Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Analysis of Protein Kinase A Activity in Insulin Secreting Cells Using a Cell Penetrating Protein Substrate and Capillary Electrophoresis

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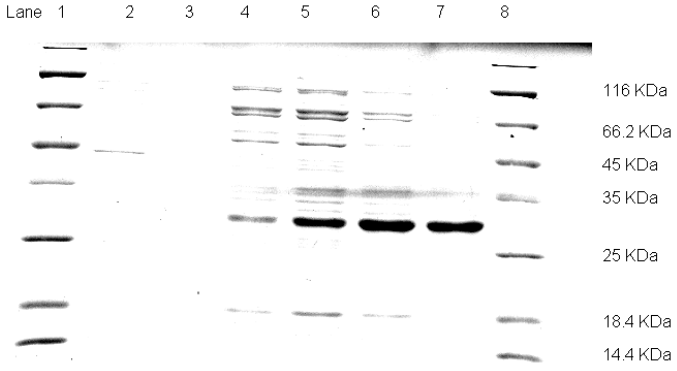

Fig. S1. 10% SDS-Polyacrylamide gel of PKAS purified in Ni-NTA agarose column. Lane 1,8 Molecular weight marker; lane 2-4 washes with 10,20,and 50 mM imidazole; lane 5,6, 7 elutions with 250 mM imidazole. 1X Tris Glycine was used as the running buffer

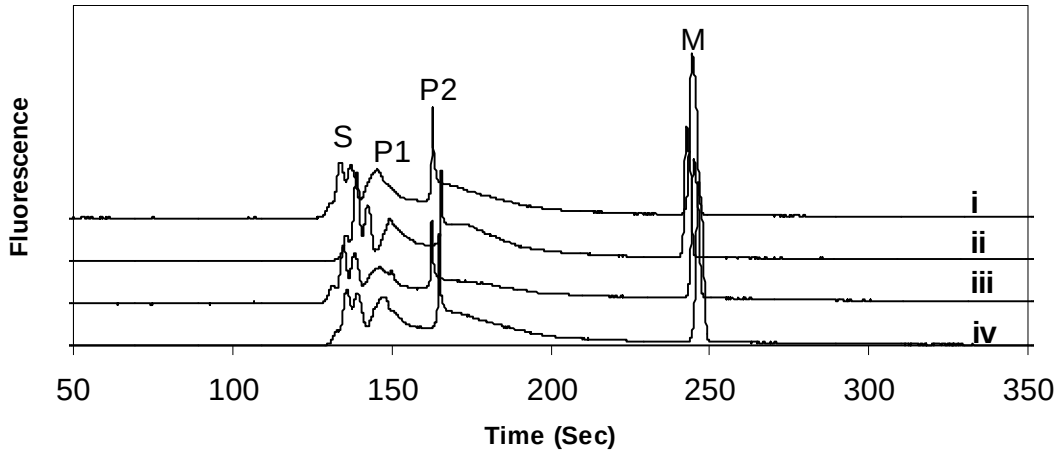

Fig. S2. Multiple trials to show the reproducibility of PKA catalyzed phosphorylation of PKAS in βTC-3 cells. Cells were loaded with PKAS and incubated in 3 mM glucose for 3 hours. Cell lysate was analyzed by CZE. Peak S is the substrate, peaks P1 and P2 are the phosphorylated products and peak M is a carboxyfluorescein internal standard. Separation conditions: 25 μm i.d. capillary, 20 mM borate, pH = 9.3, 400 V/cm

Table S-1. Comparison of data analysis methods for determining % phosphorylation

Inhibitor	% Phosphorylation (Height)	% Phosphorylation (Area)	Stimulator	% Phosphorylation (Height)	% Phosphorylation (Area)
(-) H-89	82 ± 6	80 ± 5	(-) Br-cAMP	55 ± 6	62 ± 3
(+) H-89	28 ± 4	25 ± 5	(+) Br-cAMP	71 ± 5	76 ± 4

The total percentage of PKAS molecules that exhibit phosphorylation was determined as a ratio between total peak height for all PKAS and P-PKAS peaks and for total peak area for all PKAS and P-PKAS peaks for cellular data in the presence and absence of phosphorylation inhibitor and stimulator. The data are statistically similar for both analysis methods with no bias to higher or lower levels observed