Regulation of Phospholipase C₆₁ activity by GTP-binding proteins: RhoA as an inhibitory modulator.

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Phosphatidylinositol 4,5-bisphosphate is hydrolysed by phospholipase C (PLC) to form inositol 1,4,5-trisphosphate and diacylglycerol. Each of these products acts as a second messenger in a variety of cellular responses including contraction and cell proliferation [1,2]. The PLC family of enzymes consists of three isoforms (β,γ,δ) which are differentially regulated and expressed. PLCδ has been demonstrated to be regulated by heterotrimeric G proteins of the Gq family or by βγ subunits [3,4] whereas PLCγ is tyrosine phosphorylated by growth factors [5]. The regulation of PLCδ isoforms remains obscure although there is increasing evidence to suggest the involvement of GTP-binding proteins [6,7].

Partially purified PLCδ was isolated from bovine aortic cytosol by sequential elution through Q-sepharose and heparin agarose chromatography columns, using a linear sodium chloride gradient (50-550mM) and identified immunologically by western blotting. PLC activity was measured using a method based on that described by Waldo et al [8] and using [35S]-GTPγS as a substrate. Immunoprecipitation of PLCδ from this sample resulted in a reduction in PLC activity of 89±3% (n=3) indicating that this sample is not contaminated with other isoforms of PLC, known or unknown.

In order to determine whether GTP-binding proteins have a role in the regulation of PLCδ activity samples were preincubated with GTPγS, a non-hydrolysable analogue of GTP, and PLC activity was measured. The resting PLC activity was consistently and significantly enhanced by the addition of GTPγS (Figure 1), but not by aluminium fluoride, suggesting the involvement of a monomeric GTP-binding protein.

![Figure 1. Effect of GTPγS on PLCδ activity.](image)

**To confirm the presence of a GTP-binding protein in the PLCδ fraction, GTP-binding of column fractions were measured using [35S]-GTPγS. These results demonstrated that the [35S]-GTPγS binding profile generated by the elution of the sample from heparin-agarose correlated exactly with that of PLCδ activity.**

\[\text{Figure 2. Effect of exoenzyme C3 on PLCδ activity.} \]

![Figure 2.](image)

**Clostridium botulinum exoenzyme C3 (exoenzyme C3), which selectively ADP-ribosylates and inactivates rho GTPases also produced a significant increase in PLCδ activity. (Figure 2), whereas incubation with NAD alone had no effect on PLC activity.**

**The idea of PLCδ being regulated by GTP-binding proteins is one which is gaining increasing support [6,7,9,10] and the interaction of PLC with small GTP-binding proteins has been implied by a number of reports [6,10,11]. Indeed Homma and Emori isolated a novel 122kDa protein with both rhoGAP and PLCδ stimulatory activity [11]. The data obtained in this study indicates that monomeric GTP-binding proteins have a role in the regulation of PLCδ activity and we propose that rhoA exerts a negative modulatory influence on aortic PLCδ activity. Release from this negative influence, through inhibition of rhoA by exoenzyme C3, results in a significant increase in aortic PLCδ activity.**

We would like to thank Professor Sir David Weatherall for his moral and financial help through difficult circumstances.

References: