c) EDCI method. 3 ml of DNase (4000 units/ml) plus 0.1 ml of a 50% erythrocytes in PBS were incubated for 1 h at room temperature with 100 mg of 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDCI)[4]. The cells were washed 5 times and suspended in PBS.

Control studies were performed with same procedure without immobilizing agents. 1 ml of DNA (200 mg Na-DNA/dl containing 0.05 M $MgSO_4$ in PBS) plus 1 ml of PBS were incubated with 1 ml of the erythrocyte suspension (Ht 50%) at 37 °C for 1 h and centrifuged. 1 ml of 3M trichloroacetic acid was added to the supernatants. The mixtures were left in an ice water bath for 15 min then filtered through Whatman No.42 paper. Aliquots of the filtrate were analyzed by the diphenylamine procedure.

Results and discussion. DNase immobilized on the erythrocytes by chromic chloride showed DNase activity in vitro, but other immobilizing procedures inhibited the DNase activity. About 10% of added DNase activity was immobilized on the erythrocytes. No significant hemolysis was observed and DNase activity did not decrease after several incubations (table).

DNase activities of erythrocyte immobilized DNase

	Units/0.1 ml of erythrocytes
Control	90
Immobilized DNase	
$CrCl_3$ method, 1st incubation	430
2nd incubation	450
3rd incubation	400
DIDS method	110
EDCI method	80
Free DNase (4000 units/ml)	390 (0.1 ml of DNase solution)

Considerable evidence exists to suggest that complexes of DNA and anti-DNA antibodies play a role in the pathogenesis of nephritis in SLE. A significant correlation exists between the occurrence of high titers of DNA-anti-DNA antibody complex and the severity of clinical disease and immunoglobulin deposit in the kidney. Some sera from SLE patients with nephritis, when treated with DNase, show an increase in anti-DNA antibody levels. This fact suggests that DNA has been bound in vivo to the antibody as an immune complex.

Pancreatic DNase degrades DNA mainly to a mixture of oligonucleotides, and in vivo administration of DNase to the patients with SLE may be useful to digest not only free DNA but also antibody-bound DNA. Digestion of free DNA may prevent the anti-DNA antibody formation and digestion of antibody bound DNA may decrease the immune complex.

For clinical use in future, human pancreatic deoxyribonuclease must be purified to prevent immunological reactions.

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Tissue survey of mammalian modulator-dependent protein kinases

W.N. Kuo[1]

Division of Science and Mathematics, Bethune-Cookman College, Daytona Beach (Florida 32015, USA), 20 May 1980

Summary. The levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) in various tissues of adult mice were compared. Cerebellum contained the highest levels of both modulator-dependent protein kinases and protamine-dependent protein kinase(s), whereas skeletal muscle contained no detectable enzymes. The lung and the ileum were also rich in modulator-dependent protein kinases, while other tissues were poor sources of these enzymes.

At least 2 protein kinases[2-4] from mammalian sources have been shown to be stimulated in vitro by either crude protein kinase modulator (PKM)[5-8] or by partially purified stimulatory modulator[7,8]. While investigating these 2 modulator-dependent protein kinases, other investigators have reported protamine- or histone-stimulable protein kinase(s)[9], which will be called protamine-dependent protein kinase(s). In this paper, we have addressed the question of whether modulator-dependent protein kinases and protamine-dependent protein kinase(s) are the same enzymes by comparing the levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) in various tissues of adult mice.

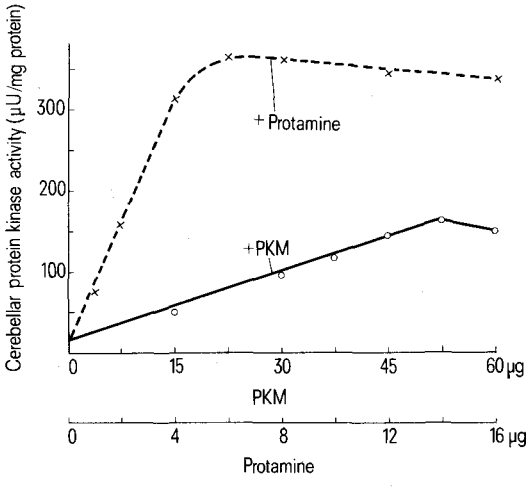
Materials and method. [γ-^{32}P] ATP was purchased from New England Nuclear; protamine chloride (grade V) was obtained from Sigma. Adult male ICR mice (each weighing 25.0±2.9 g) were used exclusively. Crude protein kinase modulator was prepared from liver extracts of ICR mice by boiling and trichloroacetic acid-precipitation[5-8]. Crude protein kinase preparations were as follows. Appropriate amount of fresh tissues from ICR mice were homogenized in 3 volumes 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 appropriately diluted with potassium phosphate (50 mM, pH 7.0) before use as an enzyme source. The standard assay system[10] for protein kinase activity was modified over the past studies in a final volume of 0.2 ml to contain phosphate buffer, pH 7.0, 10 µmoles; varied amount of endogenous substrate protein; $MgCl_2$, 2 µmoles; [γ-^{32}P] ATP, 1 nmole containing about 1.2×10^6 c.p.m. with or without stimulator, protein kinase modulator (50 µg) or protamine (4 µg); appropriate amount of crude protein kinase preparations. The reaction was carried out for 10 min at 30 °C. 1 µU of the enzyme was defined as that amount of enzyme that transferred 1 pmole of ^{32}P from [γ-^{32}P] ATP per min to recovered substrate protein under the assay conditions.

Results. The levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) were examined in 8 different tissues (table). The cerebellum contained by far the highest modulator-dependent protein kinases and protamine-dependent protein kinase(s) activites. In con-

Comparison of tissue levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s)

Tissue	Basal	+PKM (μU/mg protein)	+Protamine	Ratio +PKM/basal	+Protamine/basal	+PKM/+protamine
Lung	26	47	56	1.81	2.15	0.84
Cerebellum	17	160	301	9.41	17.70	0.53
Skeletal muscle	42	45	42	1.07	1.00	1.07
Kidney	27	38	48	1.41	1.78	0.79
Ileum	17	31	22	1.82	1.29	1.41
Epididymal fat Pad	19	23	23	1.21	1.21	1.00
Liver	13	15	18	1.15	1.38	0.83
Testis	20	25	-	1.25	-	-

Assay conditions were described in the text except for the use of 50 μg of protein kinase modulator (PKM) and 4 μg of protamine chloride. Each value shown represents the mean of triplicate samples.



trast, skeletal muscle had no protein kinase modulator or protamine stimulable enzymes, despite its highest basal enzyme activity. The lung also had an abundance of both classes of enzymes. The ileum was rich in modulator-dependent protein kinases but not in protamine-dependent protein kinase(s). Other tissues, such as epididymal fat pad, liver, testis, and kidney, were poor sources of modulator-dependent protein kinases and protamine-dependent protein kinase(s). The ratio of protein kinase modulator-stimulating activity to protamine-stimulating activity varied from tissue to tissue. A linear dependence of protein kinase activity upon the amount of crude enzyme prepared was observed with or without a stimulator, protein kinase modulator (50 μg) or protamine (4 μg) (data not shown). The maximal amounts of crude enzyme, assayed within the limit of such a linear relationship, were quite different from tissue to tissue, for example, 8 μg protein for crude cerebellar enzymes, while 14 μg protein for crude lung enzymes.

The protein kinase activity of 8 μg crude cerebellar enzymes was observed in the presence of varied amounts of stimulator (figure). The stimulation by protamine was higher than that by protein kinase modulator. A linear relationship was noted up to the doses of 50 μg protein kinase modulator and 4 μg protamine. Similar linearity was also noted in other tissues (data not shown).

Discussion. The great variation in the ratio of modulator-dependent protein kinases to protamine-dependent protein kinase(s) in different tissues might be due to the difference in types of these enzymes. We have since observed similar variations in the ratio of enzyme activity levels in various tissues in the presence of appropriate exogenous substrate protein, such as bovine serum albumine (unpublished data). This proves that the difference in various tissues is mainly due to difference in enzyme level, and not substrate protein level. The difference was not due to the difference in tissue level of protein kinase modulator either, because under modulator-dependent protein kinases assay conditions, a substantially high concentration of exogenous crude mouse liver protein kinases modulator was added. In addition, protein kinases modulator is known to contain acidic stimulatory modulator[4], while protamine is a basic protein. The stimulation of their target enzymes might therefore be through different mechanisms. In our another most recent study, it provides direct evidence that these 2 classes of enzymes are different since they can be separated (unpublished data).

We found it much easier to observe crude modulator-dependent protein kinases activity by using crude protein kinase modulator but not partially purified stimulatory modulator, since crude protein kinase modulator contained both stimulatory modulator and inhibitory protein kinase modulator[7,8], which would effectively inhibit contaminant cyclic AMP-dpendent protein kinase and thus minimize the interference from this contaminant enzyme.

It is very interesting that there is a coincidence in the levels of modulator-dependent protein kinases and stimulatory modulator in some tissues. For example: the cerebellum is rich in both modulator-dependent protein kinases and stimulatory modulator; in contrast, the skeletal muscle has almost no detectable modulator-dependent protein kinases or stimulatory modulator[7]. The physiological significance of such a coincidence is still unclear.

1 Acknowledgments. This work was supported by grants (RR-08119-PK project from the National Institutes of Health, USA, and CDP-8004200 from the National Science Foundation, USA.

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