SUMMARY

REGULATION OF THE ENZYMATIC ACTIVITY AND VESICULAR TRANSPORT OF THE ANTI-THROMBOTIC ECTO-NUCLEOTIDASE CD39: THE ROLE OF MEMBRANE COMPARTMENTALIZATION AND TRANSMEMBRANE DOMAINS.

DOCTORATE THESIS BY
AGATHI PAPANIKOLAOU

Thromboregulation is a physiological function, which ensures normal blood flow in the vessels. CD39 is a key thromboregulatory plasma membrane ecto-enzyme, which is expressed by endothelial cells that line the blood vessel wall. The importance of CD39 lies in its high ADPase hydrolytic activity, since ADP is the most potent platelet activator.

CD39 is localized in caveolae, which are specialized plasma membrane invaginations. Caveolae are considered specialized lipid microdomains, called rafts that differ from the rest of the plasma membrane being enriched in cholesterol and sphingolipids. Caveolin-1 is the marker protein of caveolae and the chasacteristic bending of membrane is attributed to the high oligomerization state of caveolin. In the present study we examined whether the localisation of CD39 in caveolae plays a role in its anti-thrombotic function. Using caveolin-1 gene disrupted mice, we show that caveolae are not essential either for the enzymatic activity of CD39 or for its targeting to plasma membrane. On the other hand, sucrose flotation experiments using detergent-based approaches indicate that CD39 associates, at least in part, with distinct lipid rafts and that cholesterol, which is enriched in rafts, regulate the enzymatic activity of CD39. Interfering with cholesterol levels using drugs like MβCD that depletes membrane cholesterol results in a strong inhibition of the enzymatic and anti-platelet activity of CD39. The mechanism of CD39 regulation by cholesterol was investigated further and we provide evidence that the effect of cholesterol on CD39 is not due to biophysical changes of membrane fluidity but

rather to a direct interaction between cholesterol and CD39. Consequently, the localization of CD39 in such cholesterol-rich domains warrants a high hydrolytic activity of this enzyme.

Given the association of CD39 with rafts and the well-known role of rafts in polarized transport, we tested whether CD39 is transported to the plasma membrane in a polarized manner and dissected the responsible targeting signals. Using polarized Madin-Darby canine kidney (MDCK) cells, we showed that indeed CD39 is polarized and in particular it is preferentially targeted to the apical side of the plasma membrane. Besides an expected apical signal in the ecto-domain, which is most likely due to glycosylation, we identified an additional unexpected apical sorting signal in the N-terminal transmembrane-cytosolic domain, which functions as an independent sorting domain.

Apart from the role of the N-terminal transmembrane/cytosolic domain in apical targeting of CD39, it is required for CD39 enzymatic activity, since deletion of this particular domain results in 50 % inhibition of the ADPase hydrolytic activity, while loss of the C-terminal transmembrane/cytosolic domain causes almost complete loss of CD39 activity. Furthermore, deletion of the C-terminal transmembrane/cytosolic domain results in inefficient transport to the membrane due to improper glycosylation. Glycosylation of CD39 takes place post-translationally in a conformation-dependent manner, which requires the presence of C-terminal transmembrane/cytosolic domain. Inhibition of CD39 activity by deletion of either transmembrane/cytosolic domain is due to loss of the ability of CD39 to engage into a cholesterol-mediated mechanism of activation.

J Biol Chem. 2005 Jul 15;280(28):26406-14. Epub 2005 May 12.

Cholesterol-dependent lipid assemblies regulate the activity of the ecto-nucleotidase CD39.

Papanikolaou A, Papafotika A, Murphy C, Papamarcaki T, Tsolas O, Drab M, Kurzchalia TV, Kasper M, Christoforidis S.

Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110 Ioannina, Greece.

CD39 (ecto-nucleoside triphosphate diphosphohydrolase-1; E-NTPDase1) is a plasma membrane ecto-enzyme that regulates purinergic receptor signaling by controlling the levels of extracellular nucleotides. In blood vessels this enzyme exhibits a thromboregulatory role through the control of platelet aggregation. CD39 is localized in caveolae, which are plasma membrane invaginations with distinct lipid composition, similar to dynamic lipid microdomains, called rafts. Cholesterol is enriched together with sphingolipids in both rafts and caveolae, as well as in other specialized domains of the membrane, and plays a key role in their function. Here, we examine the potential role of cholesterol-enriched domains in CD39 function. Using polarized Madin-Darby canine kidney (MDCK) cells and caveolin-1 gene-disrupted mice, we show that caveolae are not essential either for the enzymatic activity of CD39 or for its targeting to plasma membrane. On the other hand, flotation experiments using detergent-free or detergent-based approaches indicate that CD39 associates, at least in part, with distinct lipid assemblies. In the apical membrane of MDCK cells, which lacks caveolae, CD39 is localized in microvilli, which are also cholesterol and raft-dependent membrane domains. Interfering with cholesterol levels using drugs that either deplete or sequester membrane cholesterol results in a strong inhibition of the enzymatic and anti-platelet activity of CD39. The effects of cholesterol depletion are completely reversed by replenishment of membranes with pure cholesterol, but not by cholestenone. These data suggest a functional link between the localization of CD39 in cholesterol-rich domains of the membrane and its role in thromboregulation.

PMID: 15890655 [PubMed - indexed for MEDLINE]