Phosphorylation of apoproteins in VLDL and LDL by protein kinases in vitro.

CLAIRE E HARRISON and MICHAEL R MUNDAY

Dept. of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK.

Elevated plasma levels of cholesterol-rich low density lipoprotein (LDL) are a major risk factor in the development of atherosclerotic and coronary heart disease [1]. LDL is formed from intermediate density lipoprotein (IDL) derived from the peripheral catabolism of very low density lipoprotein (VLDL) originally assembled and secreted by liver [1]. VLDL consists of a triacylglycerol and cholesterol-ester core which is surrounded by a monolayer of phospholipid interspersed with cholesterol. Embedded in this monolayer is apoprotein E, a 34 kDa protein whose function is to bind to LDL-receptors and increase the plasma clearance of the LDL [2, 3]. Apoprotein B100 a 514 kDa protein that provides a structural framework for the particle with alternating hydrophilic and hydrophobic domains that interface with the aqueous medium and the lipid core of the particle, respectively [3]. Apo B100 also forms the receptor recognition site in LDL particles since these have lost apoE during their formation from IDL [3].

Functional apo B100 is essential for the hepatic assembly and secretion of VLDL which is under hormonal control, notably that of insulin and glucagon [4]. There is evidence that inhibition of VLDL production is not always matched by a decrease in apo B synthesis suggesting a possible post-translational regulation of apo B function [4]. It has been reported that in newly-secreted VLDL from rat liver or hepatocytes apo B is a phosphoprotein [5, 6]; that this phosphorylation is hormonally regulated by insulin [6, 7] and is important for apo B function [7].

We have observed that cAMP-dependent protein kinase (cAMP-PK), AMP-activated protein kinase (AMP-PK) and casein kinase 2, stoichiometrically phosphorylate apo B100 in vitro in preparations of human LDL where it is the only apoprotein present (M R Munday, D Carling, S Takhar and C E Harrison, unpublished results). Incubation of human VLDL with [γ-32P] ATP and the catalytic subunit of bovine heart cAMP-PK in vitro results in the 32P-labeling of a number of protein bands separated directly by SDS PAGE (Figure 1a). One of these is apo B100 in confirmation of the results obtained with LDL. The major phosphorylated band migrated with an apparent Mr of 35 KDa and minor phosphorylated bands ran at 19 and 17 KDa. Other 32P-labelled proteins are a result of phosphorylation of proteins within the protein kinase preparation particularly the autophosphorylated C-subunit of cAMP-PK at 40 KDa (Figure 1a).

Figure 1a) Separation of VLDL apoproteins phosphorylated by cAMP-PK in vitro on 8-15% SDS PAGE.

Cleveland V8 map on 15% SDS PAGE of apo B100 from LDL phosphorylated by cAMP-PK or AMP-PK in vitro.

Cleveland mapping of these protein bands using Staphylococcus aureus V8 protease showed different patterns of 32P-labelled peptides for each suggesting that they were distinct apoproteins and not proteolytic degradation products of apo B100 (Figure 2). The 35 KDa band is suggestive of apo E whereas the apparent 19 and 17 KDa bands fall midway between the quoted Mr for apo A and apo C classes. Preliminary experiments suggest that phosphorylations of VLDL by AMP-PK and casein kinase 2 result in 32P-labelling of apo B100 but not the other apoproteins (data not shown).

Figure 2 Cleveland V8 map of 32P labelled apoprotein bands from VLDL shown in Figure 1a.

apo B100 17 KDa (apo F) 17 KDa doublet

Cleveland V8 mapping of the apo B100 band from the SDS PAGE of LDL showed a different pattern of 32P-labelled peptides for apo B100 phosphorylated by cAMP-PK compared with AMP-PK (Figure 2). This indicates that these two kinases phosphorylate different sites. This has also been observed by reversed phase HPLC separation of tryptic peptides from apo B100 phosphorylated by either kinase and will be confirmed when these phosphorylation sites are identified by amino acid sequencing.

The physiologic significance of these phosphorylations is unclear. We have observed that 32P-labelled apoproteins in VLDL or LDL phosphorylated by cAMP-PK are rapidly dephosphorylated on subsequent incubation with plasma (known to be rich in phosphatase activity). Therefore, if they are to be physiologically relevant, these phosphorylations are likely to be intracellular. Apo A, C and E are all functionally important in the circulation, but apo B is the most important in intracellular VLDL assembly. Investigations of the topography of VLDL assembly poses questions as to where apo B100 may be exposed to protein kinases [5]. However, we have observed the presence of up to 0.5 mol of alkaline labile phosphate per mol of apo B in freshly isolated human LDL. Phosphorylation of apo B100 could inhibit or augment VLDL assembly and secretion. The phosphorylation of a number of proteins such as casein [9] and vitelligenin [10] is essential for their successful secretion.

Furthermore, hepatic cAMP-PK, AMP-PK and casein kinase 2 have all been observed to be under the hormonal control of glucagon and/or insulin [11, 12, 13]. Identification of apoprotein kinase sites of phosphorylation and their presence in the lipoproteins in vivo in different physiological situations will reveal the importance of this phenomenon to VLDL assembly, secretion and metabolism.

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