INHIBITION OF A Ca^{2+}-DEPENDENT PROTEIN KINASE FROM IRANIAN RICE LEAVES BY pH AND TEMPERATURE

REYHANEH SARIRI and AMIR ARASTEH
Department of Biology, Gilan University, Rasht, Iran, sariri@guilan.ac.ir

Protein kinase C (PKC) has been in the spotlight since the discovery two decades ago that it is activated by the lipid second messenger diacylglycerol (DAG). Despite Protein kinase C's enduring stage presence, the regulation and specific roles of its isozymes in defined cellular processes are still under intense investigation. PKC is an 80-KD polypeptide with four conserved domains and five variable regions. The conserved regions include an ATP-binding domain, a substrate-binding domain, a calcium binding domain, and a (DAG) binding domain. The DAG-binding domain is often referred to as the "pseudosubstrate domain" because it has an amino acid sequence that closely resembles protein substrate for the enzyme. PKC phosphorylates serine and threonine residues on a wide range of protein substrates. DAG is the key 'on' switch for most protein kinase Cs. In addition, conventional protein kinases Cs are also modulated by Ca^{2+}; these isozymes are particularly sensitive to hydrolysis of phosphatydilinositol biphosphate because it generates an additional signal, inositol triphosphate, which mobilizes intracellular Ca^{2+}.

A role of protein kinase C in cellular growth and division is demonstrated by its strong activation by phorbol esters. These compounds, from seeds of Croton tiglium, are tumor promoters, agents that do not themselves cause tumorigenesis but that potentiate the effects of carcinogens. The phorbol esters mimic DAG, bind to regulatory pseudosubstrate domain of the enzyme, and activate protein kinase C.

Many factors that might affect the biological activity of different protein kinases such as various drugs, chemicals and ethanol have been studied [1-3]. It has been shown that the external factors such as pH and temperature affect the protein kinase release into the environment by Leishmania donovani. This behavior plays an important role in parasite survival and adaptation to host environment [4]. However, the effect of varying environmental conditions on the biological activity of protein kinase C has received less attention.

In this research we measured the biological activity of a calcium dependent protein kinase extracted from leaves of different rice grown in northern Iran by using UV spectrophotometry at 280nm. The effect of varying pH and temperature on the activity of this Ca^{2+} dependent protein kinase was then studied. It was found that the release of calcium dependent protein kinase was decreased by increasing temperature and lowering the pH values. Acidic pHs are potent inhibitors of protein kinase release and they highly affect its biological activity. The inhibition of the enzyme activity by higher temperature is predictable, as part of the three dimensional structure may have changed due to

the raised temperature. These findings are very important when considering the quality of rice and the environmental conditions for its growth.

REFERENCES

1. Hayess, K. and Benndorf, R. Effect of protein kinase inhibitors on activity of mammalian small heat-shock protein (HSP25) kinase. **Biochem. Pharmacol.** 53 (1997) 1239-1247.
2. Haviryaji, S.G. and Maharaj, K. Ethanol-mediated inhibition of mitogen-activated protein kinase phosphorylation in mouse brain. **Eur. J. Pharmacol.** 439 (2002) 53-58.
3. Tadokoro, C., Kiuchi, Y., Yamazaki, Y., Oguchi, K. and Kamijima, K. Effects of imipramine and sertraline on protein kinase activity in rat frontal cortex. **Europ. J. Pharmacol.** 342 (1998) 51-54.
4. Vieira, L., Sacerdoti, N. and Jaffe, C. Effect of pH and temperature on protein kinase release by *Leishmania donovani*. **Int. J. Parasitol.** 32 (2002) 1085-1093.