Interactions between basic proteins and stimulatory protein kinase modulator

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Summary. The interactions of a number of basic proteins with stimulatory protein kinase modulator (PKM_s) from the mouse brain were measured by absorbance at 360 nm. Such interactions were not altered in the presence of Mg^{2+} at low concentrations.

Calmodulin[2] has been shown to be an intracellular multifunctional regulatory protein. In addition, stimulatory protein kinase modulator[3-5] also has been reported as another potentially important protein factor regulating a broad spectrum of cellular activities. Some of the similarity and difference of these 2 regulatory proteins have been preliminarilly compared[5]. Nevertheless, a great deal of the mystery of stimulatory protein kinase modulator still remains to be explored. The present investigation was undertaken to seek whether there are similar interactions between protein kinase modulator and basic proteins as the interactions of calmodulin demonstrated earlier by others[6].

Materials and methods. Arginine-rich histone (HA) was purchased from Worthington. Other histones, protamine chloride (grade V) and pyruvate kinase were obtained from Sigma. Bio-gel P-6DG was from Bio.Rad. Stimulatory protein kinase modulator from the brain of BDF_1: Sch hybrid mice (Holland Sprague-Dawley) was prepared through Sephadex G-100 step[3] followed by further purification with a Bio-gel P-6DG column (6.5 × 4 cm) previously eluted with deionized water.

The standard assay system contained, in a final vol. of 2 ml, potassium phosphate buffer, pH 7.0, 10 µmoles; with or without stimulatory protein kinase modulator, 80 µg; with or without other proteins, 80 µg; with or without varied amount of $MgCl_2$. The interaction was carried out for 5 min at 25 °C and the formation of turbidity was examined immediately by absorbance measurement at 360 nm.

Results and discussion. Greatly increased absorbance was observed in these samples containing the mixture of stimulatory protein kinase modulator and basic proteins including various histones and protamine chloride (table). While basic proteins were replaced by pyruvate kinase in the mixture, such an increment of absorbance was no longer detectable. The enhancement of absorbance in the mixture containing stimulatory protein kinase modulator and arginine-rich histone was further demonstrated within a broad range of wave length of 320 up to 420 nm regardless of their maximal absorbance being around 350 nm and falling off slowly on both sides of the peak (data not shown). Moreover, such enhancement was not altered in the presence of Mg^{2+} at the concentrations lower than 5 mM (data not shown). Results so far indicate that stimulatory protein kinase modulator preferentially interacts with basic protein(s) to form complex(es) and thus generates turbidity measured by enhanced absorbance. It is very interesting that both stimulatory protein kinase modulator[7] and calmodulin[8] are acidic proteins, therefore, in general it may be true that acidic proteins interact with basic proteins to form complexes under appropriate conditions. The strongest interaction was demonstrated in the sample containing arginine-rich histone and stimulatory protein kinase modulator (table). In addition, the highest substrate preference of arginine-rich histone for modulator-dependent protein kinase II has also been shown[4]. Such coincidence suggests that the preformation of complexes, between stimulatory protein kinase modulator and substrate protein(s), is required to initiate the action of modulator-dependent protein kinases. A great deal of the physiological roles of such preformed complexes is still unclear. The recent discovery of another type of complex[5], between stimulatory protein kinase modulator and Mg^{2+}, intensified the complexity to a higher degree.

Since there was no significant alterations of the interaction of stimulatory protein kinase modulator and arginine-rich histone by Mg^{2+} at low concentration, the bindings of basic protein and Mg^{2+} on the same molecule of stimulatory protein kinase modulator are not competitive and presumably are at different sites. This was further proven by our latest experiments in which similar interactions did occur when preformed complexes of [PKM_s-Mg^{2+}] were incubated with basic protein(s) (Kuo, unpublished data).

Comparison of interaction between stimulatory protein kinase modulator (PKM_s) and various proteins

Protein	Absorbance (360 nm) $-Mg^{2+}$	$+Mg^{2}$ (2 mM)
PKM_s	0.070 ± 0.001	0.075 ± 0.002
Arginine-rich histone	0.015 ± 0.002	0.021 ± 0.001
Arginine-rich histone + PKM_s	0.262 ± 0.007	0.275 ± 0.008
Histone (type II)	0.015 ± 0.002	0.015 ± 0.001
Histone (type II) + PKM_s	0.195 ± 0.005	0.203 ± 0.004
Histone (type II-S)	0.020 ± 0.001	0.019 ± 0.002
Histone (type II-S) + PKM_s	0.195 ± 0.006	0.195 ± 0.010
Histone (type VI-S)	0.018 ± 0.001	0.017 ± 0.001
Histone (type VI-S) + PKM_s	0.195 ± 0.004	0.195 ± 0.007
Histone (type VII-S)	0.015 ± 0.001	0.016 ± 0.001
Histone (type VII-S) + PKM_s	0.214 ± 0.009	0.210 ± 0.005
Protamine chloride (grade V)	0.015 ± 0.001	0.015 ± 0.001
Protamine chloride (grade V) + PKM_s	0.228 ± 0.009	0.236 ± 0.008
Pyruvate kinase	0.005 ± 0.001	0.004 ± 0.001
Pyruvate kinase + PKM_s	0.082 ± 0.002	0.076 ± 0.002

Each value shown represents the mean (±SEM) from 3 to 5 samples.

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