Apolipoprotein CII from Chicken (Gallus domesticus)

THE AMINO-TERMINAL DOMAIN IS DIFFERENT FROM CORRESPONDING DOMAINS IN MAMMALS

(Yvonne Andersson, Solveig Nilsson, Anna Lindberg, Lars Thelander, and Gunilla Olivecrona‡)

From the Department of Medical Biochemistry and Biophysics, Umeå University, S-901 87 Umeå, Sweden

The amino acid sequence of chicken apolipoprotein CII (apoCII) was determined from cDNA sequencing and from partial protein sequencing. The chicken sequence showed an overall identity of around 30% to all the other previously known apoCII sequences. Comparison of the carboxy-terminal domain (residues 51–79, human numbering) showed at least 50% identity between species. By limiting the region to residues 51–70 the similarity was remarkably high, about 85%. This is in concert with the previous opinion that residues in the region 56–76 are directly engaged in binding to lipoprotein lipase and in activation of this enzyme. In contrast, in the amino-terminal end up to residue 50 (human numbering) less than 24% of the amino acid residues in chicken apoCII were identical to residues of any of the other species. In addition, chicken apoCII is four residues longer than human apoCII (83 versus 79 residues), probably due to an extension at the amino-terminal end. Although the sequence was completely different in the amino-terminal domain, the structures necessary for binding to lipid appear to be present in chicken apoCII. Secondary structure prediction showed that the amino-terminal domain could form two amphipathic \( \alpha \)-helices in almost similar areas of the sequence as was previously predicted for human apoCII.

Human apolipoprotein CII (apoCII)\(^1\) is a 79-amino acid residue protein that is associated with lipoproteins in plasma (1–5). It has a crucial role in lipoprotein metabolism as an activator for the enzyme lipoprotein lipase (LPL) (4). On hereditary deficiency of apoCII, plasma levels of triglycerides are highly increased due to accumulation of chylomicrons and very low density lipoproteins in blood (4, 5). Studies with fragments of human apoCII have shown that the amino-terminal domain (residues 1–50) is involved in binding to lipid, while the carboxy-terminal domain (residues 55–79) binds to and activates LPL (6, 7).

The amino acid sequences are known for apoCII from eight mammalian species: human (8), monkey (9), dog (10), cow (11), sheep (12), rat (13), mouse (14), and guinea pig (15). The gene structures are known for human (16) and mouse apoCII (14). All these sequences are highly homologous throughout most of the molecule. The LPL-apoCII system developed early in evolution (17). We speculated that activator proteins from evolutionary distant animals might retain strong sequence homology in those parts of the molecule that are directly involved in protein-protein interactions with LPL, while other parts might be less conserved (18). In a previous study, apoCII-like activity was found in chicken egg yolk (19). After partial purification the yolk activator could stimulate bovine LPL to the same extent as human apoCII, indicating that the critical parts of the molecule are indeed conserved (20). The yolk activator appeared to be present in two size variants, one larger at about 10 kDa and one smaller at about half that size, each of which was present in several charge variants (19).

The aim of the present study was to resolve the amino acid sequence for the chicken activator and compare its structure with that of mammalian apoCII variants. For this we have isolated activator from rooster plasma, determined partial amino acid sequences, and used these to identify cDNA clones from a chicken liver cDNA library. The results show a highly conserved region of 20 amino acid residues in the carboxy-terminal domain, flanked by regions with little or no homology at the amino acid level.

EXPERIMENTAL PROCEDURES

**Materials—** Rooster blood (Gallus domesticus) was mixed with two-thirds volume of 0.1 M glucose, 72 mM NaCl, 27 mM sodium citrate (pH 6.1) to prevent coagulation. Plasma was collected after centrifugation and was stored at −20 °C. Immobilon filters were from Millipore, Bedford, MA. Polyvinylidene difluoride filters, PVDF (0.2 micron) were from Bio-Rad. Nitrocellulose filters were from Schleicher & Schuell, Dassel, Germany. Restriction endonucleases, the cDNA synthesis kit, Sephadex G-75 superfine, and DEAR-Sepharose were from Pharmacia LKB Biotechnology, Uppsala, Sweden. T4 polynucleotide kinase was from MBI Fermentas Molecular Biology, Vilnius, Lithuania. Cloned Pfu DNA polymerase was from Strategene, La Jolla, CA. Oligonucleotides were synthesized on an Applied Biosystems 392 RNA/DNA synthesizer. Oligo(dT)-cellulose was from Sigma. [\( \alpha \text{-32P} \)]dCTP, [\( \gamma \text{-32P} \)]ATP, and nucleic acid transfer membrane Hybond N+ and rainbow colored protein molecular weight markers were from Amersham Corp. The DNA Sequencing kit, version 2, was from U. S. Biochemical Corp. Intralipid\(^2\) is a commercial emulsion of soybean triacylglycerols in egg yolk phosphatidylcholine used for parenteral nutrition (Intralipid, 20%, Pharma- cia Hospital Care, Stockholm, Sweden). Microcon-3 was from Amicon Division, Beverly, MA. Trypsin-TPCK was from Worthington. Lipoprotein lipase was purified from bovine milk as described (21). Computer-assisted analyses were accomplished with the programs Lasergene DNA Star Inc, Madison, WI; and with Wisconsin sequence analysis package, Genetics computer group (GCG). Animal procedures were approved by the local ethical committee.

**Isolation of Lipid-binding Proteins from Rooster Plasma—** The excess of phospholipids that are present as lysosome-like structures was removed from Intralipid\(^2\) as described previously (22). The emulsion was then mixed with rooster plasma, recovered, and washed according to the method previously developed for bovine apoCII (22). Delipidation was done by mixing the emulsion with acetone/ethanol (1:1) in the ratio 1:20 (v/v). The mixture was stirred for 30 min on ice and was then left overnight at −20 °C. The precipitated proteins were recovered by centrifugation (2000 rpm, 15 min at −10 °C) and mixed again with acetone/ethanol (1:1) and left for 90 min at −20 °C. The centrifugation and

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\( ^1 \)The abbreviations used are: apoCII, apolipoprotein CII; LPL, lipoprotein lipase; PVDF, polyvinylidene difluoride; PCR, polymerase chain reaction; Tricine, N\(^2\)-hydroxy-1,1-bis(hydroxymethyl)ethyl glycine.

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\( ^\ddagger \)To whom correspondence should be addressed. Tel.: 46-90-165234; Fax: 46-90-167840; E-mail: guilla.olverona@medchem.umu.se.

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Preparation of cDNA Library—Total RNA was isolated as described previously (15). Poly(A) RNA was recovered after two cycles of adsorption to an oligo(dT)-cellulose column. The cDNA library was constructed from rooster liver RNA following the protocol for Pharmacia cDNA synthesis kit using 5 × 10^{-6} g of poly(A) RNA. The cDNA was further processed as described previously (15). The transformation frequency was 2.6 × 10^{5} pg DNA.

Labeling of Oligonucleotides—The oligonucleotides were labeled with [γ-32P]ATP using bacteriophage T4 polynucleotide kinase as recommended by the manufacturer.

Polymerase Chain Reaction (PCR)—To amplify the apoCII cDNA, reaction mixtures were prepared using PCR with primer A and B (Fig. 3). After completion the sample was purified by electrophoresis on 8% polyacrylamide gels (26). Bands were cut out and put in 0.5 mM ammonium acetate overnight at 37°C. The cDNA was precipitated with ethanol and dissolved. It was cleaved by restriction enzymes EcoRI and XhoI, ligated into pUC18, and transformed into MC1061, and clones were then analyzed for the presence of insert.

Screening of the cDNA Library—The oligonucleotide designated C, corresponding to nucleotide 214–251 of the chicken apoCII cDNA sequence, was constructed from the PCR product obtained using primers A and B. About 150,000 colonies were screened using probe C. The screening procedure was according to the manufacturer of Hybrid N+ membranes. The filters were prehybridized for at least 4 h at 50°C in 5 × SSC, 50 mM sodium phosphate (pH 7.0), 7.5 × Denhardt’s solution (26), and tRNA (0.25 mg/ml). Hybridization was carried out overnight at 50°C in fresh hybridization solution containing 4 × 10^{9} cpm of radioactive probe/ml. The filter was extensively washed, twice in 5 × SSC for 30 min at 50°C and twice in 5 × SSC for 30 min at 60°C. Autoradiograms were obtained using Kodak XAR films.

DNA Sequencing—DNA from the positive clones remaining after a second screening was prepared for sequencing of double-stranded DNA (26). Several different primers had to be used to allow sequencing of the whole inserts. The DNA was sequenced by the dideoxy terminator method using DNA Sequencing kit, version 2 (USB).

RESULTS

Initial experiments showed that none of the apoCII cDNA probes we had available from other species (human, rat, guinea pig) or synthetic probes covering the most conserved region of apoCII were sufficiently similar to allow identification of chicken apoCII. The probes did not hybridize to mRNA on Northern blots, and they did not recognize true positive clones in the chicken liver cDNA library. Therefore, chicken apoCII was isolated from plasma to generate protein sequences for synthesis of chicken-specific probes.

Chicken plasma stimulated the activity of bovine LPL in vitro, with Intralipid® as substrate, about as efficiently as plasma from many other animal species do (data not shown). The activating material floated with the lipid emulsion on centrifugation. Thus, the lipid-binding proteins from chicken plasma could be isolated by the method that had been previously used for purification of apoCII from several other species (11, 13, 15). After delipidation, the precipitated proteins were run on a column of Sephadex G-75 (Fig. 1A). Most of the activating ability was associated with the proteins of low molecular weight. They were analyzed by electrophoresis in an alkaline polyacrylamide gel system containing urea (Fig. 1B). The protein band of fraction 24 was sequenced in 18 cycles from the amino terminus and was found to be homologous to the previously studied apovitellenin I (27). The main protein band in fraction 26 had the amino-terminal sequence AHQS. Since there were several proteins in fractions 25–27, and since it was hard to determine which of these were activating, a further separation by electrophoresis on a dense Tricine-SDS-polyacrylamide gel was done on fraction 26 (Fig. 1C). After slicing of corresponding unstacked lanes, activation was localized to three minor components with apparent molecular masses of 7–10 kDa (a–c, Fig. 1C). On amino-terminal sequence analyses the band designated a showed the sequence RP2SDPPQQ; band b showed the sequence RPTS, while band c was not sufficiently pure for sequence analysis. On repeated sequence analyses of comparable materials the sequence RP2SDPPQQ (Q) was obtained, but after 8–9 cycles it was not possible to proceed further in any of the materials. This was probably due to the high content of proline residues in the amino-terminal end, which was later found to contain 6 Pro per 14 residues. On some occasions an additional Ser residue was found in the fourth position (RPTSSDPPQQ). Protein corresponding to fractions 25–27 from the Sephadex columns was pooled and run on precipitation steps were repeated twice. Finally, the protein pellet was resuspended in diethyl ether at room temperature for 30 min and was then centrifuged (2000 rpm, 5 min). The precipitated protein was recovered, suspended in diethyl ether, centrifuged as above, and then dried under nitrogen. The recovery was about 200 mg of lipid-binding protein (by weight) from 3.25 liters of plasma (from blood diluted by two-thirds volume citrate-containing buffer).

Purification of Lipase Activating Proteins—The delipidated protein was suspended in 8 M urea, 5 mM Tris-Cl (pH 8.2) (10 mg/ml). After centrifugation to remove insoluble material, about one-third of the material was applied on a column of Sephadex G-75 superfine (25 mm inner diameter × 720 mm) equilibrated in the same buffer as the sample. Fractions with activating proteins from three columns were pooled and further purified by chromatography on a column of DEAE-Sephadex A-50 (100 mm inner diameter × 100 mm) which was eluted by a linear gradient from 5 to 80 mM Tris-Cl (pH 8.6–8.8) containing 6 M urea. Fractions containing activating proteins were analyzed by electrophoresis on polyacrylamide gels in a discontinuous alkaline system with urea (23) and on Tricine-SDS-polyacrylamide gels (24). Amino-terminal sequences were determined on proteins separated by electrophoresis after a second screening was prepared for sequencing of double-stranded DNA. Sequence analysis was made on the total mixture of fragments after lyophilization of the sample.

For cleavage with trypsin, apoCII (38 μg/ml in 6 M urea, 50 mM Tris-Cl (pH 8.8)) was diluted in water 2:1 (v/v) to reduce the concentration of urea. Ammonium bicarbonate (2 M) was added to a final concentration of 72 mM to reach pH 7.8. Trypsin-TPCK (30% w/w) was added from a stock solution of 0.5 mg/ml in 10 mM HCl. The sample was incubated at room temperature for 20°C until sequence analysis (15). Only one major band was obtained, but after 8–9 cycles it was not possible to proceed further in any of the materials. This was probably due to the high content of proline residues in the amino-terminal end, which was later found to contain 6 Pro per 14 residues.
a column of DEAE-Sephacel. Two fractions with ability to activate LPL were selected for further characterization. The fractions were analyzed by electrophoresis in the alkaline polyacrylamide gel system containing urea (Fig. 2). The protein in fraction 37 had the amino-terminal sequence RPTSDPPQ. Despite the fact that the sample was pure and could be used in satisfactory amounts for sequencing, it was not possible to proceed in the sequence after the Asn residue, as was previously found with some of the materials from the Tricine gels.

The protein with slowest migration in fraction 26 (Fig. 2) started with the sequence RPTSD, but the material was not sufficiently pure for continued sequencing.

The 8–10 residue amino-terminal sequence found in the activating proteins was not suitable for generation of nucleic acid probes due to the high number of possible codons for each amino acid residue and due to the sequence inconsistency between different variants. Furthermore, the sequence obtained was different from all previously known apoCII variants, with the possible exception of the last two residues (PQ). To ensure that this was still the right protein, it was necessary to obtain internal protein sequences. ApoCII from three of the previously known species have only one methionine residue (Met⁶⁰). We therefore attempted to cleave the protein in fraction 37 from the DEAE-Sephacel column (Fig. 2) with cyanogen bromide. Due to the limited amount of material available, the cleavage was done on protein adsorbed to a PVDF filter. Sequencing of the mixture of fragments revealed two parallel sequences. One was identical with the previously obtained amino-terminal sequence (RPTSDPPQ), which as before stopped after the Asn residue. The other sequence was identified as TYTGILTDQLYH of which the first 9 residues were identical to residues 62–70 in guinea pig apoCII. This finding strengthened the case that, despite the unusual amino-terminal sequence, the isolated protein corresponded to chicken apoCII. Furthermore the results indicated that chicken apoCII had only one methionine residue.

Two primers were designed. The upstream antisense primer A was designed from the amino-terminal sequence (TSDP-PQQ). The downstream sense primer B was designed from the internal amino acid sequence (GILTDQ). It was simplified based on the comparison of the nucleotide sequences encoding the amino acid residues GIL(F/T)DQ in all known apoCII variants (human (8), monkey (9), dog (10), rat (13), mouse (12), guinea pig (15)). To limit the number of variants only codons used in these species were selected, which decreased the oligonucleotide mix from 512 variants to 24. Primer A was then used together with primer B to amplify chicken apoCII cDNA from a mixture of chicken liver cDNA. Even though conditions were selected to be as stringent as possible, several DNA fragments were obtained. All fragments were analyzed, and one of them showed a sequence similar to the expected one. The deduced
amino acid sequence was, however, too short to correspond to the whole peptide. Only 18 amino acid residues were obtained flanked by the primer sequences, QSILDAYEKGTAAVMTYT. The TYT sequence was the same as the one found after cleavage with cyanogen bromide. In addition we were able to obtain the amino-terminal sequence of a tryptic fragment of chicken apoCII, LRPPTTPQSILDA. This confirmed the sequence obtained from the PCR fragment and provided 7 additional amino acid residues toward the amino terminus.

At this point a homologous oligonucleotide designated C, spanning nucleotides 214–251 of chicken apoCII, was made for screening of the chicken cDNA library. After a second screening, five positive, independent candidate clones were isolated for sequencing. From one of the longest cDNA inserts (T1) 404 nucleotides were determined, corresponding to a signal peptide and the apoCII sequence. The amino-terminal Arg residue was known from previous protein sequencing of several chicken apoCII variants, as described above. Chicken apoCII is 4 residues longer in the amino-terminal end than its human counterpart and consists of 83 amino acid residues. The only methionine found upstream in the signal peptide was 13 amino acid residues from the amino-terminal end, and therefore, the T1 insert probably did not contain the initiation Met codon. None of the inserts contained the common polyadenylation signal (AATAAA) but two copies of the more rare sequence ATTAAA at 17 and 28 nucleotides upstream from the poly(A) sequence, respectively. Sequences from the other positive clones confirmed the T1 sequence.

A comparison of the deduced amino acid sequence of chicken apoCII with apoCII sequences from eight different mammals was done (Fig. 4). Interestingly, there are few sequence identities in the signal peptide or in the sequence of the amino-terminal domain coded for by exon 3 in the human gene (up to residue 50, according to the human numbering). Starting from the exon border between exon 3 and exon 4 (amino acid residue 51) to the end of the coding sequence, the identities for chicken apoCII compared with the other species is 50–60%. If the area is limited to residues 51–70, the identity with human apoCII is 70%, and the identity with any of the mammalian variants is 85% (17 residues out of 20). Table I shows comparisons of identity of the entire alignable chicken apoCII sequence and of the amino-terminal and the carboxyl-terminal domains, respectively, with corresponding parts of apoCII variants from all mammals and birds.
presently known animal species. The calculated isoelectric point for chicken apoCII is alkaline (pI 5 7.7) and much higher than those for apoCII variants from the other species (range 4.2–5.9, for human apoCII pI 5 4.4, calculations made by the GCG program). This was in accord with the migration of chicken apoCII activity on the alkaline urea gels (Fig. 2).

The role of the amino-terminal region of apoCII appears to be in binding of the protein to lipid/water interfaces (28, 29). Datta et al. (10) had previously predicted that residues 14–33 and 44–63 of human apoCII can form amphipathic helices with the potential to bind to lipid. Chicken apoCII has no clear sequence identity with other apoCII variants in the amino-terminal domain. Furthermore, it contains 20% of helix-breaking proline residues compared with less than 10% in human apoCII. Still the predicted amphipathic α-helical regions in chicken apoCII are almost superimposable to those of human apoCII (using the Eisenberg algorithm in the DNAStar program). According to these calculations the following sequence areas are predicted to form α-amphipathic regions in human apoCII: 14–36, 43–56, and 69–74. In chicken apoCII the corresponding areas are 21–40, 47–60, and 73–78. The difference is explained by the fact that chicken apoCII is 4 residues longer in the amino-terminal end. Fig. 5 shows helical wheel structures for the first amphipathic region in human apoCII and for the corresponding region in chicken apoCII. It is evident that the helices have two sides, one hydrophobic and one polar, and that the chicken helix has a more extended hydrophobic face than the human.

**DISCUSSION**

This is the first report on the structure of an LPL activator from a non-mammalian species. Chicken apoCII was found to have the same overall design as its mammalian counterparts, with a lipid-binding amino-terminal domain and a segment in the carboxyl-terminal domain with high homology to the amino acid sequences previously shown to be involved in activation of LPL. It was previously known that the lipases and their activator proteins react across species. Thus, human apo-CII was shown early to activate chicken LPL (30), and the chicken apoCII that we isolated here fully activated human LPL. Sequence alignment with the mammalian apoCII variants shows that the chicken protein contains four additional amino acid residues in the amino-terminal domain and thus has 83 residues, as compared to 79 for human apoCII. In the region from residue 51 to residue 70 (human numbering) 14 out of 20 residues were identical, and most of the other six were functionally analogous. In contrast, there was no significant homology in the amino-terminal 50 residues, and the chicken protein showed some unusual features here. Six of the 14 amino-terminal residues are prolines, and in the sequence 5–17 (human numbering) every third amino acid residue is a glutamine (a total of 5 Gln residues). The overall sequence identity between chicken and human apoCII is around 30%, which is lower than that found between chicken apoAI and human apoAI (about 50%) (31). The rapid evolution of the apoCII amino-terminal domain indicates that the structural requirements on this part of the molecule are not very specific. This is in concert with its presumed function in lipid binding. In contrast, the part of apoCII that interacts with LPL appears to be strictly conserved.

In the mammalian apoCIIIs that have been studied, residues 51–77 (human numbering) are highly conserved. The chicken sequence limits the region of high homology to 20 residues, 51–70. Only one of the nine residues beyond that, Gly77, is fully conserved. Within the homology region 13 residues are identical in all eight mammalian CII structures. Of these, 12 are also

**TABLE I**

| Animal species | Percent identity to sequence of chicken apoCII |
|----------------|-----------------------------------------------|
|                | Residues 1–79 | Residues 1–50 | Residues 51–79 |
| Human          | 26.6          | 18.0          | 51.7           |
| Monkey         | 26.6          | 18.0          | 51.7           |
| Sheep          | 27.8          | 18.0          | 51.7           |
| Bovine         | 27.8          | 18.0          | 51.7           |
| Dog            | 32.9          | 22.0          | 58.6           |
| Rat            | 28.0          | 23.3          | 50.0           |
| Mouse          | 29.3          | 23.4          | 50.0           |
| Guinea pig     | 30.7          | 17.4          | 58.6           |

**FIG. 5.** Predicted amphipathic α-helices for human apoCII and for chicken apoCII as shown schematically by the helical wheel model. The Eisenberg algorithm (see “Experimental Procedures”) was used to predict α-amphipathic regions in apoCII. Three regions were found both for human and for chicken apoCII of which the first is shown here. A shows human apoCII, residues 14–36. B shows chicken apoCII, residues 21–40. The figures were produced by the helical wheel subroutine of the GCG program. The view is from the top of the helices down through their centers. The lines connect the amino acid residues that are depicted by single letter abbreviations in each corner. , hydrophobic amino acid residues.
present in the chicken sequence. The only new difference is that Ser^54 is substituted by a Glu. Among the 20 residues in the high homology region, there are only three positions where the chicken has a residue that is not used in any of the mammalian sequences. The pattern of the critical functional region that emerges from these sequence comparisons is in concert with the information from studies with synthetic peptide fragments. These have shown that the shortest fragment that gives full activation in appropriate model systems is 61–79. This fragment can be shortened at the carboxyl-terminal end down to residue 69, provided that residues are added at the aminoterminal end (51–69 or 44–69) (32). The flanking sequences at either side of the high homology region may be necessary to stabilize the active conformation.

Early studies suggested that the last two residues in human apoCII, Glu^-^7^-^Glu^-^9, were essential for activation of LPL (33), and it has been suggested that these residues interact with a pair of Lys residues at positions 147 and 148 in (human) LPL (34). None of these Glu residues or a functional equivalent are present in chicken apoCII, or in the dog (10), the guinea pig (15), or the bovine apoCII proteins (11). Furthermore, one of the proposed Lys residues is lacking in guinea pig LPL even though this enzyme is activated by human apoCII (35). Overall the hypothesis that these ion pairs are critical for the activation of LPL by apoCII now appears unlikely, also taking into account that apoCII activates LPL at 1 m NaCl (36, 37).

The start of the high homology region in apoCII coincides with the border between exons 3 and 4 in the human and mouse apoCII genes (14, 16, 38). Hence, the preceding part of the chicken apoCII gene may originate from a different source than Exon 3 in mammalian apoCII. A computer-assisted search for sequences homologous to the aminoterminal part of chicken apoCII did not give any clue to this point. Mammalian apolipoproteins, with the exception of apoB, are built up of repetitive blocks of 11/22 amino acids arranged in a specific order (39). Similar repeats are evident in the amino-terminal part of chicken apoCII (not shown), indicating that this part of the molecule has also evolved from an apolipoprotein ancestor.

In some mammals a hexapeptide is split off from the aminoterminal end of apoCII and of apoAI at a conserved cleavage site involving a paired Gln-Gln. A similar cleavage site, Gln-Gln, is present in chicken apoAI (40). More than 90% of the apoAI in chicken plasma is cleaved which demonstrates that the proteolytic machinery is operational in chickens (31). In contrast, no corresponding proteolytic cleavage site was found in the chicken apoCII sequence. This is in concert with the finding that none of the apoCII variants isolated from plasma were truncated at the amino terminus. The functional importance of the cleavage of apoCII in some species is not known. It does not appear to involve activation of LPL since the pro- and mature forms of human apoCII activate LPL to the same extent (11).

Both previous (20) and present studies show that there exist several charge variants of chicken apoCII. We do not yet have any explanation for this heterogeneity. There is no site for N-glycosylation. Therefore, oligosaccharides, if present, must be O-linked. A minor fraction of apoCII in human plasma has been reported to be glycosylated (38). On aminoterminal sequencing of chicken apoCII variants, separated by electrophoresis, we usually found the sequence RPTSSDPQ instead of the RPTSSDPQQ sequence predicted from the cDNA. This may indicate the presence of carbohydrate at one of the Ser residues. Furthermore, on Tricine-SDS-polyacrylamide gels, we observed chicken apoCII variants that differed slightly in molecular weight. These data are compatible with differently glycosylated forms of the peptide. In the material isolated from plasma we found no indication of an apoCII variant with a molecular size of about 5 kDa. Thus, this form of chicken apoCII, which was consistently found in egg yolk (19), is probably created by proteolysis on transfer of the lipoproteins into the oocyte (41), but we cannot rule out the possibility that the low molecular weight form is present in blood of the laying hen. Judging from its molecular weight the shorter form probably arises from cleavage around amino acid residue 40 (human numbering), where there are two Arg and one Glu residues in the chicken sequence, providing possible cleavage sites for proteases. A possibility is that the short form of chicken apoCII does not bind to lipid as efficiently as the full-length variant and therefore was not recovered by adsorption to Intralipid® in the initial step of the purification.

In summary we have demonstrated that chicken apoCII shows strong similarity to apoCII variants from mammals in a short area (20 amino acid residues) of the carboxyl-terminal domain. This area contains the previously demonstrated regions for interaction with LPL and for activation (7, 32). The amino-terminal domain was less conserved, indicating a rapid divergence of this part of the apoCII molecules. Of special interest is, however, that secondary structure predictions show potential for amphipathic helix formation located at almost identical sites in the chicken apoCII sequence as previously reported for human apoCII. Thus, it seems as if the structural basis for the lipid-binding ability is preserved.

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