LIPOPROTEIN LIPASE INTERACTION WITH SYNTHETIC *N*-DANSYL FRAGMENTS OF APOLIPOPROTEIN C-II

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Apolipoprotein C-II (apoC-II), a 78-amino acid residue protein component of plasma lipoproteins, enhances lipoprotein lipase-dependent hydrolysis of long chain fatty acylglyceryl esters in triacylglycerol-rich lipoproteins. The amino acid sequence region required for activation of the enzyme is found in the carboxyl terminal third of the protein between residues 55 and 78 (1, 2). To assess possible protein-protein interaction with lipoprotein lipase and the activator, a series of synthetic fragments of apoC-II that contain a single dansyl group on the amino terminal residue was prepared by solid phase peptide synthesis. Resonance energy transfer from the tryptophan residues of lipoprotein lipase to the apoC-II-DNS-peptides, which do not contain tryptophan, demonstrated specific enzyme-activator association in the absence of a lipid substrate.

METHODS

Bovine milk lipoprotein lipase was purified by the method of Kennunen (3). Fractions that contained only one band by electrophoresis on SDS gels were used. Peptides were synthesized by the method of Sparrow (4). To introduce the DNS probe, the amino terminal protecting group of individual peptides was removed with 50% TFA/CH_2Cl_2. After neutralization of the reaction mixture with 5% diisopropylethylamine in CH_2Cl_2, the α-amino group was reacted with dansyl chloride and 1% diisopropylethylamine in CH_2Cl_2. Peptides were cleaved from the resin with simultaneous removal of protecting groups by treatment with HF. Initial peptide purification was done by chromatography on DEAE-Sephadex in 6 M urea. In some cases, DNS-peptides were further purified by reversed phase HPLC on octadecylsilyl-silica in 1% triethylammonium phosphate utilizing a gradient of 0–50% 2-propanol. Criteria for homogeniety were: the correct amino acid analysis, the presence of a single band by isoelectric focusing, and a single peak on reversed phase HPLC. Fluorescence measurements were made at 20°C on a SLM single photon-counting spectrofluorimeter. Excitation wavelength was 280 nm. The increase in fluorescence intensity at 490 nm was the measure of resonance energy transfer from tryptophan to the dansyl group. Individual DNS-peptides were added to 1.9 μM lipoprotein lipase in 0.05 M Tris, pH 8.3, containing 0.15 M NaCl and 0.01 M $CaCl_2$. Peptide concentrations ranged from 0.2–5.0 μM.

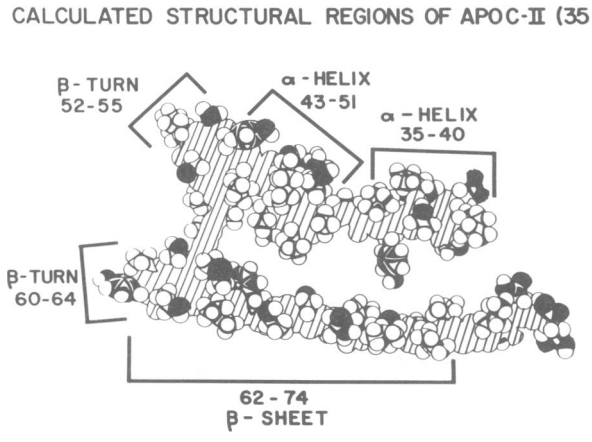

FIGURE 1 Proposed secondary structure of ApoC-II (35–78).

RESULTS AND DISCUSSION

The peptides apoC-II-DNS-(64–78), apoC-II-DNS-(60–78), apoC-II-DNS-(55–78), apoC-II-DNS-(50–78) and apoC-II-DNS-(43–78) gave activation of lipoprotein lipase that was 0, 60, 80, 90, and 100%, respectively, of that produced by native apoC-II. A double reciprocal analysis of the changes in fluorescence intensity as a function of DNS-peptide concentrations gave association constants of 0.25, 1.25, 4.6, 0.5, and 2.0 $\times$ 10^6 M^{-1}, respectively, for these peptides. Energy transfer from apoC-II-DNS-(55–78) was abolished by guanidium chloride. No interaction could be detected between bovine serum albumin and apoC-II-DNS-(43–78) by this technique. Competitive displacement of apoC-II-DNS-(43–78) by apoC-II-(55–78) indicated that the association of lipoprotein lipase and the unsubstituted peptide was about one order of magnitude lower affinity than that between the fluorescent peptide and the enzyme. Energy transfer also occurred from the enzyme presence of a nonhydrolyzable substrate, single walled vesicles of 1-oleyl-2-palmityl-2-phosphoryl choline glyceryl ether, that contained 2 mol % trioleylglyceryl ether and 10 mol % cholesterol.

Bengtsson and Olivecrona (5) have reported a k_a of $\sim 10^6$ M^{-1} for lipoprotein lipase-apoC-II interaction in the presence of a phosphatidylcholine-trioleoylglycerol emulsion. Our data, determined in the absence of a substrate, gave values for k_a that were the same order of magnitude.

The putative protein secondary structure deduced by the Chou and Fasman analysis (6), can be divided into discrete regions: a β-sheet between residues 62 and 74, β-turns involving residues 60–64 and 52–55 and α-helixes consisting of residues 51–43 and 35–40 (Fig. 1). Thus, we propose that the enhancement of lipoprotein lipase catalysis by apoC-II is primarily due to direct protein-protein interactions involving residues 55–74, rather than an effect of apoC-II on the substrate surface. The specific protein-protein interaction between lipoprotein lipase and apoC-II may also serve as a mode of attachment of lipoprotein lipase to its substrate at the endothelial surface.

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THERMODYNAMICS OF LIPID-PROTEIN ASSOCIATION IN HUMAN PLASMA LIPOPROTEINS

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The plasma lipoproteins are water-soluble macromolecules which are the primary transport vehicles for lipids in blood (1). We have been working on a thermodynamic model of lipid-protein association that would correctly predict the free energy of this process. That the major polar components are water soluble and freely exchange between lipoproteins can be taken as tacit evidence for their equilibrium distribution between lipoproteins (1–3).

In aqueous solutions, the unitary free energy of association, ΔG_a, of an amphiphile with a lipoprotein may be

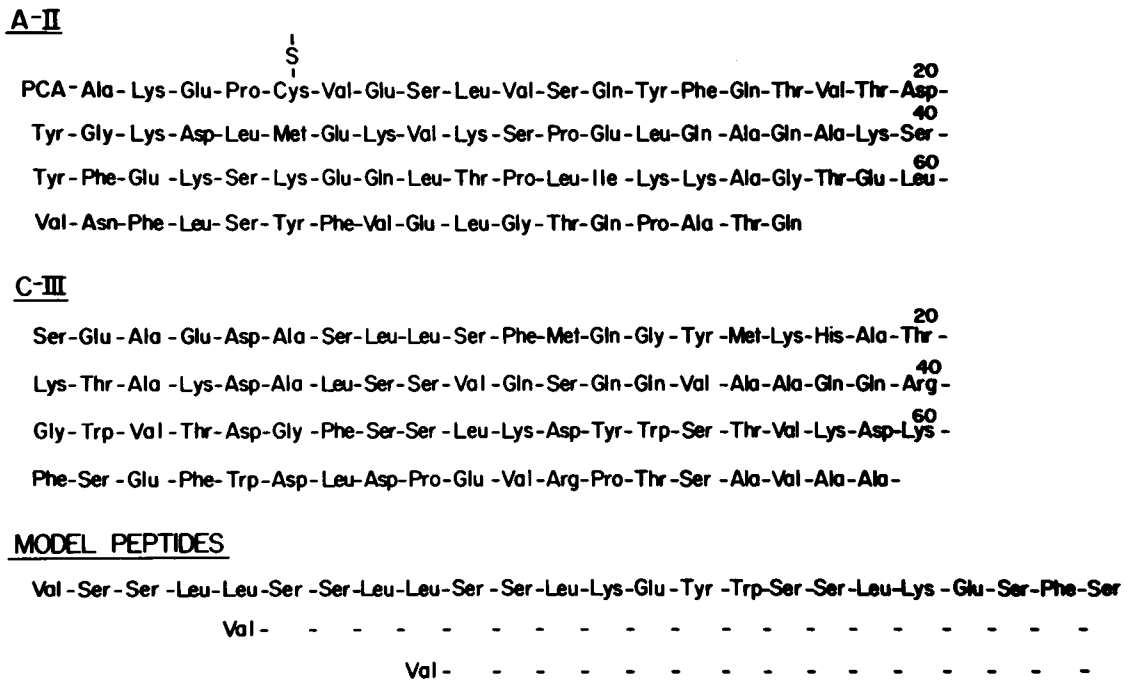

FIGURE 1 Primary structure of the polypeptides of this investigation. Reduced apoA-II monomer is carboxymethylated at Cys-6. The model peptides are, from top to bottom, designated in the text as LAP-24, LAP-20, and LAP-16.