Amino acid sequence of rat apolipoprotein A-II deduced from the nucleotide sequence of cloned cDNA

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Abstract A rat apolipoprotein A-II cDNA clone was isolated from a rat liver cDNA library by in situ hybridization of bacteriophage plaques using a ^{32}P-labeled human apoA-II cDNA as a probe. The cDNA insert from this clone was characterized by DNA sequencing. The amino acid composition derived from the DNA sequence data matched well with that of rat apoA-II reported earlier (Herbert et al. 1974. *J. Biol Chem.* **249:** 5718–5724), indicating that the cDNA insert coded for rat apoA-II. Further evidence was provided by a comparison of the amino acid sequence of rat apoA-II obtained here with that of human apoA-II (Brewer et al. 1972. *Proc. Natl. Acad. Sci. USA.* **69:** 1304–1308). While the rat apoA-II cDNA insert did not code for the entire presegment, it had the same COOH-terminal residues of the presegment as well as the same prosegment (Ala-Leu-Val-Arg-Arg) as in human preproapoA-II, suggesting that rat apoA-II was also synthesized initially as preproapoA-II. Mature rat apoA-II contains 79 amino acids. Residue 6 of mature rat apoA-II is Asp, while it is Cys in human apoA-II, and this would account for the absence of dimeric forms of rat apoA-II in plasma. While the overall amino acid sequence homology between rat and human apoA-II is about 50%, the amphipathic α-helical structures, which are responsible for lipid-binding, seem to be conserved in the two proteins. The size of rat apoA-II mRNA was estimated to be about 600 nucleotides. Analysis of mRNA levels in different tissues demonstrated that the liver appeared to be the chief site of synthesis of rat apoA-II. —**Nagashima, M., G. Morris, G. Howlett, N. Fidge, and G. Schreiber.** Amino acid sequence of rat apolipoprotein A-II deduced from the nucleotide sequence of cloned cDNA. *J. Lipid Res.* 1986. **27:** 706–712.

Supplementary key words rat apoA-II mRNA • rat preproapoA-II

Apolipoprotein A-II is the second major protein component of high density lipoprotein in man. The plasma concentration of apoA-II in man is in the range of 30–50 mg/dl (1). Apolipoprotein A-II exhibits polymorphism in plasma, and several forms of this protein including a sialylated form have been reported (2, 3). Human apoA-II consists of two identical chains of 77 amino acids linked together by a disulphide bridge at residue 6. The amino acid sequence of human apoA-II obtained by direct Edman degradation (4) has been confirmed by DNA sequence analysis of apoA-II cDNA (5–8). Furthermore, genomic structural organization of human apoA-II and its chromosome locations have been reported (9, 10).

It has been demonstrated that human apoA-II is initially synthesized as a preproprotein both by the liver and small intestine (11). The 18 amino acid-long presegment with a sequence typical of a signal peptide is cleaved intracellularly to produce proapoA-II. Although the apoA-II prosegment has COOH-terminal Arg-Arg residues, the processing of proapoA-II seems to differ from that of other propeptides in that the prosegment of apoA-II is removed extracellularly (for review see ref. 12). The exact site of this conversion of proapoA-II to the mature protein remains to be elucidated.

The physiological function of apoA-II is also uncertain. In vitro studies have demonstrated that apoA-II may regulate the activities of hepatic lipase (13). Recently, it has been reported that apoA-II, together with apoA-I, may be involved in the binding of HDL particles to specific HDL-receptors (14). Whether or not apoA-II has any physiological function(s) or is simply a structural protein of HDL, it remains interesting that the occurrence of plasma apoA-II appears to be species-dependent (15). For example, while apoA-II is one of the major protein components of human HDL, its counterpart is not found in plasma HDL of rabbit or dog. Apolipoprotein A-II with an amino acid composition similar to that of human apoA-II is present in rat HDL as a minor component (16).

In the present study, we elucidate the primary structure of rat apoA-II by the isolation and sequencing of a rat

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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apoA-II cDNA clone. The comparison of the amino acid sequence of rat apoA-II with that of human apoA-II revealed a number of interesting points as to the possible mechanism of its synthesis and evolutionary changes.

MATERIALS AND EXPERIMENTAL PROCEDURES

Antiserum to human apoA-II

Antiserum to human apoA-II was prepared as described elsewhere (14).

Construction of liver cDNA libraries and identification of apoA-II clones

The λgt10 and λgt11Amp3 libraries were constructed from either human or rat liver polyadenylated RNA by the methods of Young and Davis (17) and Kemp et al. (18) with the modification described by de Jong et al. (19). Colonies of *E. coli* BTA282 infected with recombinant λgt11Amp3 containing human liver cDNA were grown on nitrocellulose filters and screened for clones expressing human apoA-II antigenic determinants (18). Bacteriophage λDNA was isolated by ultracentrifugation followed by extraction with formamide (20). The DNA insert was isolated from recombinant bacteriophage λDNA digested with restriction enzyme *Eco*RI by electrophoresis in 1.2% agarose gel.

In order to identify clones containing rat apoA-II cDNA from rat liver cDNA libraries constructed in λgt10, plaques were screened by hybridization (20). The host cells used were *E. coli* C600. Culture plaques of bacteriophage λ were transferred to nitrocellulose filters. The DNA bound to the filters was denatured with 0.5 M NaOH/1.5 M NaCl, neutralized with 1.5 M NaCl/0.5 M Tris, pH 8, and washed in 30 mM sodium citrate containing 300 mM NaCl. The filters were baked for 2 hr at 80°C under vacuum, and prehybridized for 2 hr at 42°C in 20 mM phosphate buffer, pH 6.5, containing 50% formamide, 750 mM NaCl, 1 × Denhardt's solution, 1 μg/ml of poly A, and 100 μg/ml of heat-denatured herring sperm DNA. The filters were then hybridized in the same solution at 42°C overnight with the entire human apoA-II cDNA insert which had been nick-translated (20) in the presence of [^{32}P]dATP. The filters were washed three times in 30 mM sodium citrate containing 300 mM NaCl and 0.1% SDS at room temperature, and then twice in 1.5 mM sodium citrate containing 15 mM NaCl and 0.1% SDS at 55°C, and exposed to X-ray film.

These experiments were performed according to the C1 containment conditions of the Committee on Recombinant DNA Molecules of the Australian Academy of Science.

Isolation and Northern blots of total RNA

Total RNA was prepared from tissues by initial homogenization in 4 M guanidine thiocyanate followed by three cycles of homogenization in 7.5 M guanidine HCl and precipitation with ethanol as described (21). Total RNA was denatured with formamide/formaldehyde, separated by electrophoresis in 1% agarose/formaldehyde gel (20), and transferred to nitrocellulose filter by the method of Thomas (22). Apolipoprotein A-II RNA species were detected by hybridization to ^{32}P-labeled rat apoA-II cDNA.

DNA sequencing

DNA sequencing was performed by the dideoxy chain termination method of Sanger, Nicklen, and Coulson (23). The whole cDNA insert as well as fragments of the insert obtained by digestion with restriction enzyme *Alu*I were cloned into M13mp8 vector. The recombinant single-stranded phage DNA was used as a template for the sequencing with the M13 pentadecamer sequencing primer purchased from New England Biolabs. In some cases, DNA sequence of the complementary strand was obtained using the double-stranded replica form as a template with the M13 reverse sequencing primer.

RESULTS

Isolation of clones expressing human apoA-II

A human liver cDNA library with a total of 10^6 recombinants was prepared in λgt10 vector from polyadenylated RNA. The cDNA amplified in λgt10 was isolated and inserted into the *Eco*RI site of λgt11Amp3 vector, producing a cDNA library with a total of 7.5×10^4 recombinants. Ten thousand colonies of *E. coli* BTA282 infected with recombinant λgt11Amp3 were screened for clones expressing human apoA-II by an immunochemical procedure (17, 18) using antiserum against human plasma apoA-II (diluted 1:500). Four positive signals were detected. The cDNA insert was isolated from one of the putative human apoA-II clones, termed λampAPAII-4, and characterized. Digestion of this 480 bp cDNA insert with restriction enzymes *Bst*NI, *Dde*I, *Ava*II, *Fnu*4HI and *Xba*I led to construction of a restriction map that was identical to that deduced from the nucleotide sequence of human apoA-II (5–8). This 480 bp cDNA insert contained the entire coding sequence of human preproapoA-II. When proteins from λampAPA-II-4 were analyzed by Western blots (24), the results demonstrated that the human apoA-II-like protein was synthesized as a 130,000-dalton chimeric polypeptide containing antigenic determinants of both human apoA-II and β-galactosidase.

Identification of a rat apoA-II clone

When Northern blots of rat liver polyadenylated RNA were probed with nick-translated human apoA-II cDNA, a rat RNA species with an electrophoretic mobility very

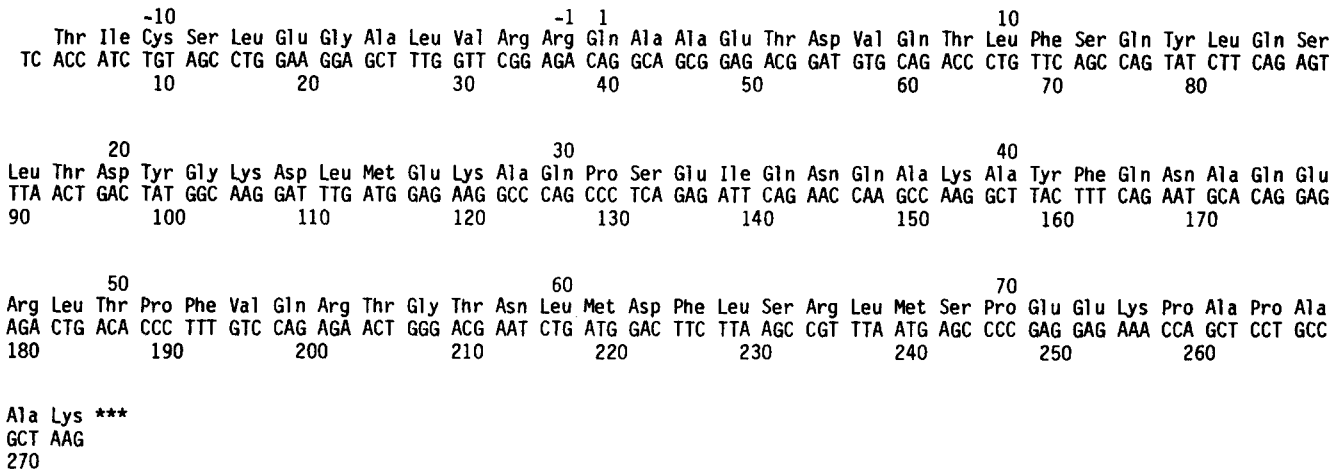


Fig. 1. Nucleotide sequence of the coding region of rat apoA-II cDNA and the derived amino acid sequence. The nucleotide sequence was derived from sequencing of both strands. Numbering of amino acids begins at the N-terminus of the mature protein.

similar to that of human apoA-II mRNA was detected (data not shown) indicating structural homology between apoA-II of human and rat origin. Therefore, rat liver cDNA libraries constructed in λgt10 were screened by in situ plaque hybridization using a ^{32}P-labeled human apoA-II cDNA as a probe. The hybridization was carried out as described under Experimental Procedures. Screening of 10^4 plaques resulted in the detection of one positive signal.

A cDNA insert of about 475 bp was isolated from the putative rat apoA-II clone, and subcloned into M13mp8 for DNA sequence analysis. The DNA sequence of the coding region, and the derived amino acid sequence are shown in **Fig. 1.** The amino acid composition of rat apoA-II deduced from its DNA sequence was found to be almost identical to that reported earlier from direct amino acid analyses (16) as shown in **Table 1.** The result strongly suggested that the cDNA insert coded for rat apoA-II. The lack of histidine residues is also characteristic for apoA-II. Further evidence was given by the comparison of rat apoA-II amino acid sequence to that of human apoA-II (**Fig. 2**). The amino acid sequence of human apoA-II (5–8) is positioned to maximize homology between the two sequences. While the overall sequence homology is about 50%, the NH_2-terminal domain has higher homology. Furthermore, hydrophobic residues in corresponding positions are well conserved between apoA-II of two species.

It has been shown that human apoA-II is initially synthesized as a preproapoA-II. It contains an NH_2-terminal signal peptide of 18 amino acids followed by a pentapeptide prosegment with a sequence Ala-Leu-Val-Arg-Arg (11). The part of the presegment found had the same amino acid sequence as that of the corresponding section of human apoA-II. Rat apoA-II also has the same prosegment (Ala-Leu-Val-Arg-Arg) as that of human apoA-II. It is most likely that rat apoA-II is also synthesized initially as a preproapoA-II.

Human apoA-II consists of two identical chains, each 77 amino acids long, linked together by a Cys6-Cys6 disulfide bridge (4). However, in rat apoA-II, Cys is replaced by Asp due to a single base change. This is consistent with the observation that only the monomeric form of apoA-II is found in rat plasma HDL (15). It seems that the disulfide bond is not essential in lipid–protein interactions.

TABLE 1. Comparison of rat apolipoprotein A-II amino acid composition calculated from DNA sequence with that reported earlier by direct amino acid analysis

	Apolipoprotein A-II	
Amino Acid	Calculated from DNA Sequence	Direct Amino Acid Analysis[a]
	mol/100 mol of amino acid	
Lys	6.3	5
His	0	0
Arg	3.8	4
Asp + Asn	8.9	9
Thr	7.6	7
Ser	6.3	6
Glu + Gln	20.2	21
Pro	6.3	6
Gly	2.5	3
Ala	11.4	12
Val	2.5	3
Met	3.8	3
Ile	1.3	1
Leu	10.1	11
Tyr	3.8	4
Phe	5.1	5
Trp	0	0
Cys	0	0

[a]Values were taken from ref. 16.

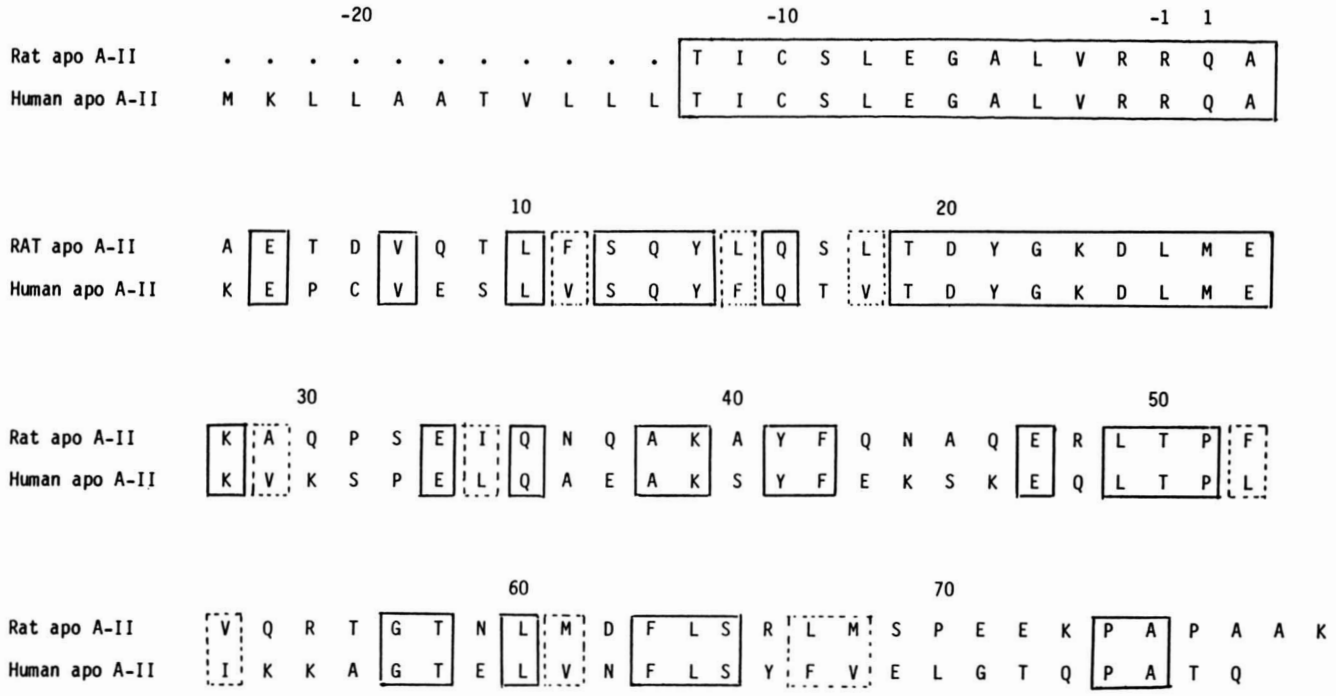
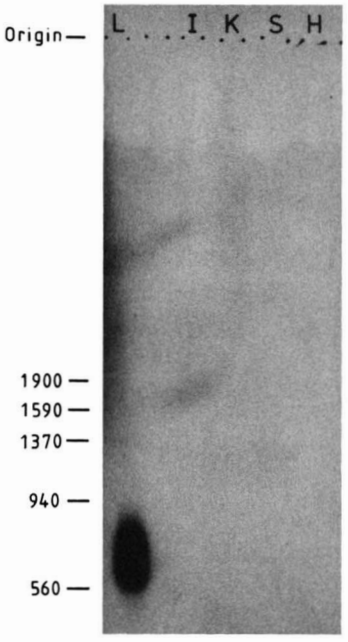


Fig. 2. Comparison of the derived amino acid sequence of rat apoA-II with the amino acid sequence of human apoA-II. The amino acid sequence of human apoA-II (5–8) was positioned to optimize homology, and numbers refer to this sequence. Closed boxes indicate identical sequences while open boxes represent sequences in which hydrophobicity is conserved.



Size and tissue distribution of rat apoA-II mRNA

Using rat apoA-II cDNA as a probe, the size and the tissue distribution of rat apoA-II mRNA were studied by the techniques of Northern blotting. The radioautograph of the Northern blot of total RNA from rat liver and of various extrahepatic tissues is shown in **Fig. 3.** The size of rat apoA-II mRNA from the liver is estimated to be about 600 nucleotides (lane 1).

Unlike rat apoA-I and apoA-IV which are synthesized by the liver and small intestine in comparable amounts (25), rat apoA-II is not synthesized in significant amounts by the small intestine as shown by the failure to detect apoA-II mRNA in this tissue under the conditions used (lane 2). Even using 45 μg of rat total intestinal RNA, apoA-II mRNA was not detected. Other extrahepatic tissues such as kidney, stomach, and heart do not contain any detectable amounts of apoA-II mRNA. Among the adult rat tissues examined, liver appears to be a major site of the synthesis of apoA-II.

Fig. 3. Size and tissue distribution of rat apoA-II mRNA. Fifteen μg of total rat RNA isolated from liver (L), intestine (I), kidney (K), stomach (S), and heart (H), and the molecular markers were denatured and fractionated on a 1% agarose gel containing 2.2 M formaldehyde in gel buffer (5 mM sodium acetate, 20 mM HEPES, pH 7.0, 0.5 mM EDTA). Prior to loading on the gel, samples were denatured by heating at 55°C for 15 min in gel buffer containing 50% formamide and 2.2 M formaldehyde. After electrophoresis, the gel was stained with 0.3 μg/ml of ethidium bromide and the RNA was then transferred overnight to nitrocellulose by blotting. Filters were baked for 2 hr at 80°C under vacuum, prehybridized, and then hybridized to ^{32}P-labeled rat apoA-II cDNA in 20 mM phosphate buffer, pH 6.5, containing 50% formamide, 750 mM NaCl, 1 × Denhardt's solution, 1 μg/ml of polyA, and 100 μg/ml of heat-denatured herring sperm DNA at 42°C. Filters were washed first in 30 mM sodium citrate containing 300 mM NaCl and 0.1% SDS at room temperature and then in 1.5 mM sodium citrate containing 15 mM NaCl and 0.1% SDS at 50°C, and exposed to X-ray film. The positions of the molecular weight markers (^{32}P-labeled HindIII and *Eco*RI restriction endonuclease fragments of λDNA) are indicated on the left.

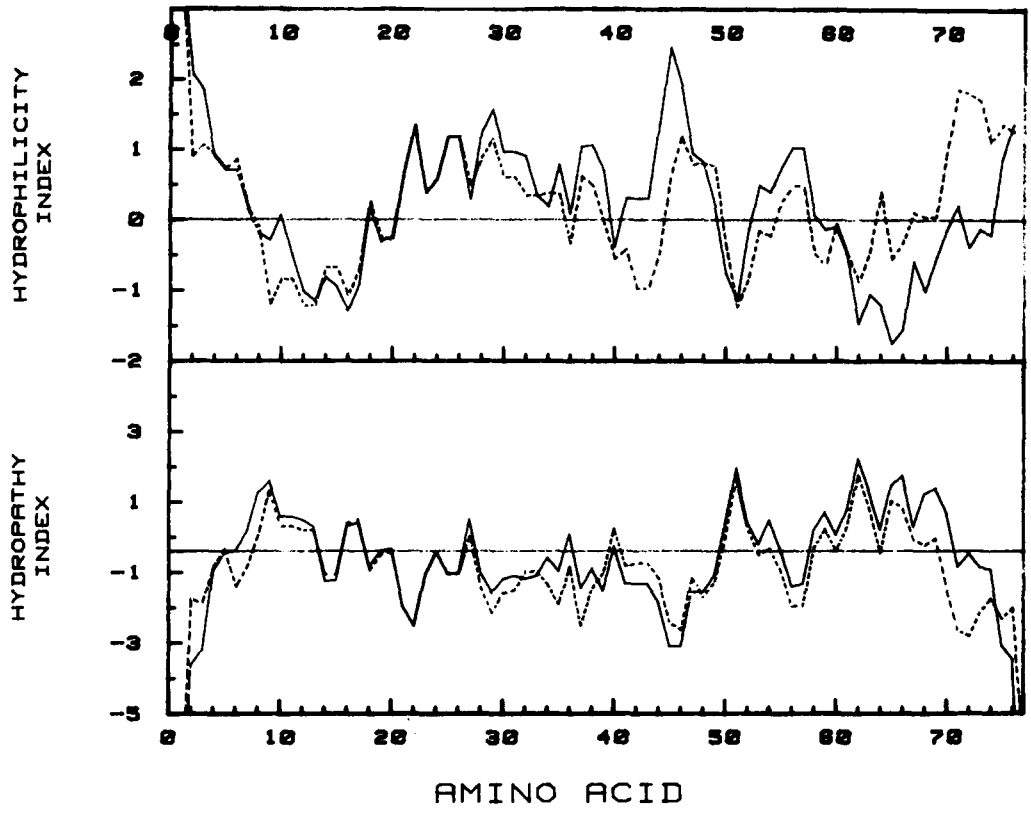


Fig. 4. Hydrophilicity and hydropathy indices of mature rat and human apoA-II. Hydrophilicity (upper panel) and hydropathy (lower panel) indices of rat apoA-II (-----) and of human apoA-II (—) were computed according to the methods described by Hopp and Woods (31) and Kyte and Doolittle (32), respectively. For the comparison, only residues 1–77 of rat apoA-II were used in the determination.

DISCUSSION

In this study we have reported cloning and structural studies of rat apoA-II. While the rat apoA-II cDNA clone isolated here contained a 3' non-coding region as well, we have focused our attention on the coding region in order to elucidate the amino acid sequence of rat apoA-II. Comparison of the amino acid sequence data of rat and human apoA-II indicated a number of interesting points. Firstly, it is very likely that rat apoA-II is also synthesized as a larger precursor protein containing a preprosegment, and its presegment is cleaved off prior to its secretion. Gordon et al. (11) have reported that, in a human hepatocellular carcinoma cell line (HepG2), apoA-II prosegment was cleaved extracellularly, and hypothesized that the apoA-II prosegment could play a role in the incorporation of this protein into HDL particles. Studies of the turnover and the rate of incorporation into HDL particles of *E. coli*-produced rat apoA-II molecules that have altered sequences in their prosegments by mutagenesis may lead to the understanding of the function of the apoA-II prosegment.

Secondly, rat apoA-II exists only in a monomeric form since residue 6 of the mature protein is Asp instead of Cys as in human and chimpanzee apoA-II (26).

Thirdly, while the overall amino acid sequence homology between apoA-II of rat and human is only about 50%, at the nucleic acid level the two sequences are 70% homologous. The amino acid sequence of the preprosegment is well conserved. Within the mature protein where amino acid substitutions have occurred, it appears that the chemical nature of the residues has been generally conserved in corresponding positions. This is demonstrated by the comparison of the hydropathy and hydrophilicity indices of two mature apoA-II (**Fig. 4**). Apart from the more hydrophilic nature of rat apoA-II at its C-terminus, both hydrophilicity and hydropathy indices of the two proteins are reasonably similar. Furthermore, secondary structural analyses of rat and human apoA-II conducted according to the method of Chou and Fasman (27) predict overall structural similarities of these two proteins, including α-helical regions consisting of residues 25–30 and 33–49 (results not shown). Both α-helices of human apoA-II are amphipathic, and may be involved in lipid binding (28). In the case of rat apoA-II, while one of the α-helices (residues 25–30) contains an amphipathic amino acid sequence, the other helix has a sequence in which many of the charged amino acids have been replaced by either glutamine or asparagine. In this regard it is noteworthy that Boguski et al. (29) have observed a high frequency of asparagine and glutamine in charged regions of the repeat units in rat apoA-IV. As they pointed out, it is conceivable that these amino acids are function-

ally interchangeable with their charged counterparts in these proteins. This would preserve the formation of a structural pattern which is characteristic for apolipoproteins (28–30).

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