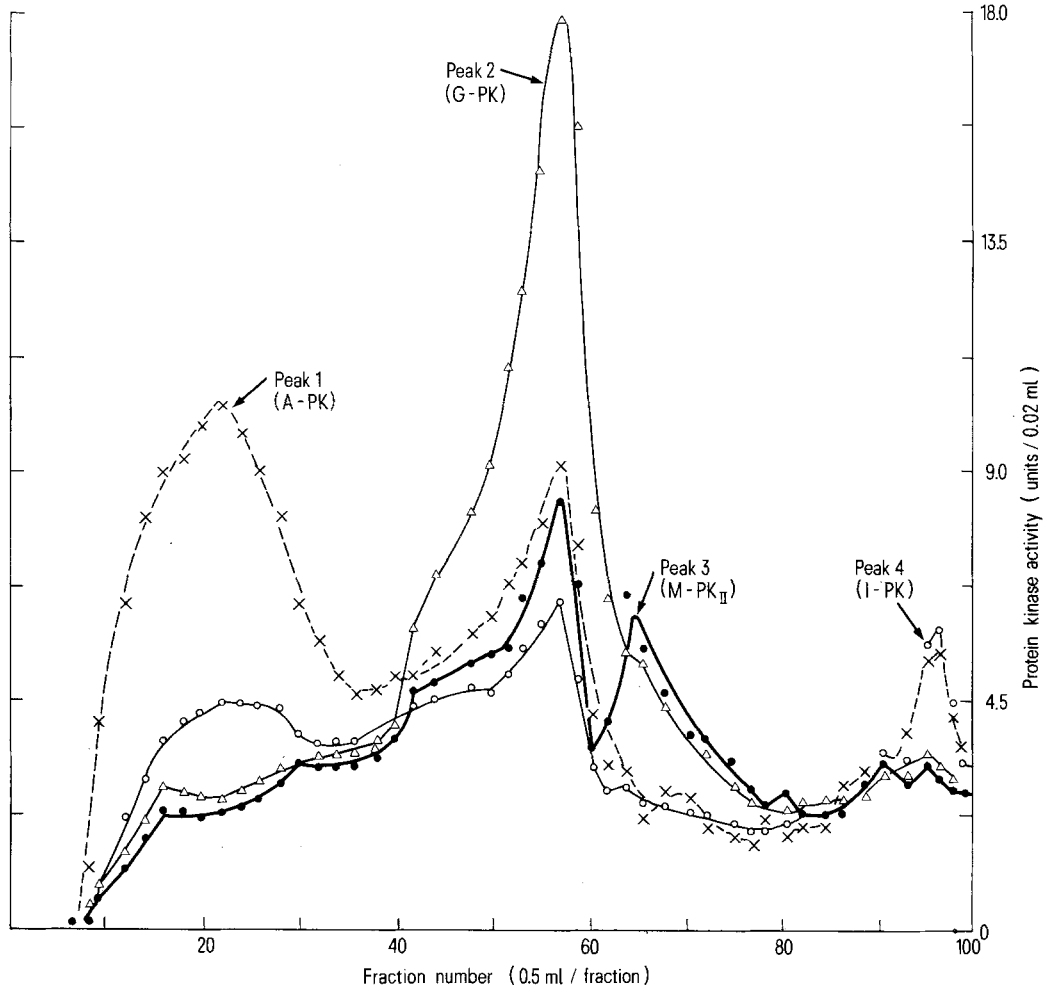
Results. The concentrated G-PK obtained by Sephadex G-200 filtration was slightly contaminated with A-PK activity. The contaminant A-PK was more stable than the G-PK because the ratio of G-PK/A-PK activity declined tremendously after 8 months of storage at −20 °C (data not shown). By using histone (type II-S) as the substrate, A-PK, G-PK, M-PK_{II} and cyclic nucleotide-independent protein kinase (I-PK) peaks were observed in the reapplied Sephadex G-200 chromatography (figure). Maximal activity of G-PK (peak 2) depended on the presence of both cGMP and PKM, but that of MPK_{II}, (peak 3) depended only on the presence of PKM. A-PK (peak 1) was stimulated by cAMP but was inhibited by PKM. Like MPK_{II}, I-PK (peak 4) was cyclic nucleotide-independent, but unlike MPK_{II}, I-PK was inhibited by PKM. There was no detectable PKM_s[7,8] activity in any of the reapplied Sephadex G-200 fractions (data not shown).

3 substrate proteins, Histone (type II-S), Arginine-rich histone, and Protamine chloride (grade V), were examined for their specificity to the protein kinases obtained from repeated gel filtration (table). G-PK (peak 2) lost most of its

Substrate protein specificity of protein kinases from peak fractions of repeated gel filtration of Sephadex G-200

Substrate	PK	None		+cGMP	
		−PKM	+PKM	−PKM	+PKM
		Units			
Histone, type II-S	A	1.3	1.4	1.4	2.9
	B	0.4	1.3	0.4	1.2
	C	0.9	2.0	0.9	2.1
Arginine-rich histone	A	2.5	2.9	2.7	6.2
	B	1.0	4.0	1.2	4.0
	C	1.9	4.0	1.8	3.8
Protamine chloride, grade V	A	0.6	0.7	0.6	0.9
	B	0.5	0.8	0.5	0.9
	C	1.8	2.1	1.8	2.0

Assay conditions were as described in the text, except for the variation of substrates and 1.1 µg of enzymes used. Each value shown represents the mean of triplicate samples. A, G-PK (peak 2) activity using fresh enzyme; B, G-PK (peak 2) activity using enzyme restored 7 days at −20 °C; C, M-PK_{II} (peak 3) activity using fresh enzyme.



Protein kinase activity observed in reapplied Sephadex G-200 chromatography.
○—○, Basal;
●—●, PKM;
△—△, PKM +cGMP;
×---×, cAMP.

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