Biol. Chem. Hoppe-Seyler
Vol. 369, pp. 1045–1054, September 1988

Expression of Normal and Mutagenized Apolipoprotein CII in Procaryotic Cells

Structure-Function Relationship

Cordula HOLTFRETER and Wilhelm STOFFEL

Institut für Physiologische Chemie der Universität zu Köln

(Received 6 June 1988)

Summary: A full-length human apo CII cDNA clone has been constructed by completing the 5′ end of an incomplete cDNA with a 44 bp long synthetic oligonucleotide. This apo CII cDNA insert was cloned into the pSP19 expression vector and transcribed and translated in vitro. Its N-terminal signal sequence (23 amino-acid residues) was accurately cleaved during cotranslational translocation through endoplasmic reticulum membranes to yield the mature apo CII.

Mature apo CII was expressed on a preparative scale as fusion protein apo CII-β-galactosidase with the full-length apo CII cDNA integrated into the pUR291 vector. Furthermore it was expressed in *E. coli* transformed with the pKK233-2 apo CII clone. The preform was accurately processed by the host cell.

C-Terminal apo CII deletion mutants generated by partial *Bal*31 digestion of the pKK233-2 apo CII vector yielded well-defined truncated apo CII polypeptides on a preparative scale which allowed the determination of the polypeptide domain responsible for the activation of the serum lipoprotein lipase.

Expression von normalem und mutagenisiertem Apolipoprotein CII in procaryotischen Zellen.
Struktur-Funktionsbeziehungen.

Zusammenfassung: Durch Vervollständigung des 5′-Endes eines humanen Apo-CII-cDNA-Klons mit Hilfe eines 44 bp langen synthetischen Oligonucleotids wurde ein kompletter cDNA-Klon konstruiert. Diese Apo-CII-cDNA wurde in den pSP19-Expressionsvektor kloniert und in vitro transkribiert und translatiert. Ihre N-terminale Signalsequenz (23 Aminosäurereste) wurde während der cotranslationalen Translokation durch Membranen des endoplasmatischen Retikulums korrekt zum reifen Apo CII abgespalten.

Das reife Apo-CII konnte im präparativen Maßstab als Fusionsprotein mit β-Galactosidase nach Insertion der vollständigen Apo-CII-cDNA in den pUR291-Vektor erhalten werde.

Weiterhin wurde der cDNA-Klon in *E.-coli*-Zellen exprimiert, die mit dem pKK233-2-Apo-CII-Klon transformiert waren. Die Präform wurde durch die Wirtszellen exakt prozessiert.

C-Terminale Apo-CII-Deletionsmutanten, die durch partiellen *Bal*31-Abbau des pKK233-2 erzeugt wurden, führten im präparativen Maß-

---

Abbreviations:
apo CII, human serum apolipoprotein CII; ELISA, enzyme-linked immuno-sorbent assay; HTGL, human hepatic triacylglycerol lipase; LPL, human serum lipoprotein lipase; $NaDodSO_4$, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein class; dsDNA, double-stranded DNA; cDNA, complementary DNA; BSA, bovine serum albumin; ER, endoplasmic reticulum; Mops, 4-morpholinepropanesulfonic acid.

stab zu definiert verkürzten Apo-CII-Polypeptiden. Hierdurch war eine Bestimmung derjenigen Polypeptid-Domäne im Apo-CII möglich, die für die Aktivierung der Serum-Lipoproteinlipase verantwortlich ist.

Key words: Apo CII cDNA/in vitro transcription-translation/cotranslational translocation and processing/expression in *E. coli*/mutagenized apo CII and LPL activation properties.

Serum apolipoproteins function as lipid binding, solubilizing and carrier proteins. In addition they are responsible for other important functions such as extracellular lipid homeostasis, cellular lipid metabolism and maintenance of lipid structural membrane elements. Apo C polypeptides, particularly apo CII, play a key role in the regulation of the enzymatic activity of serum lipoprotein lipase (LPL)[1,2]. Unlike the hepatic triacylglycerol lipase (HTGL)[3,4] the lipoprotein lipase (LPL) reaches its optimal lipolytic activity only in the ternary complex consisting of the VLDL or chylomicron particle, LPL and apolipoprotein CII. Although the details of the enzymatic mechanisms within this complex are not understood certain domains of the 79 amino-acid residues long mature apo CII polypeptide[5] seem to be involved in triacylglycerol, fatty acid and heparin binding whereas the C-terminal region between residues 43 to 79 has been suggested as the LPL-binding and activation domain[6,7]. The isolation and characterization of apo CII-specific cDNA clones[8–11] and the subsequent elucidation of the apo CII gene organization and its allocation to chromosome 19q13[12,13] confirmed the amino-acid sequence of the mature form and in addition revealed that the primary translocation product carries a 23 amino acid N-terminal signal sequence.

Protein engineering of apo CII by site-directed mutagenesis, expression of the mutated apo CII gene in procaryotic cells, its isolation and purification should permit the detailed study of the afore-mentioned functions of the apoprotein with its key function in the lipolysis of chylomicron and VLDL triacylglycerols.

In this report we describe the construction of a full-length apo CII-specific cDNA clone, the in vitro expression of the apo CII cDNA cloned into the pSP19 vector, the expression of apo CII as fusion protein with β-galactosidase when cloned into the pUR291 vector and of the mature apo CII cloned into the pKK233-2 vector and its isolation on a preparative scale. *Bal*31 exonuclease digestion yielded several apo CII mutants truncated at the C-terminus. Six of these mutant apo CII cDNAs were characterized and expressed on a preparative scale in *E. coli*. In structure-function correlation studies the apo CII polypeptides were compared with respect to their serum lipoprotein lipase activating properties in the in vitro assay.

Materials and Methods

Apo CII was isolated from human serum VLDL by HPLC and isoelectric focussing[14] followed by electroelution and used for antibody production in rabbits. The IgG fraction was isolated by $(NH_4)_2SO_4$ precipitation and DEAE Sephacel chromatography[15].

The decapeptide Asp^7-Thr^{16} of the mature human apo CII was synthesized by the Merrifield solid-phase synthesis method[16], coupled to hemocyanin[17] and used as antigen. Antibody titers were determined with the ELISA[18].

Lipoprotein lipase activity was assayed following a modified procedure described by Ehnholm et al.[19] and Schotz and Garfinkel[20]. Radiolabelled triolein was used as substrate.

Recombinant DNA techniques

Standard techniques such as subcloning, the treatment of DNA with restriction endonucleases, *Bal*31 exonuclease, DNA polymerase, DNA ligase and the screening of λgt11 libraries, colony screening, large scale phage DNA preparation and plasmid isolation were applied[21].

A human liver λgt11 cDNA library was screened and the positive apo CII clone rescreened with a 45^{mer} oligonucleotide coding Met^{58}-Val^{74} as hybridization probe.

Oligonucleotides were synthesized by the solid phase phosphoamidite method on an Applied Biosystems model 380A oligonucleotide synthesizer[22].

The double-strand sequencing technique with the appropriate synthetic primers[23,24] and the dideoxy chain-terminating method for DNA fragments subcloned into M13 phage vectors[25] were used.

The five synthetic oligonucleotides required for the completion of the apo CII cDNA at the 5′ end were mixed in an equimolar ratio, 5′-phosphorylated, hybridized (5 min 100 °C, 15 min 65 °C, 1 h room temperature) and ligated.

This dsDNA sequence was ligated to the *Ava*II 5′ end of the incomplete apo CII cDNA (bp 65-442) with its 3′ *Hpa*II end. The resulting *Bam*HI-*Hpa*II fragment comprising the coding region of apo CII was cloned into the pUC13 vector, Fig. 1.

The (Δ pre)apo CII cDNA mutant was generated for in vivo expression from the pSP19 apo CII clone by

linearization with *Ava*II, partially filled in with dTTP and dGTP and then restricted with *Hin*dIII. The truncated apo CII cDNA was isolated and purified by electrophoresis through 1% agarose onto a NA 45 membrane (Schleicher & Schüll, Düren) and after elution ligated into the pKK233-2 plasmid which had been restricted with *Nco*I before and partially filled in with dATP, dCTP, dTTP.

Southern blot hybridization

DNA fragments, separated by electrophoresis through agarose gels, were transferred to a Gene Screen membrane (NEN) and hybridized under conditions recommended by the manufacturer[26].

Hybridization analysis of RNA

RNA synthesized in vitro was denatured in 50% formamide/2.2M formaldehyde at 68 °C for 5 min and electrophoresed through a 1% agarose gel containing 2.2M formaldehyde[27]. RNA was transferred to Gene Screen Plus membranes for hybridization or direct autoradiography when a labelled precursor was used.

In vitro expression

a) Transcription

Transcription in vitro with the pSP system[28] was carried out as described before[29]. pSP19 apo CII was linearized with *Pst*I and transcribed in the presence of ^{m7}G (5′) ppp(5′)G with SP6 polymerase (Boehringer, Mannheim) to the capped mRNA.

b) Translation

Aliquots of the apo CII-specific mRNA were used for priming the reticulocyte lysate or wheat germ system (Amersham) for in vitro translation in the presence of [^{35}S]methionine under the conditions used before[30–32]. The in vitro synthesized polypeptides were analysed by $NaDodSO_4$ PAGE and viewed by fluorography and autoradiography.

c) Immunoprecipitation

For immunoprecipitation of apo CII from in vitro translation assays either monospecific antisera, the IgG fraction isolated by $(NH_4)_2SO_4$ and DEAE Sephacel chromatography or anti apo CII decapeptide IgG were used. Anti apo CII antibodies were purified by affinity chromatography on a decapeptide Sepharose CL-4B column.

In vivo expression of the apo CII gene in E. coli

An overnight culture of the pKK233-2 apo CII clone in JM105 or W3110 in LB medium was grown after 1 : 100 dilution in M9 minimal medium (200 μg/m*l* Ampicillin) to A_{600} = 0.5, then induced with IPTG (final concentration 10^{-4}M). The labelled precursor [^{35}S]methionine (15 μCi/m*l*) was added and cells harvested after 5 min, 4 and 16 h. The reaction was stopped by the addition of NaN_3 (0.1M) and methionine (10^{-3}M).

Protein isolation

Cells were sedimented by centrifugation, washed three times with buffer (0.5M NaCl, 0.5% Nonidet P40, 5mM EDTA, 50mM Tris/HCl, pH 7.5, 1 mg/m*l* BSA), resuspended in 25mM Tris/HCl, pH 8.0, 50mM glucose, 10mM EDTA and after addition of lysozyme (4 mg/m*l*) incubated for 5 min, briefly sonified and treated with DNAse I (1 mg/m*l*) for 30 min at room temperature.

The lysate was heated in the sample buffer for 5 min. 2 m*l* lysate was dialysed against 0.1M MOPS buffer pH 7.5 at 4 °C and adsorbed to 2 m*l* anti apo CII Ig coupled to Affigel-10, filled into a 5 m*l* plastic syringe, washed with 0.1M Mops buffer and 40 m*l* NaCl/$PO_4^{3\ominus}$. Apo CII was eluted with 4 m*l* 1M NH_4OH, the eluent dialysed against 20mM sodium phosphate buffer pH 7.0 and lyophilised for $NaDodSO_4$ PAGE.

Results

Construction of vectors for in vitro and in vivo expression of apo CII

A 45^{mer} oligonucleotide was used for screening approximately 6 × 10^5 recombinant phages of a λgt11 human liver library[33]. An apo CII cDNA clone which missed 44 bp at the 5′ terminus was isolated and completed with five overlapping synthetic oligonucleotides as outlined in Fig. 1.

Since the *Eco*RI site at the 3′-terminal end of the 350 bp long cDNA insert was defect, a *Hpa*II site 12 bp downstream from this *Eco*RI site was

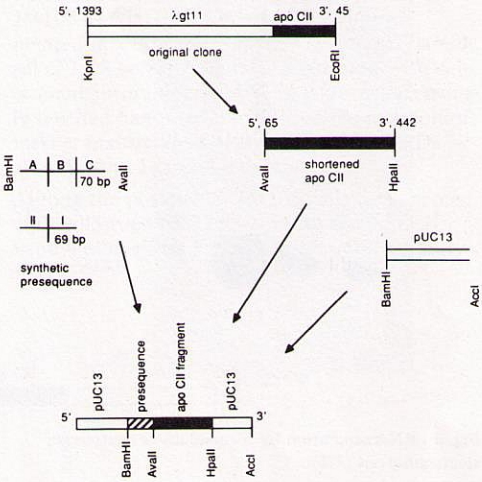


Fig. 1. Completion and characterisation of human apo CII cDNA clone.

The 5′-overlapping oligonucleotides resembling the presequence of apo CII were first ligated and then linked with the 5′ end to the pUC13 vector and with the 3′ end joined to the 5′*Ava*II-3′*Hpa*II-truncated apo CII fragment.

chosen for further subcloning e.g. into the compatible *Acc*I site of pUC13. DNA sequence analysis confirmed the 440 bp long apo CII insert.

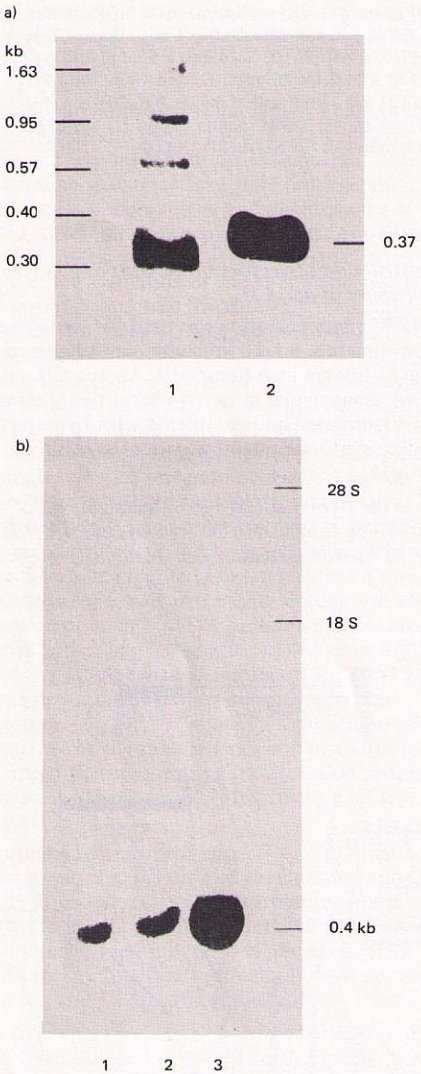


Fig. 2. RNA separation by formaldehyde agarose gel electrophoresis (1%).

a) Lane 1: transcript of apo CII cDNA cloned into the *Bam*HI-*Pst*I sites of the multicloning sequence of pSP19. Radioactive precursor: [α-^{32}P]UTP; lane 2: pBR/*Hin*fI DNA marker.
b) Northern blot hybridization of RNAs. Hybridization probe: labelled 45^{mer} oligonucleotide. Lane 1: human liver RNA (A_{260} = 40); lane 2: human liver RNA (A_{260} = 32); lane 3: apo CII transcript in pSP19 vector.

The 3′ terminus ends at position 426. Eight bp of the poly A$^+$ sequence are missing but it contains 12 bp of the λgt11 sequence.

In vitro expression of apo CII cDNA

Apo CII cDNA was subcloned into the pSP19 vector[28,34]. The vector was linearized with *Hin*dIII and transcription carried out in the presence of capping reagent and either [α-^{32}P] UTP or cold NTPs. The apo CII-specific mRNA was separated by 1% agarose gel electrophoresis and visualized either by autoradiography (Fig. 2a) or Northern blot hybridization analysis with the labelled apo CII-specific 45^{mer} oligonucleotide, Fig. 2b. The size of the in vitro synthesized transcript was identical with the apo CII mRNA present in liver RNA.

Priming the reticulocyte lysate or the wheat germ translation system with the apo CII-specific mRNA led to a 11 kDa primary translation product. Translation in the presence of dog pancreatic ER membranes resulted in products of approximately 9 kDa. These [^{35}S]-methionine-labelled products were immunoprecipitated with anti apo CII IgG for separation by $NaDodSO_4$ polyacrylamide gel electrophoresis (20%) and subsequent autoradiography, Fig. 3.

The electrophoretic mobilities of the primary and processed translation products proved that the presequence had been removed during co-translational translocation by the signal peptidase.

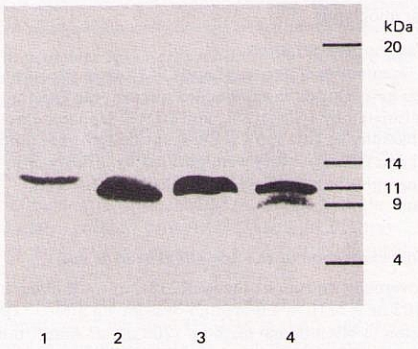


Fig. 3. Autoradiogram of in vitro transcribed and translated apo CII cDNA.

20% $NaDoSO_4$ polyacrylamide gel electrophoresis of immunoprecipitated products. Lane 1: primary transcript synthesized with reticulocyte lysate system; lane 2: addition of canine pancreatic membranes; lane 3: primary transcript synthesized with wheat germ system; lane 4: addition of canine pancreatic membranes; numbers at the right indicate positions of marker polypeptides.

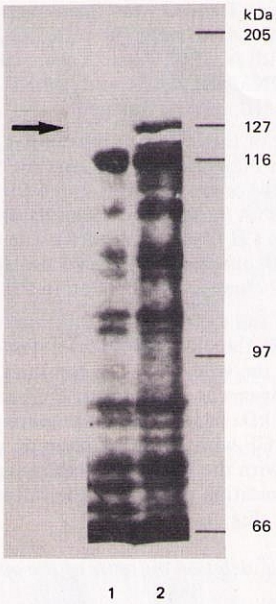


Fig. 4. $NaDodSO_4$ polyacrylamide gel electrophoresis of *E. coli* proteins (7.5%), Commassie blue staining.

Lane 1: *E. coli* JM105 transformed with pUR291; lane 2: *E. coli* JM105 transformed with pUR291 apo CII. The arrow indicates the position of the apo CII-β-galactosidase fusion protein at 127 kDa.

This was confirmed by radio sequencing of the [^{35}S]methionine labelled synthesis products.

Expression of apo CII cDNA as fusion protein

Major amounts of apo CII and its mutant derivatives were required for activation studies of serum lipoprotein lipase which could not be prepared by in vitro synthesis.

We therefore cloned the apo CII cDNA insert first into the *Bam*HI-*Pst*I sites of the procaryotic expression vector pUR291[35] which leads to the expression of a fusion protein of apo CII and β-galactosidase in *E. coli* and studied its stability against endogenous proteases of *E. coli* and the toxicity of this lipid binding apoprotein in the host cell. Fig. 4 shows the $NaDodSO_4$ polyacrylamide gel electrophoretic separation (7.5%) of the *E. coli* proteins.

It is apparent that besides the 116 kDa β-galactosidase the fusion protein at 127 kDa is also present in the protein extract of the pUR291 apo CII-transformed JM105 cells. The cleavage of the chimeric protein after secretion through the cytoplasmic membrane has been postulated for this phenomenon which has been observed also with the lysozyme-β-galactosidase fusion protein[35].

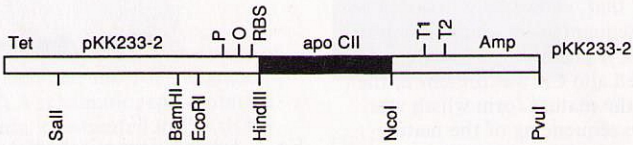
Expression of apo CII cDNA as mature polypeptide chain

A suitable vector for the expression of eucaryotic genes in procaryotic cells is the pKK233-2 vector described by Amann and Brosius[36] with the strong IPTG-inducible tac-promoter.

Fig. 5 outlines the organization of the pKK233-2 apo CII vector with the apo CII cDNA insert in the *Nco*I-*Hin*dIII restriction site.

Pulse labelling experiments of IPTG-induced JM105 (lac I^q) in [^{35}S]methionine-supplemented medium and $NaDodSO_4$ polyacrylamide gel (17.5%)-electrophoretic separation with subsequent autoradiography (Fig. 6) revealed strongly labelled bands in lanes 4–6 at the position of marker mature apo CII from VLDL at 9 kDa after 5-min-, 1- and 4-h pulses.

Among the proteins of JM105 cells transformed with wild-type pKK233-2 vector the apo CII band was missing. The electrophoretic mobility



Abbreviations:

P	promoter
O	operator
RBS	ribosomal binding site
T1, T2	terminators of transcription
Tet	tetracyclin sensitivity
Amp	ampicillin resistance

Fig. 5. Organization of the pKK233-2 apo CII plasmid.

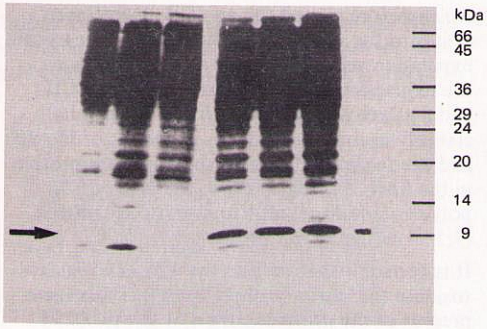


Fig. 6. Autoradiogram of $NaDodSO_4$ PAGE (17.5%) of pulse-labelled JM105 cells transformed with pKK233-2 vector for 1: 5 min; 2: 1 h; 3: 4 h; of JM105 cells transformed with pKK233-2 apo CII for 4: 5 min; 5: 1 h; 6: 4 h.

The arrow indicates the position of the mature apo CII at 9 kDa.

of apo CII within the protein mixture suggested that the presequence of apo CII was lost in a bacterial processing of preapo CII. This was further proven by the purification of the apo CII fusion protein from the *E. coli* lysate. The isolation and purification of apo CII from the abundant *E. coli* proteins was achieved by immunoaffinity chromatography. Superior to the purification of apo CII-specific polyclonal antiserum from anti *E. coli* protein antibodies via *E. coli* protein Sepharose 4B chromatography was the purification of the anti apo CII IgG fraction by selective adsorption to an apo CII peptide Affigel-10 matrix.

The results of the two purification methods and the identification of the product as the mature apo CII with a molecular mass of 9 kDa is summarized in Fig. 7. The yield of purified apo CII was in the range of 10–15 μg/m*l* of the stationary bacterial culture.

It should be noted that neither by immunoprecipitation and subsequent autoradiography nor by ELISA any apo CII protein could be detected. All the radiolabelled apo CII was present in the bacterial lysate in the mature form which was confirmed by radio sequencing of the mature form ([^{35}S]Met in cycle 9, not shown).

Construction of an apo CII cDNA clone with deleted signal sequence and its expression in E. coli

Full length apo CII cDNA was restricted with *Ava*II which cuts at position 65 and deletes the presequence. T and G were filled in the recessed 3′ end of the *Ava*II site. The pKK233-2 vector was cut with *Nco*I. C, A and T were filled in with T_4 DNA polymerase and ligated with the *Ava*II-*Hin*dIII apo CII fragment.

The result of these cloning procedures are summarized in Fig. 8a which presents the Southern blot analysis of the pKK233-2 apo CII (Δ pre) mutated DNA (lane 1) and of the *Bam*HI-*Hin*dIII (Δ pre)apo CII fragment (lane 2). The nucleotide sequence of the 5′ end of the (Δ pre)apo CII-pKK233-2 clone is documented in Fig. 8b.

Induction and expression of this mutant apo CII DNA in the pKK233-2 vector was carried out as for the wild-type. The resulting apo CII protein appears as a prominent [^{35}S]methionine-labelled 9 kDa band in autoradiography of the 17.5% PAGE with an electrophoretic mobility identical with the in vivo synthesized and processed translation product of the full length apo CII cDNA, Fig. 9.

3′-Terminal deletion mutants of the apo CII gene

The pKK233-2 apo CII DNA was linearized at the 3′ terminus with *Hin*dIII and partially

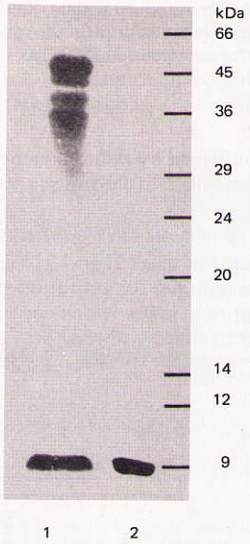


Fig. 7. Autoradiogram of $NaDodSO_4$ PAGE of ^{35}S-Met labelled JM105 cells transformed with pKK233-2 apo CII.

Lane 1: Proteins of JM105 cells transformed with pKK233-2 apo CII, immunoprecipitated with anti apo CII IgG purified by *E. coli* protein Sepharose 4B chromatography; lane 2: as in 1, but IgG were purified by apo CII peptide affinity chromatography.

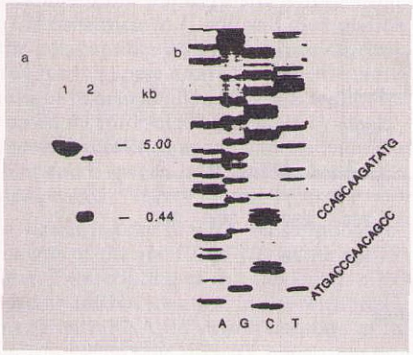


Fig. 8. a) Southern blot analysis of 1: pKK233-2 apo CII (Δ pre)DNA and 2: pKK233-2 apo CII (Δ pre)DNA restricted with *Bam*HI-*Hin*dIII.

b) Nucleotide sequence of the 5′-end of pKK233-2 apo CII (Δ pre) DNA.

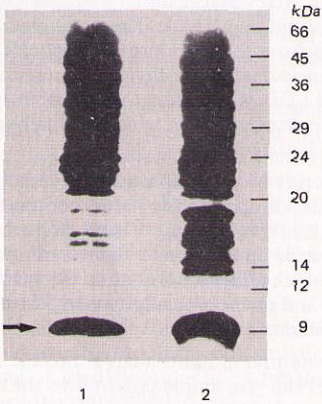


Fig. 9. *Expression of full-length apo CII cDNA (lane 1)* and apo CII cDNA-deleted signal sequence in pKK233-2 vector (lane 2).

Autoradiogram of [^{35}S]methionine-labelled proteins of *E. coli* JM105 proteins, 17.5% PAGE.

digested with *Bal*31 exonuclease. Some parts of the vector DNA which had also been degraded had to be replaced. A schematic representation of this reconstitution is presented in Fig. 10a. The degraded DNA ranges between 5.0 and 3.8 kb as compared to 5.044 kb of the undigested pKK233-2 apo CII clone, Fig. 10b.

492 colonies with mutated pKK233-2 apo CII were investigated. Those with deletions useful for the structure-function studies were selected with three different 24^{mer} oligonucleotides hybridizing to nucleotide sequences coding Ser^{56}-Thr^{63}, Thr^{64}-Val^{71}, Leu^{72}-Gln^{79} of the C-terminus of the mature apo CII sequence and a 30^{mer} probing the sequence downstream the stop codon. Six deletion mutants were characterized as schematically presented in Fig. 11.

These mutants were used for in vivo expression in *E. coli* JM105 and the proteins assayed for their activation of serum lipoprotein lipase.

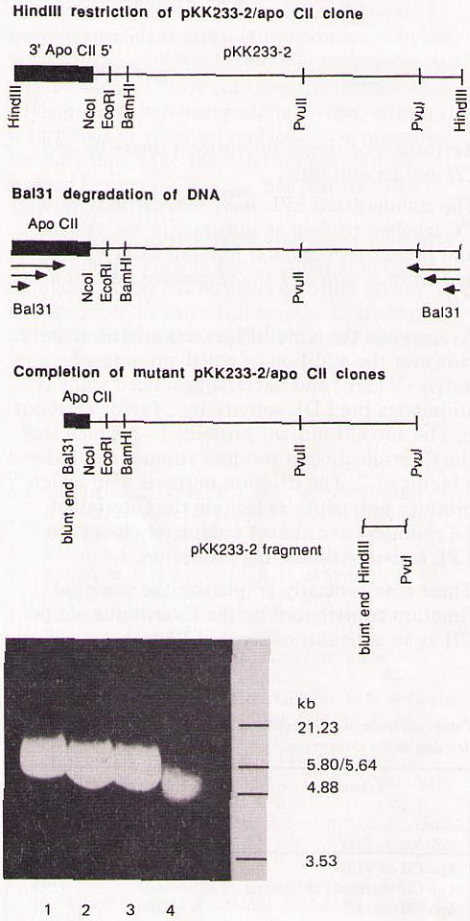


Fig. 10.

a) Deletion mutants of pKK233-2 apo AII with 3′-terminal truncated coding sequences obtained by partial *Bal*31 exonuclease digestion.

b) Agarose gel electrophoresis (1%) of 3′ deletion mutants linearized with *Hin*dIII. 1: pKK233-2 apo CII linearized with *Hin*dIII; 2: *Hin*dIII-*Bal*31 30 s; 3: 45 s; 4: 60 s.

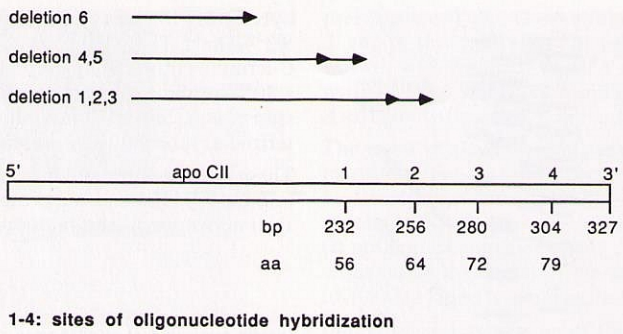


Fig. 11. 3′ Deletion mutants of apo CII cDNA.

Activation of serum lipoprotein lipase by apo CII and its mutants

The standardized LPL assay was carried out with ^{14}C-labelled triolein as substrate in the absence and presence of apo CII isolated from VLDL and from *E. coli* and the respective mutant apo CII.

As shown in the table LPL exerts a basic activity. However the addition of equal amounts of native (VLDL) and bacteria-produced apo CII stimulates the LDL activity by a factor of about 6. The apo CII mutant proteins 1–3 which lack the C-terminal eight residues stimulate only by a factor of 2. The deletion mutants 4–6 which produce polypeptides lacking the C-terminal 16 residues have almost completely lost their LPL activity-stimulating properties.

These results clearly emphasize the essential function contributed by the C-terminus of apo CII as an activator of serum LPL.

Table. Activation of lipoprotein lipase by apo CII proteins with deletion at the C-terminus.

Activator	Free fatty acids [nmol × ml^{-1} × h^{-1}]*	Factor
Apo CII of VLDL	649	6.4
Apo CII (purified) of bacteria	601	5.8
Apo CII del. 1	209	2.1
Apo CII del. 2	236	2.3
Apo CII del. 3	179	1.8
Apo CII del. 4	147	
Apo CII del. 5	161	
Apo CII del. 6	161	
pKK233-2 wild-type lysate (100–400 μl)	75	
Test without activator	102	

* Released from triolein.

Discussion

Apo CII, a 79 amino-acid residues polypeptide, plays an essential role in lipid transport particularly of triacylglycerol-rich particles such as chylomicrons and VLDL. The rapid clearing of serum from these lipoprotein particles and their transformation to chylomicron remnants, IDL and LDL, is dependent on the activation of serum lipoprotein lipase which normally works at a low basic activity.

The key role of apo CII in triacylglycerol metabolism is apparent in natural mutants of apo CII e.g. apo $CII_{Toronto}$ [37] and two other preliminarily analysed gene defects of apo CII[38] which all lead to functionally inactive apo CII and phenotypically to a hypertriglyceridemia.

A set of natural mutants analysed to the molecular level can give ample insight into the functional domains of a protein depending on the location of the mutation.

However site-directed mutagenesis allows the probing of functional domains at defined sites and even the design of apolipoproteins with particular functional domains or their deletion.

It has been postulated that apo CII normally isolated from VLDL and recently obtained by solid-phase synthesis[39,40] forms a ternary complex with the triacylglycerol-rich chylomicron or VLDL particle and lipoprotein lipase and therefore should have a lipoprotein lipase binding and/or activating site, a triacylglycerol- and fatty acid-binding site and presumably a heparin-binding site.

We describe here a first approach to unravel the functional domain of apolipoprotein CII by site-directed mutagenesis, the LPL-activating domain of apo CII. Essential for these studies was the

expression of the wild-type and mutagenized apo CII constructs in *E. coli* and the isolation of the polypeptides on a preparative scale required for the lipase assay. Site-specific mutagenesis of human apolipoprotein E within the proposed receptor-binding domain by single amino-acid substitution and expression of the mutant apo E species in *E. coli* has been reported recently[41].

First we carried out in vitro transcription experiments of the apo CII cDNA in the pSP19 system. Transcription of this full length cDNA yielded an mRNA identical in size with that in human liver mRNA. In vitro translation of this transcript yielded the primary translation product preapo CII. In the presence of canine pancreatic membranes processing to the mature sequence occurred.

Apo CII cDNA inserted into the expression vector pUR291 produced a fusion protein of apo CII-β-galactosidase in *E. coli* JM105 cells. This indicates that the apo CII moiety with high lipid affinity does not interfere with the metabolism of the host cell to an extent which would prohibit its expression. Activation studies of the lipoprotein lipase require the mature forms of the wild-type and mutants of the apolipoprotein. The pKK233-2 vector proved to be a suitable vector for the production of recombinant apo CII polypeptides. We succeeded in the in vivo expression of the apo CII cDNA with the signal sequence which however is proteolytically cleaved to the mature form by the plasma membrane of the bacterial host cell as the canine pancreatic ER membrane does in vitro. This is an additional example for the comparable events and specificities in the cotranslational processing of signal peptides in bacterial and the eucaryotic cells[42–45].

Expression of the apo CII cDNA in which the signal peptide is deleted resulted in the same product, the mature 79 residues apo CII. Interestingly the apolipoprotein is apparently secreted into the periplasmic space, since neither the medium nor the supernatant of the lysed bacteria but exclusively the pellet of the lysed host cells contained the translation products which could be isolated in homogenous form by immunoaffinity chromatography. Secretion into the periplasmic space has also been described for chicken albumin[46]. Purification of the IgG fraction of the antiserum by chromatograpny over *E. coli* protein Sepharose 4B is helpful but the best result was obtained with anti apo CII antibodies selectively extracted from the antiserum by specific adsorption to apo CII peptide Sepharose 4B or Affigel-10 and subsequent desorption.

With the affinity-purified recombinant apo CII polypeptides as activator proteins in the serum lipoprotein lipase assay we could demonstrate that the activation of lipoprotein lipase by recombinant wild-type apo CII is only slightly lower than with apo CII isolated from VLDL. Deletion of the C-terminal six amino-acid residues reduced the activation of lipoprotein lipase to less than one third. Apo CII truncated by 16 C-terminal residues has completely lost its activator properties.

Our in vitro deletion mutants of apo CII at the C-terminal part of the molecule with gene products from residues 1–72, 1–64 and 1–56 of normal apo CII found a correspondence in the apo $CII_{Toronto}$, isolated from a homozygous apo CII deficiency[37]. It contains the normal apo CII sequence between residues 1–68, with its C-terminal 11 residues replaced by a missense hexapeptide. Like the deletion mutants described here apo $CII_{Toronto}$ has lost its LPL-activating activity.

The approach described in this paper may be helpful in the elucidation of other functional domains of apo CII but also of other serum apolipoproteins since full length-cDNA clones of most of them are available for site-directed mutagenesis.

Literature

1 La Rosa, J.C., Levy, R.J., Herbert, P.N., Lux, S.R. & Fredricks, D.S. (1970) *Biochem. Biophys. Res. Commun.* **41**, 57–62.
2 Smith, L.C., Voyta, J.C., Catapano, A.L., Kinnunen, P.K.J., Gotto, A.M. & Sparrow, J.I. (1980) *N.Y. Acad. Sci.* **2**, 213–223.
3 Schrecker, O. & Greten, H. (1979) *Biochim. Biophys. Acta* **572**, 244–256.
4 Clarke, A.R. & Holbrook, J.J. (1985) *Biochim. Biophys. Acta* **827**, 358–368.
5 Jackson, R.L., Baker, H.N., Gilliam, E.B. & Gotto, A.M. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 942–945.
6 Musliner, A.T., Church, E.C., Herbert, P.N., Kinston, M.J. & Shulman, R.S. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5258–5262.
7 Kinnunen, P.K.J., Jackson, R.L., Smith, L.C., Gotto, A.M. & Sparrow, J.T. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4848–4851.
8 Jackson, C.L., Bruns, G.A.P. & Breslow, J.L. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2945–2949.
9 Myklebost, O., Williamson, R.B., Markham, A.F., Myklebost, S.R., Rogers, J., Woods, P.E. & Humphries, S.E. (1984) *J. Biol. Chem.* **259**, 4401–4404.
10 Sharpe, C.R., Sidoli, A., Shelley, C.S., Lucero, M.A., Shoulders, C.C. & Baralle, F.E. (1984) *Nucleic Acids Res.* **12**, 3917–3932.
11 Fojo, S.S., Law, S.W. & Brewer, H.B., Jr. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6354–6357.

12 Bruns, G.A.P., Karathanasis, S.K. & Breslow, J.L. (1984) *Arteriosclerosis* **4**, 97–102.
13 Wei, C.F., Tsao, Y.K., Robberson, D.L., Gotto, A.M., Brown, K. & Chan, L. (1985) *J. Biol. Chem.* **260**, 15211–15221.
14 Catapano, A.L., Jackson, R.L., Gilliam, E.B., Gotto, A.M. & Smith, L.C. (1978) *J. Lipid Res.* **19**, 1047–1052.
15 Garvey, J.S., Cramer, N.E. & Sussdorf, D.H. (1977) *Methods of Immunology*, 3rd ed., Benjamin Inc., Reading, Massachusetts.
16 Stewart, J.M. & Young, J.D. (1984) Pierce Chemical Company, 2nd ed.
17 Goodfield, T.L., Levine, L. & Fasman, G.D. (1964) *Science* **144**, 1344–1346.
18 Hudson, L. & Hay, F.C. (1980) *Practical Immunology*, 2nd edition, Blackwell Scientific Publishers, Oxford.
19 Ehnholm, C.H., Shaw, W., Greten, H. & Brown, W.V. (1975) *J. Biol. Chem.* **250**, 6756–6761.
20 Schotz, M.C. & Garfinkel, A.S. (1972) *J. Lipid Res.* **11**, 68–70.
21 Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.
22 Beaucage, S.L. & Caruthers, M.H. (1981) *Tetrahedron Lett.* **1981**, 1859.
23 Chen, E.J. & Seeburg, P.H. (1985) *DNA* **4**, 165–170.
24 Heinrich, P. (1986) *Guidelines for Quick and Simple Sequencing*, Boehringer Mannheim, Biochemica.
25 Sanger, F., Nicklen, S. & Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463–5467.
26 Southern, E.M. (1975) *J. Mol. Biol.* **98**, 503–517.
27 Lehrach, H., Diamond, D., Wozney, J.M. & Broedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
28 Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zim, K. & Green, M.R. (1984) *Nucleic Acids Res.* **12**, 7035–7055.
29 Konarska, M.M., Padgett, R.A. & Sharp, P.A. (1984) *Cell* **38**, 731–736.
30 Erickson, A.H. & Blobel, G. (1930) *Methods Enzymol.* **96**, 38–50.
31 Stoffel, W., Blobel, G., Walter, P. (1981) *Eur. J. Biochem.* **120**, 519–522.
32 Walter, P. & Blobel, G. (1983) *Methods Enzymol.* **96**, 84–93.
33 De Wet, J.R., Fukushima, H., Dawji, N.N., Wilcox, E., O'Brien, J.S. & Helinsky, D.R. (1984) *DNA* **3**, 437–447.
34 Krieg, P.A. & Melton, D.A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
35 Rüther, U. & Müller-Hill, B. (1983) *EMBO J.* **2**, 1791–1794.
36 Amann, E. & Brosius, J. (1985) *Gene* **40**, 183–190.
37 Conelly, P.W., Maguire, G.F., Hofmann, T. & Little, J.A. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 270–273.
38 Humphries, S.E., Williams, L., Myklebost, O., Stalenhoef, A.F.H., Demarker, D.N.M., Baggio, G., Crepaldi, G., Galton, D.J. & Williamson, R. (1984) *Hum. Genet.* **67**, 151–155.
39 Fairwell, T., Hopattankar, A.V., Brewer, H.B. & Khan, S.A. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4796–4800.
40 Balasubramaniam, A., Chopra, Y.M., Yates, M.T. & Jackson, R.L. (1987) *Bioinorg. Chem.* **15**, 141–151.
41 Lalazar, A., Weisgraber, K.H., Rall, S.C., Giladi, H., Innerarity, T.L., Lervanon, A.Z., Boyles, J., Amit, B., Gorecki, M., Mahley, R.W. & Vogel, T. (1988) *J. Biol. Chem.* **263**, 3542–3545.
42 Inouye, S., Wang, S., Sekizawa, J., Halegona, S. & Inouye, M. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1004–1008.
43 Talmadge, K., Stahl, S. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3369–3373.
44 Mercereau-Puijalon, O., Royal, A., Carni, B., Garapin, A., Krust, A., Gannon, F. & Koutilsky, P. (1978) *Nature (London)* **275**, 505–507.
45 Fraser, T.H. & Bruce, B.J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5936–5940.
46 Baty, D., Mercereau-Puijalon, O., Perrin, D., Kourilski, P. & Lazdunski, C. (1981) *Gene* **16**, 79.

C. Holtfreter and W. Stoffel, Institut für Physiologische Chemie der Universität zu Köln, Joseph-Stelzmann-Str. 52, D-5000 Köln 41.