The Enzymatic Activation of Autotaxin by Divalent Cations
Without EF-Hand Loop Region Involvement

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Background Autotaxin (ATX) is a recently described member of the nucleotide pyrophosphatase/ phosphodiesterase
(NPP) family of proteins with potent tumor cell motility-stimulating activity. Like other NPPs, ATX is a glyco-
protein with peptide sequences homologous to the catalytic site of bovine intestinal alkaline phosphodiesterase
(PDE) and the loop region of an EF-hand motif. The PDE active site of ATX has been associated with the
motility-stimulating activity of ATX. In this study, we have examined the roles of the EF-hand loop region and
of divalent cations on the enzymatic activities of ATX.

Methods To find out the effects of divalent cations on ATX-mediated tumor cell motility, we performed Boyden
chamber tumor cell motility assay using human melanoma cell line, A2058 in the presence or absence of divalent
cations. To identify the significance of the EF-hand loop region of ATX on tumor cell motility-stimulation and
PDE activity, we constructed three site-directed point mutants and a deletion mutant within the EF-hand loop of
ATX and compared the PDE activity of these mutants with that of wild type recombinant ATX.

Results Ca++ or Mg++ were each demonstrated to increase the PDE activity of ATX in a concentration
dependent manner, whereas incubation of ATX with chelating agents abolished this activity, indicating a
requirement for divalent cations. Non-linear regression analysis of enzyme kinetic data indicated that addition of
these divalent cations increases reaction velocity predominantly through an effect on Vmax. Three mutant
proteins, Ala306, Ala242, and Ala351-ATX, in the EF hand loop region of ATX had comparable enzymatic activity
to wild type protein. A deletion mutation of the entire loop region resulted in slightly reduced PDE with normal
motility stimulating activity. However, the PDE activity of this same deletion mutant remained sensitive to
augmentation by cations.

Conclusion These data strongly imply that cations increase ATX mediated tumor cell motility stimulation by
interactions outside the EF hand loop region of ATX.