Lipoprotein lipase: molecular interactions of the enzyme

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Lipoprotein lipase (EC 3.1.1.34), as the extraplastic enzyme responsible for the hydrolysis of plasma lipoprotein triacylglycerol, plays a pivotal role in the metabolism of circulating lipids. The occurrence and action of the enzyme in lipoprotein metabolism has been the subject of a number of recent reviews (Cryer, 1981; Quinn et al., 1982; Hamash & Hamash, 1983) and this aspect will not be considered here. Rather, the molecular interactions that occur in the exercise of the function of the enzyme will be considered, particularly as these are reflected by studies in vitro.

From studies of lipoprotein lipase action in vitro it is clear that the enzyme may interact with a number of different molecular species and that the relationships which exist between these various binding events contribute to the control of functional activity. Taking the events separately, the first is associated with the catalytic or active site of the enzyme.

The active site of lipoprotein lipase is relatively non-specific in that it hydrolyses tri-, di- and mono-acylglycerols as well as phospholipids. However, the positional specificity of hydrolysis is strict for all substrates with a preferential cleavage of the sn-1(3) ester bonds with subsequent isomerization of the 2-monoylglycerol for complete breakdown (Scow & Olivecrona, 1977). The enzyme will also hydrolyse water-soluble model substrates, e.g. p-nitrophenylphosphate. Quinn et al. (1982) have indicated that a 'serine esterase-like' mechanism appears best able to accommodate observations from both the pH rate and the covalent modification experiments on lipoprotein lipase.

The catalytic efficiency of the enzyme is high, and with long-chain acylglycerols as substrate each lipoprotein lipase can hydrolyse 1000 ester bonds/s. In order to accommodate the observed rate at which, for example, a chylomicron of 0.16 μm diameter containing 1.3 x 10^6 triacylglycerol molecules is degraded, a number of enzyme molecules must act together on each lipoprotein particle. It can be shown that, in general, the rate of triacylglycerol hydrolysis is proportional to the number of enzyme molecules bound to each lipoprotein particle and for a chylomicron of the size indicated above, approx. 30-40 enzyme molecules would be required to act in concert to bring about the maximal rate of hydrolysis observed in vitro (see below also).

The second discernible site on the lipoprotein lipase molecule is that which may be described as the lipid (interfacial)-binding site. This site is distinct from the active site and has been proposed because of the observed ability of the enzyme to bind to lipoproteins, lipid emulsions and liposomes in the absence of the activator protein or hydrolysis. The binding through this site is reversible and accounts for the observed rapid movement of the enzyme between lipoprotein particles in vitro.

A third site, distinct from those already described, on the enzyme is responsible for the interaction between lipoprotein lipase and its activator protein, apo C-II. The presence of the activator protein is obligatory for the hydrolysis of lipoproteins or triacylglycerol emulsions by the enzyme but may not be required when non-physiological water-soluble substrates are studied. It is clear that a distinct binding site is involved because, not only may the enzyme and activator form a complex in solution but the affinity between them is enhanced greatly when both are present at a lipid interface, with which either or both may interact independently. This increased affinity in the presence of lipid may reflect the fact that both concentrate at the interface or may indicate that on binding to lipid one or both of the reactants undergoes a conformational change which may facilitate binding and subsequent catalytic activation. Certainly, it is the case that apo C-II undergoes a conformational change on lipid-binding, although whether the enzyme does is not certain.

The activation of the enzyme by apo C-II is mediated by a direct 1:1 protein-protein interaction in a manner which is not dependent on any increase in the binding of the enzyme to the substrate-containing surface. The mechanism of apo C-II action probably involves a facilitation of the interfacial formation of the enzyme triacylglycerol complex by making the orientation of enzyme and substrate more favourable for this interaction. The catalytic enhancement is probably mediated through the interaction of the C-terminal one-third of the 78 residue apo C-II, which exists as a β-sheet region that may complement a similar region on the enzyme. The remaining part of the apo C-II molecule (N-terminal sequence 1-49) contains the lipid-binding domain necessary for its interaction with the lipoprotein particle.

In addition to the sites described above and which are involved directly in the hydrolysis of triacylglycerol, the enzyme is also able, via a fourth distinct region, to bind to a variety of polyanions (Bengtsson et al., 1980). Of the polyanions studied the glycosaminoglycans have been the group to receive most attention. Indeed the use of heparin covalently linked to agarose has become a widely used affinity chromatographic step in the purification of enzyme from many sources. The enzyme-heparin interaction is ionic although it is not related merely to charge density or to the presence of N-sulphate groups. The common structural feature significant to the interactions, and which is present in the three glycosaminoglycans that bind lipoprotein lipase (heparan sulphate and dermatan sulphate) and which is absent from those that do not (chondroitin sulphate and hyaluronic acid), is the L-iduronic acid moiety. It has been suggested many times, on the basis of circumstantial evidence (reviewed by Cryer, 1981, 1983), that the binding of the enzyme to the luminal surface of the endothelium is via interaction with such glycosaminoglycan polymers. The recent more direct evidence in favour of this contention is discussed below.

As far as the binding of glycosaminoglycans to the enzyme in vitro is concerned, the other functional sites on the lipoprotein lipase molecule are unaffected by the event. Thus, the active and apo C-II-binding sites are unaffected since, among other things, lipoprotein lipase bound to immobilized heparin is active catalytically and can be stimulated fully by apo C-II. Furthermore, the enzyme will bind to lipid/water interfaces, via its lipid-binding site, equally well whether heparin is present or not.

The final binding site discernible on the lipoprotein lipase has been characterized by its ability to interact with either detergents, like deoxycholate, or long-chain fatty acids. The binding of deoxycholate or fatty acids has, for example,
been shown to stabilize this otherwise unstable enzyme in solution. As far as enzyme function is concerned, deoxycholate or fatty acids in the absence of a suitable acceptor like serum albumin, which appears to have a higher affinity for them than does the enzyme. These observations can be correlated with the binding of the enzyme to hepatic-agarose and interfere with the interaction between lipoprotein lipase and its activator protein, apo C-II. The fatty-acid-binding site is distinct from the interfacial (lipid-binding) site and the interaction of fatty acids with the enzyme can be reversed by the addition of albumin, which may be purely coincidental, however, that most heparan-sulphate proteoglycans carry approx. four glycosaminoglycan chains.

Work with endothelial cells grown in culture has also implicated heparan-sulphate-like molecules in the cellular binding of lipoprotein lipase. Both Cheng et al. (1981) and Shimada et al. (1981) have shown rapid, specific and saturable binding to occur between the enzyme and isolated endothelial cells and showed that reversal of the binding was only induced by adding glycosaminoglycans which contained N-acetylgalactosamine. Cheng et al. (1981) showed that specific activity of the enzyme was unchanged upon cell binding and that pretreatment of the cells with platelet-derived endoglucoaminidase (proteinase-free) reduced subsequent enzyme binding by up to 90%. Shimada et al. (1981) provided further evidence for the involvement of heparan sulphate in the cellular binding of enzyme by showing that both crude and highly purified heparinase from Halobacterium heparinum, but not chondroitinase ABC, caused a large decline in the interaction.

The binding of enzyme to the glycosaminoglycan chains of endothelial cell-surface proteoglycans produces a situation where the enzyme is anchored at a distance from the plasma-membrane surface of the cell (up to 40 nm). The consequences of this spatial separation have been reviewed elsewhere (Cryer, 1981, 1983).

The enzyme sequestered at the endothelial cell surface has a much shorter half-life than that in the tissue parenchymal cells. Recently, using hearts from starved rats perfused in the absence of heparin in vitro, Bagby (1983) estimated that the t½ for enzyme at the endothelium was approx. 10 min. The enzyme at this site had a fractional release rate constant (Kₚ) of 0.009 compared with 0.002 and 0.003 for alkaline phosphatase and creatine kinase respectively. Thus with hearts containing in total 150 units of activity/g, 2.1 units were released per min. It is clear from other experiments (Wallinder et al., 1979) that any released enzyme would be removed from the circulation rapidly and would therefore not interfere with the delicate balance of lipoprotein metabolism which is directed by endothelial-bound lipoprotein lipase.