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Sequence specific ^{1}H-NMR assignments and secondary structure of a carboxy-terminal functional fragment of apolipoprotein CII

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The structural properties of a synthetic fragment of human apolipoprotein CII (apoCII) has been studied by circular dichroism and proton nuclear magnetic resonance. The fragment corresponds to the carboxy-terminal 30 amino acid residues and retains the ability of apoCII to activate lipoprotein lipase. Like native apoCII, the fragment has a tendency to self-associate in pure aqueous solution. Addition of 1,1,1,3,3,3-hexafluoro-2-isopropanol to aqueous solvent dissolves the aggregates and leads to an increase in the α-helical content of the peptide, probably by stabilizing transient helical structures. The resonances in the ^{1}H-NMR spectrum of the fragment in 35% $(CF_3)_2CHOH$ were assigned through standard procedures from nuclear Overhauser enhancement spectroscopy, correlated spectroscopy and total correlated spectroscopy experiments. The NMR data indicates the formation of a stable α helix spanning Ile66–Gly77. Another α helical turn may be formed between Lys55 and Ala59 and possibly span even further towards the carboxyl terminus. These structural elements are different from those previously predicted for this part of the sequence of apoCII.

Human apolipoprotein CII (apoCII) is a 79 amino acid residue protein (Jackson et al., 1977; Hospattankar et al., 1984), which is reversibly bound to certain plasma lipoproteins (Havel et al., 1973). Its function is to activate the enzyme lipoprotein lipase, which hydrolyses triacylglycerols and phospholipids contained in the lipoprotein particles (Smith and Pownall, 1984). ApoCII, like several other apolipoproteins, has low solubility in water and a tendency to self-associate at elevated concentrations (Brown et al., 1970; Mantulin et al., 1980). It readily associates with natural or synthetic lipid aggregates. This induces conformational changes in the apolipoprotein (Morrisett et al., 1977).

From studies of natural and synthetic fragments of apoCII, it is apparent that the protein contains two functional segments (Smith and Pownall, 1984). The carboxy-terminal third of the protein, approximately residues 56–79, contains the structures needed for activation of lipoprotein lipase (Kinnunen et al., 1977; Smith et al., 1980). The detailed mechanism for this activation is not known, but it involves protein-protein interaction between apoCII and the lipase (Smith and Pownall, 1984; Quinn et al., 1983; Olivecrona and Bengtsson-Olivecrona, 1987). The amino-terminal segment, up to approximately residue 50, contains the structures needed for interaction with lipids (Sparrow and Gotto, 1980), though no continuous hydrophobic segments are evident. Molecular-structure calculations, based on Chou-Fasman rules and Edmundson Wheel analyses, predict that there are two or three helical regions in this part of the molecule (Mantulin et al., 1980; Datta et al., 1987). Like in other apolipoproteins (Segrest et al., 1973), these helical regions are amphipathic in nature and probably serve to bind apoCII to lipid/water interfaces. In accord with this, the helical content of apoCII increases on binding to lipid (Morrisett et al., 1977; Sparrow and Gotto, 1980).

There are no detailed data on the folded structure of apoCII or any other mammalian apolipoprotein. Molecular-structure data for an apolipoprotein determined by X-ray crystallography at 0.25 nm resolution was recently reported (Breiter et al., 1991) and shows an overall architecture of five long α helices connected by short loops. X-ray diffraction studies on human apolipoprotein E are in progress (Aggerbeck et al., 1988; Maulik et al., 1990).

ApoCII has a strong potential to rapidly attain its functional conformation, even after delipidation and treatment with denaturing agents. Since the carboxy-terminal part, on its own, is able to activate lipoprotein lipase, we have here initiated studies on the solution structure of the synthetic apoCII fragment, residues 50–79 by NMR and circular dichroism. A mixed aqueous solvent containing 35% 1,1,1,3,3,3-hexafluoro-2-isopropanol $[(CF_3)_2CHOH]$ was used to give conditions for well resolved ^{1}H-NMR spectra.

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Abbreviations. ApoCII, apolipoprotein CII; $(CF_3)_2CHOH$, 1,1,1,3,3,3-hexafluoro-2-isopropanol; DQF-COSY, correlated spectroscopy with double-quantum filtering; $Ole_2GroPCho$, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; COSY, correlated Overhauser spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, total correlated spectroscopy.

MATERIALS AND METHODS

The carboxy-terminal fragment of human apoCII, corresponding to residues 50–79 (Jackson et al., 1977; Hospattankar et al., 1984), was synthesized and purified to homogeneity. The sequence is 50RDLYSKSTAAMSTYTGI-FTDQVLSVLKGEE (numbering refers to residue numbers in intact apoCII). The composition was confirmed by amino-acid analysis and mass spectrometry. For the NMR studies, approximately 6 mg polypeptide was dissolved in 550 µl solution containing 58% ^1H_2O, 7% ^2H_2O, 35% $(CF_3)_2C^2HO^2H$ and 20 mM deuterated acetic acid, giving a final peptide concentration of approximately 3 mM. A similar sample was prepared in which ^1H_2O was exchanged for ^2H_2O.

ApoCII was purified from human delipidated very-low-density lipoproteins by gel filtration in 6 M guanidium chloride and chromatography on diethylaminoethyl Sephacel (Pharmacia Biotechnology) in 5 M urea (Jackson and Holdsworth, 1986). Bovine lipoprotein lipase was purified from milk as described (Bengtsson-Olivecrona and Olivecrona, 1991). For detection of lipoprotein-lipase-stimulating activity, ^3H-labeled trioleoylglycerol was sonicated into a commercial phospholipid-stabilized emulsion of soy bean triacylglycerols (Intralipid, AB KABI Nutrition, Stockholm, Sweden). The composition of the incubation system was previously described (Peterson et al., 1985) but rat serum was omitted here and the indicated amounts of apoCII or fragment were added. The peptides were dissolved to a concentration of 0.1 mM in 10 mM Tris/HCl, pH 8.5, with 3 M guanidinium chloride. Stock solutions, or logarithmic dilutions in guanidinium chloride thereof, were added to the incubation mixtures (1%, by vol.). Incubations were for 20 min at 25 °C. The released fatty acids were extracted and their radioactivity was determined as described (Peterson et al., 1985). 1 U of activity represents the release of 1 µmol fatty acid/min.

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (Ole_2Gro-PCho) was purchased from Avanti Polar Lipids (USA). The purity was higher than 99% when checked by thin-layer and gas chromatography. The unilamellar lipid vesicles were prepared by sonication (Kalman et al., 1989 and references cited therein). The sonicator used was a Soniprep 150 (MSE, Scientific Instruments, England) supplemented with an exponential microtip.

CD spectra were recorded on a Jasco J-600 spectropolarimeter at 25 °C. Spectra were corrected with respect to baseline and for dilution in the titration experiments. From the measured CD signal, the ellipticity Θ_{obs} at a particular wavelength in degrees, the mean residue molar ellipticity $[\Theta]_\lambda$ (degrees · cm^2 · dmol^{-1}) was calculated (Campbell and Dwek, 1984);

$$[\Theta]_\lambda = \frac{\Theta_{obs} \cdot 10}{LC'},$$

where L is the lightpath (decimeters) and C' is the molar concentration of amino acid residues (mol/l) based on an average residue molecular mass.

Two-dimensional proton NMR spectra were recorded at 25 °C in the phase-sensitive mode via time-proportional phase incrementation (Redfield and Kunz, 1975; Marion and Wüthrich, 1983). Total correlated spectroscopy (TOCSY) spectra were measured on a Bruker AM600 spectrometer using a MLEV-17 spin-lock pulse sequence (Bax et al., 1987) with a mixing time of 96 ms. Correlated spectroscopy with double quantum filtering (DQF-COSY) (Shaka and Freeman, 1983; Rance et al., 1984) and nuclear Overhauser enhancement spectroscopy (NOESY) spectra (Macura and Ernst, 1980; States et al., 1982) were recorded on a Bruker AM500 spectrometer. NOESY spectra for assignment purposes were recorded using a mixing time of 400 ms. In all experiments, relaxation delays of 1.7–2.5 s were used with prior saturation of the water resonance. Typically, the spectra were measured with a spectral width of 10 ppm with the carrier frequency placed at the water-resonance frequency. 32–48 transients with two or four dummy scans were recorded/t_1 increment. In the case of TOCSY and NOESY spectra, 1024 real data points were collected in the t_2 domain and 512 increments in the t_1 domain. Prior to Fourier transformation, the data were zero filled to 1024 points in the t_1 dimension and shifted sine bell windows were applied for resolution enhancement. For the DQF-COSY 2048 real points were collected in the t_2 domain and 1024 increments in the t_1 domain. The data were zero filled to 2048 points in the t_1 dimension.

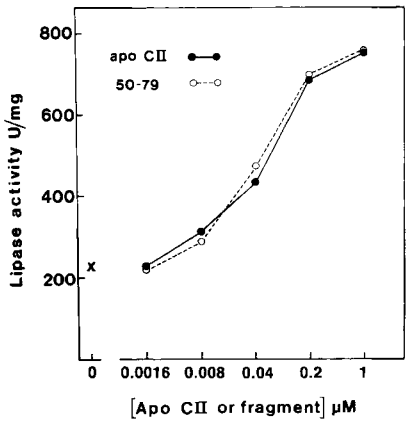

Fig. 1. Lipoprotein lipase activity as a function of the concentration of apoCII (●) and of apoCII fragment 50–79 (○). The substrate used was ^3H-labeled trioleoylglycerol in a phospholipid-stabilized emulsion of triacylglycerols. Each data point represents the mean of duplicate incubations. X = activity in the absence of apoCII or fragment.

RESULTS

Comparison of the ability of apoCII and of the synthetic carboxy terminal fragment 50–79 to activate lipoprotein lipase

Fig. 1 shows that equimolar amounts of apoCII and of the fragment stimulated the activity of lipoprotein lipase to a similar degree. The substrate used was a phospholipid-stabilized emulsion of long-chain triacylglycerols. The maximal stimulation factor was about 3.5-fold. In other systems, where the activity of the lipase alone was lower and the dependence of apoCII was more marked, the fragment was less effective. This is in agreement with Balasubramanian et al. (1986) who showed that with certain substrates the lipid-binding ability of apoCII is required for maximal efficiency.

CD studies of apoCII and the carboxy-terminal fragment

Since the choice of solvent for structural studies of apoCII was a critical question, we undertook an initial CD study to investigate the effect of adding $(CF_3)_2CHOH$ to the solvent. An original aqueous solvent of 20 mM acetic acid had been

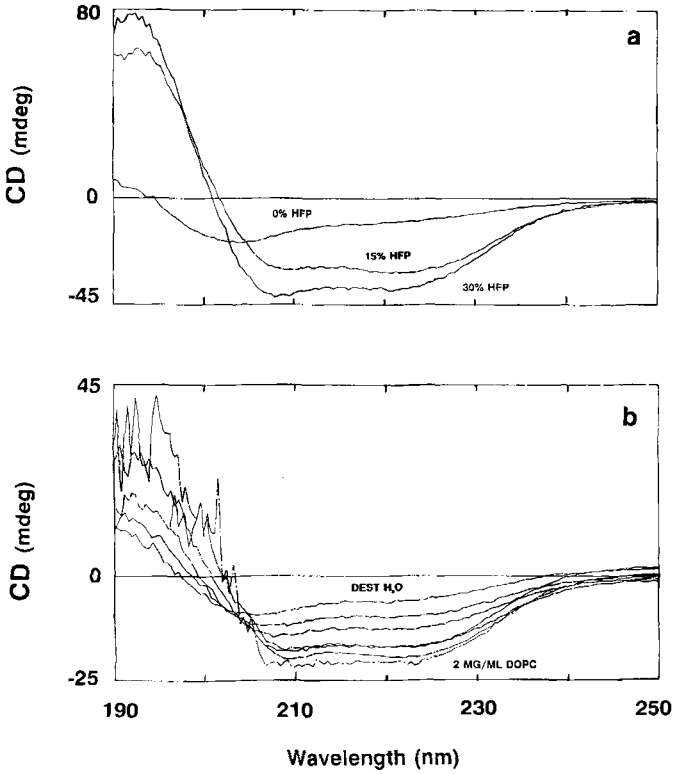

Fig. 2. **Circular dichroism spectra of apoCII.** (a) Circular dichroism spectra at 25°C of apoCII (100 μg/ml; light path 2 mm) in solutions containing 20 mM acetic acid with varying concentrations of $(CF_3)_2CHOH$ (HFP). The concentrations of $(CF_3)_2CHOH$ used were 0, 15 and 30% (by vol.). (b) Circular dichroism spectra at 25°Ç of apoCII (400 μg/ml; light path 0.5 mm) in aqueous solutions with varying concentrations of $Ole_2GroPCho$ vesicles. The $Ole_2GroPCho$ concentrations used were 0 (= DEST H_2O), 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 mg/ml.

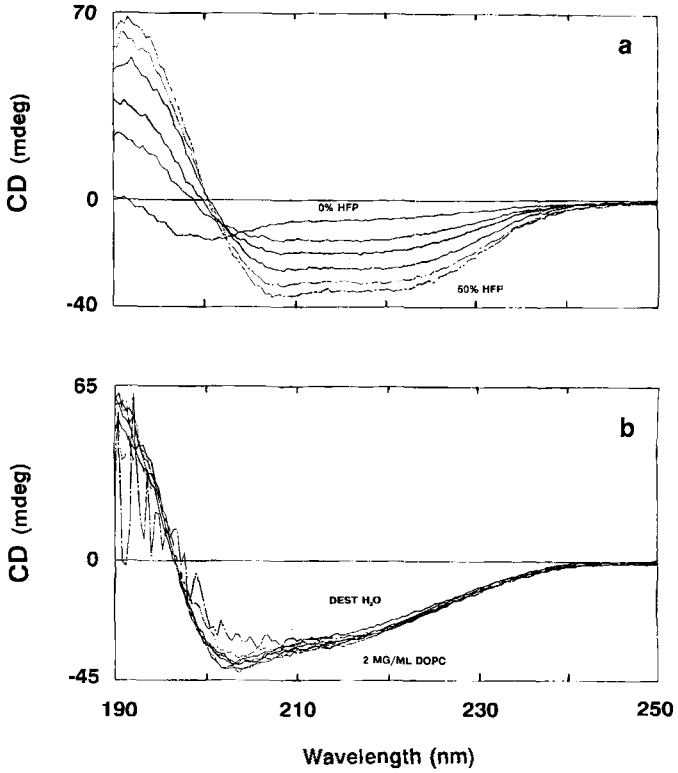

Fig. 3. **Circular dichroism spectra of apoCII fragment 50–79.** (a) Circular dichroism spectra at 25°C of apoCII fragment 50–79 (100 μg/ml; light path 2 mm) in solutions containing 20 mM acetic acid with varying concentrations of $(CF_3)_2CHOH$ (HFP). The $(CF_3)_2CHOH$ concentrations used were 0, 10, 15, 25, 35 and 50% (by vol.). (b) Circular dichroism spectra at 25°Ç of apoCII fragment 50–79 (400 μg/ml; light path 0.5 mm) in aqueous solutions with varying concentrations of $Ole_2GroPCho$ vesicles. The $Ole_2GroPCho$ (DOPC) concentrations were 0 (= DEST H_2O), 0.05, 0.1, 0.2, 0.5, 1.0 and 2 mg/ml.

chosen to give a pH of about 3.5, which is optimal for a minimum hydrogen-exchange rate of the amide protons in the peptide backbone (Wüthrich and Wagner, 1979). Preliminary one-dimensional proton NMR spectra had shown greatly increased resolution of the proton resonances, particularly for the carboxy-terminal fragment but also of native apoCII, when $(CF_3)_2CHOH$ was added to the aqueous solvent (data not shown).

Fig. 2 shows CD spectra of native apoCII when $(CF_3)_2CHOH$ was added, as well as a series of spectra when $Ole_2GroPCho$ vesicles were added in increasing amounts. A qualitative inspection of the spectra shows that the effects of $(CF_3)_2CHOH$ and $Ole_2GroPCho$ are similar; the apparent contribution of α helix is increased. A semiquantitative evaluation of the spectra was made, based on the assumption that α helix and random coil are the dominating types of secondary structure. At 222 nm, the mean residue molar ellipticity is 3900 deg · cm^2 · dmol^{-1} for random coil (Greenfield and Fasman, 1969) and −38000 deg · cm^2 · dmol^{-1} for α helix (Toniolo et al., 1979). For apoCII, we estimate $[\Theta]_{222}$, as −6000, −22000 and −12000 deg · cm^2 · dmol^{-1} in 20 mM acetic acid, in 20 mM acetic acid with 30% $(CF_3)_2CHOH$ added and in 2 mg/ml $Ole_2GroPCho$ at neutral pH, respectively. From the $[\Theta]_{222}$ values, we estimate the average helical content to be about 20% in 20 mM acetic acid, 60% in 30% $(CF_3)_2CHOH$ and 40% in 2 mg/ml $Ole_2GroPCho$. In a previous study, $[\Theta]_{222}$ was found to be −10000 deg · cm^2 · dmol^{-1} for apoCII, leading to an estimated helical content of 30–40% in 0.1 M sodium phosphate buffer, pH 7.4 (Mantulin et al., 1980).

Fig. 3 shows the CD spectra of the carboxy-terminal fragment of apoCII, titrated with $(CF_3)_2CHOH$ and with $Ole_2GroPCho$ vesicles. Qualitatively, the effect of $(CF_3)_2CHOH$ follows that seen with the whole peptide; the contribution of α helix is increased. In contrast, $Ole_2GroPCho$ vesicles had negligible influence on the CD spectrum of the fragment. An evaluation similar to that for the whole peptide gives $[\Theta]_{222}$ as −4000 deg · cm^2 · dmol^{-1} in 20 mM acetic acid and −18000 deg · cm^2 · dmol^{-1} in 35% $(CF_3)_2CHOH$, leading to estimated average helical contents of 20% and 50%, respectively.

NMR assignments

A requisite for the elucidation of the three-dimensional structure of a protein by NMR is the sequence specific-assignment of all the resonances in the spectra (Billeter et al., 1982). The spin systems of the amino acids were identified by combining the information from TOCSY (Fig. 4) and DQF-COSY spectra (CαH-NαH region is presented in Fig. 5). The spin systems were delineated in the TOCSY spectrum. Direct connectivities were then recognized in the DQF-COSY spectrum.

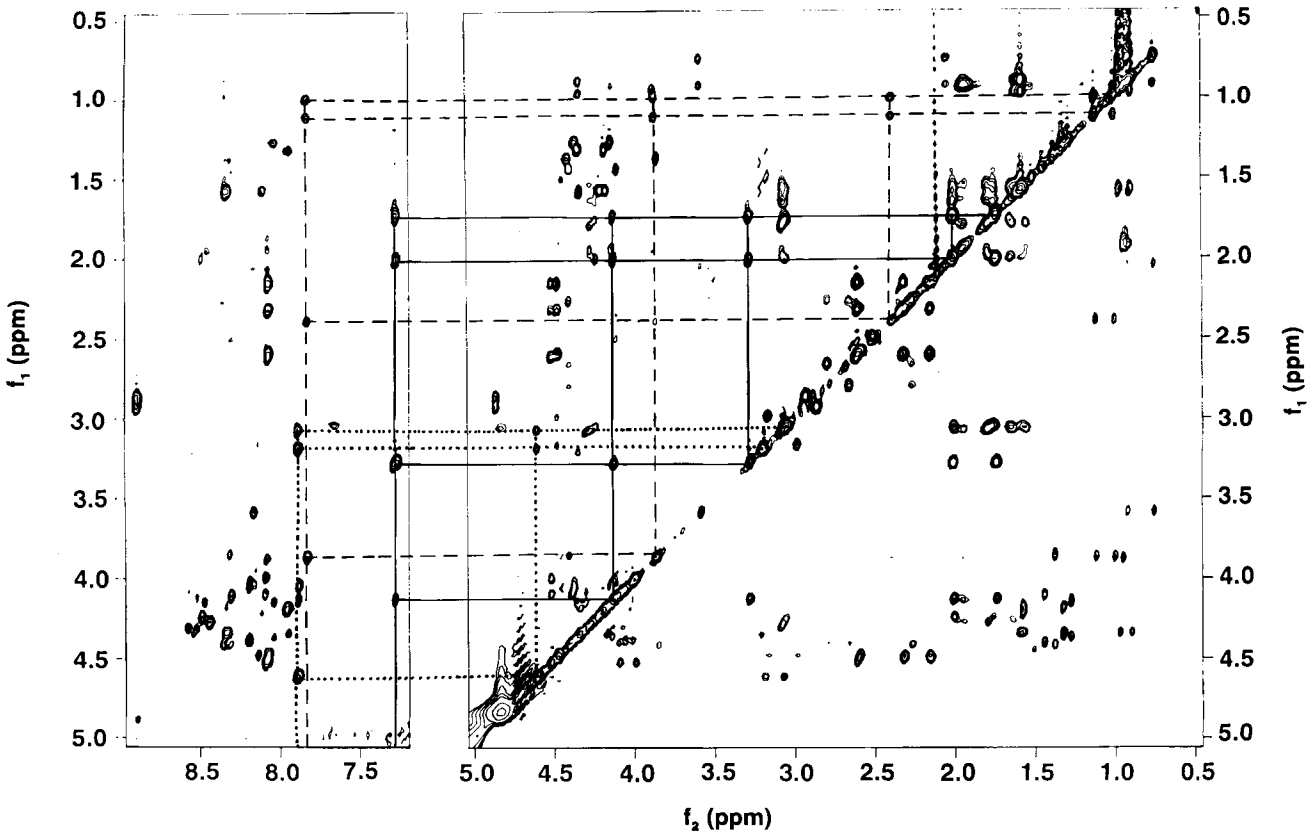

Fig. 4. TOCSY at 25 °Ç and 600 MHz of apoCII fragment 50–79 in a solution containing 55% (by vol.) ^1H_2O, 35% $(CF_3)_2CHOH$, 10% ^2H_2O, 20 mM acetic acid and 3 mM peptide. The mixing time used was 96 ms. The spin systems of Arg50 (——), Val74 (– – – –) and Tyr53 (·····) are indicated.

The spin systems were later assigned to specific amino acid residues in the primary sequence by identification of sequential connectivities in the NOESY (CαH-NαH region in Fig. 6a) spectra. Using this strategy, all proton resonances from the peptide could be assigned.

The assignment can be initiated by observing the CβH resonances around 3 ppm. These peaks are usually due to aromatic residues and aspartic acid or asparagine. In the TOCSY and DQF-COSY spectra there are CαH-CβH crosspeaks in this region, corresponding to five residues. The peptide contains three aromatic residues and two aspartic acid residues. The two tyrosines and the phenylalanine can be identified through the aromatic region around 7 ppm. Observed NOESY crosspeaks between the aromatic protons of tyrosines and the phenylalanine and their corresponding CβH establish their spin systems. Hence, also the aspartic-acid-spin systems could be identified. It could then be determined, that one aspartic acid CαH resonance is very close to the water resonance and is partially saturated during the irradiation. The presence of relayed through bond connectivities from the NαH protons to the CβH protons in the TOCSY spectrum led to the unambiguous assignment of all the resonances of the two tyrosines, the phenylalanine and the two aspartic acids.

In the TOCSY and the DQF-COSY spectra, a number of crosspeaks from around 1.7 ppm to around 3 ppm were present. These were assigned as being connectivities from methylene protons to the CεH of the two lysines and to the CδH of Arg50. The complete spin systems of nonexchangeable protons and NαH protons of the arginine and the two lysines could be identified in the TOCSY spectrum (Fig. 4). Also, TOCSY crosspeaks to one side chain NH proton could be observed.

The two glycine patterns could be recognized in the DQF-COSY spectrum due to the presence of two CαH–NαH crosspeaks for each residue (Fig. 5). These also possess a characteristic coupling pattern. It may be noted that the crosspeaks involving one glycine residue are very weak.

The apoCII fragment contains four serines and four threonines. These residues typically have CβH resonances close to or above 4 ppm. A number of CαH–CβH crosspeaks in this region were observed in the TOCSY and DQF-COSY spectra. These were consequently assigned to serine and threonine residues. The $C\gamma H_3$ of the threonines could be identified by direct NαH–CαH connectivities and relayed NαH–CβH and NαH–$C\gamma H_3$ connectivities in the TOCSY experiment.

There are six residues in the fragment containing high field aliphatic methyl protons; three leucines, two valines and one isoleucine. These residues were treated as one group in the spin-system-identification procedure. The CγH protons of two leucines and the $C\gamma H_2$ of the isoleucine could not be distinguished in the TOCSY and DQF-COSY spectra.

Sequential NOE connectivities, between the CαH of one residue and the NαH of the following residue, in the NOESY spectrum (Fig. 6a) were identified. A number of $C\alpha_iH-N\alpha_{i+1}H$ steps were ambiguous, due to spectral overlap. The different steps could then either be confirmed by the presence of sequential $C\beta_iH-N\alpha_{i+1}H$ NOE, $N\alpha_iH-N\alpha_{i+1}H$ NOE, or a particular step could be excluded due to incompatibility with the primary structure. Using this strategy, all resonances in the proton NMR spectrum could be assigned. The sequential

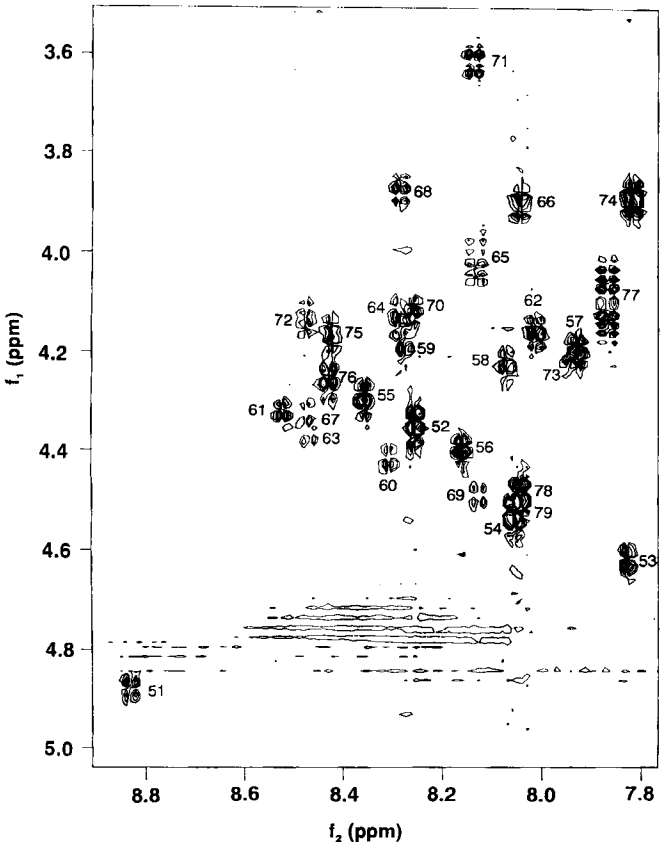

Fig. 5. DQF-COSY at 32°C and 500 MHz of apoCII fragment 50–79. The NαH–CαH part of the spectrum is shown. The solution contained 58% (by vol.) ^1H_2O, 35% $(CF_3)_2CHOH$, 7% ^2H_2O, 20 mM acetic acid and 3 mM peptide. The numbering refers to the amino acid residues giving rise to the crosspeaks.

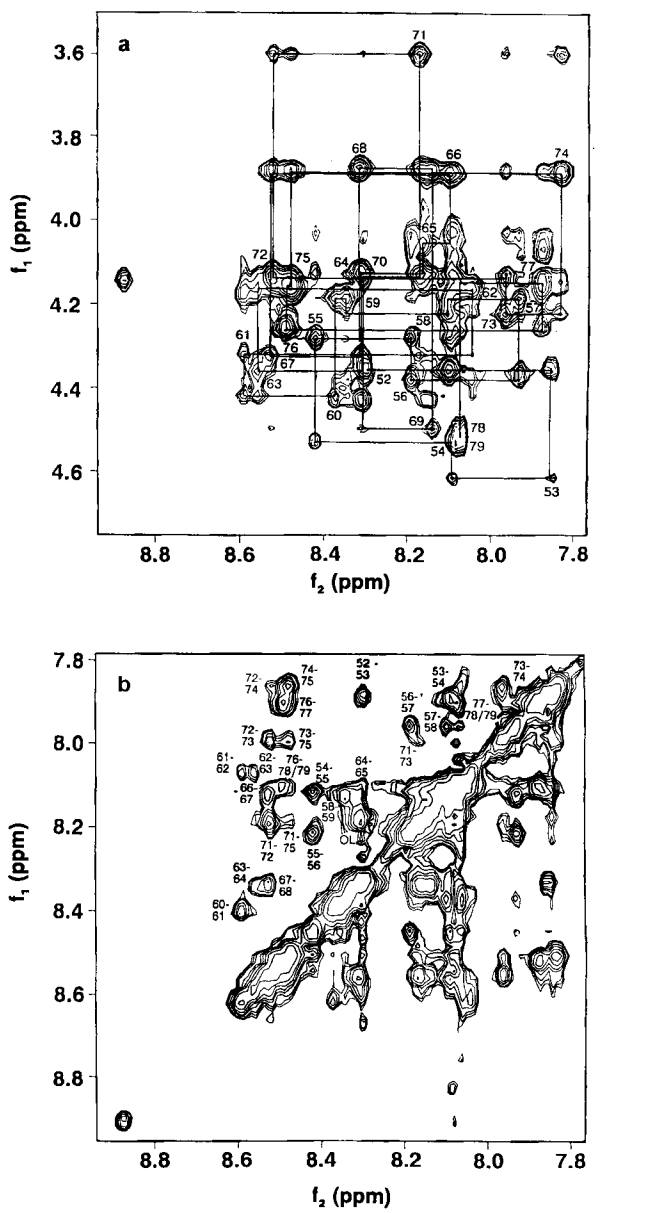

Fig. 6. Parts of a NOESY spectrum at 25°C and 500 MHz of apoCII fragment 50–79. The mixing time used was 400 ms. The solution contained 58% (by vol.) ^1H_2O, 35% $(CF_3)_2CHOH$, 7% ^2H_2O, 20 mM acetic acid and 3 mM polypeptide. (a) NαH–CαH part of the spectrum. Intra-residual crosspeaks are labeled with their corresponding residue number. The 'sequential walk' is shown, connecting consecutive amino acids in the primary structure through their sequential crosspeaks. (b) NαH–NαH region of the same spectrum as in Fig. 6a. Sequential and long-range crosspeaks are labeled. OL, crosspeaks that could not be unambiguously assigned to particular residues due to overlap.

walk in the CαH–NαH region of the NOESY spectrum is demonstrated in Fig. 6a. The assignments are summarized in Table 1.

Secondary structure

When all the resonances in the ^{1}H-NMR spectrum of the peptide are assigned, the observed crosspeaks in the NOESY spectra can be used for determination of the three-dimensional structure of the molecule (Wüthrich, 1986). Any long-range NOE is a consequence of a folded structure. After sequential assignment of the peptide resonances, a number of NOESY crosspeaks remained, which could not be assigned as being intraresidual or sequential. This fact, together with the observation of slowly exchanging backbone amide protons (see below), gives strong support for a folded structure of the apoCII fragment under the conditions that were used.

α Helices are typically manifested in NOESY spectra by $C\alpha_iH-N\alpha_{i+3}H$ crosspeaks. A number of such crosspeaks were found. Particularly in a stretch Thr68–Gly77, several consecutive $C\alpha_iH-N\alpha_{i+3}H$ connectivities could be detected. Crosspeaks for Gln70–Ser73, Leu72–Leu75 and Ser73–Lys76 could not be distinguished separately. If present, they would coincide with other intraresidual or sequential crosspeaks. Due to spectral overlap, also any $C\alpha_iH-N\alpha_{i+3}H$ involving the two carboxy-terminal glutamic acid residues would be hidden. A number of $C\alpha_iH-N\alpha_{i+2}H$ and $C\alpha_iH-N\alpha_{i+4}H$ crosspeaks could be detected in the carboxy-terminal half of the polypeptide. There are also strong $N\alpha_iH-N\alpha_{i+1}H$ and some $N\alpha_iH-N\alpha_{i+2}H$ and $N\alpha_iH-N\alpha_{i+4}H$ crosspeaks in this part of the sequence (Fig. 6b). Fig. 7 shows certain $C\alpha_iH-C\beta_{i+3}H$ and $C\alpha_iH-C\gamma_{i+3}H$ crosspeaks involving residues 63, 64, 66–71 and 74. Crosspeaks that involve Gly65 are anomalously weak in all spectra, which suggests a less-regular structure around this residue. Disorder and/or dynamic effects may be responsible for the observed loss of crosspeak inten-

Table 1. Proton resonance assignment of the carboxy-terminal fragment 50–79 of apoCII determined at 298 K in 20 mM acetic acid with 35% hexafluoroisopropanol (in ppm relative to 4,4-dimethyl-4-silapentanesulfonate). ×, resonance not observed (most likely due to spectral overlap). For γ2 of I66, the peak was only visible in NOESY.

Residue	Assignment										
	NαH	α	β1	β2	γ1	γ2	δ1	δ2	ε1	ε2	other
	ppm										
R50	×	4.12	2.01	×	1.74	×	3.27	×	7.27		
D51	8.88	4.86	2.86	2.93							
L52	8.30	4.33	1.58	×	1.60		0.89	0.97			
Y53	7.88	4.60	3.07	3.18			7.18	7.18	6.90	6.90	
S54	8.08	4.50	3.98	4.08							
K55	8.44	4.25	2.00	1.94	1.55	1.64	1.79	×	3.06	×	×
S56	8.19	4.36	4.00	4.05							
T57	7.94	4.17	4.33		1.32						
A58	8.11	4.20	1.58								
A59	8.33	4.16	1.58								
M60	8.36	4.39	2.27	×	2.66	2.79			×		
S61	8.59	4.29	4.06	4.13							
T62	8.03	4.13	4.37		1.28						
Y63	8.54	4.33	3.21	×			7.15	7.15	6.79	6.79	
T64	8.30	4.09	4.37		1.44						
G65	8.15	3.94, 4.03									
I66	8.09	3.87	1.95		0.95	1.19	1.85				
F67	8.52	4.29	3.09	×			7.22	7.22	7.30	7.30	7.25
T68	8.31	3.84	4.40		1.38						
D69	8.13	4.47	2.99	3.18							
Q70	8.30	4.10	2.21	2.35	2.47	2.52			6.61	6.40	
V71	8.16	3.58	2.05		0.76	0.92					
L72	8.52	4.11	1.95	×	1.52		0.95	×			
S73	7.95	4.20	3.96	4.04							
V74	7.83	3.86	2.37		1.00	1.12					
L75	8.46	4.13	1.96	×	1.60		0.93	×			
K76	8.48	4.23	2.01	×	1.58	1.65	1.76	×	3.04	×	7.65
G77	7.87	4.03, 4.13									
E78	8.07	4.51	2.15	2.32	2.61	×					
E79	8.07	4.47	2.15	2.32	2.61	×					

sity. A summary of the observed connectivities is presented in Fig. 8. Taken together, these results strongly suggest a relatively stable helical structure for residues 66–77.

In a separate NMR experiment, the exchange rates for the NαH protons were studied. Lyophilized polypeptide was dissolved in $^2H_2O/(CF_3)_2C^2HO^2H$ solvent and the disappearance of the NαH resonances was followed. 1 h after dissolving the peptide in ^2H_2O, a number of resonances still remained (Fig. 9), probably due to hydrogen-bond formation involving these NαH protons. The well-resolved resonances of Val74, Ser73, Asp69, Val71, Met60 and Ser61 could be recognized. Some resonances that could be due to several NαH protons were also present. One resonance could be assigned either to Phe67 or Leu72. Another could be due to Leu75 or Lys76, and a third could belong either to Leu52, Gln70, Thr64 or Thr68. In order to distinguish between more rapidly and slowly exchanging amide proton resonances, a NOESY spectrum was recorded over 6–18 h of exchange. Intraresidual or sequential crosspeaks in the CαH–NαH region indicated the presence of Phe67, Leu72, Leu75, Thr68 and Gln70 beside those that could be unambiguously assigned in the one-dimensional spectrum. The NαH proton resonances of Met60 and Ser61 disappeared after 6 h. After 43 h, only the resonances of Val71, Leu72, Ser73, Val74 and Leu75 still remained, indicating a particularly stable secondary structure in this part of the polypeptide.

$C\alpha_iH-N\alpha_{i+3}H$ NOESY crosspeaks could also be found for Lys55–Ala58, Ser56–Ala59, and Met60–Tyr63. The NαH protons of Met60 and Ser61 exchange relatively slowly with the solvent (complete loss of signal in a couple of hours after ^1H_2O to ^2H_2O exchange; see Fig. 9). These NOE connectivities and exchange characteristics are indications of the formation of a helical part also for Lys55–Thr64 in addition to the one over Ile66–Gly77. The formation of an α helix throughout the peptide, from Lys55 to the carboxyl terminus, cannot be excluded by the NMR data.

DISCUSSION

The CD spectrum of both the carboxy-terminal fragment and of native apoCII shows that addition of $(CF_3)_2CHOH$ to the aqueous solution leads to an increased amount of α helical secondary structure.

$(CF_3)_2CHOH$ is a solvent of the same nature as trifluoroethanol, which is known to stabilize the α helix in peptides (Nelson and Kallenbach, 1986). Combined CD and ^{1}H-NMR studies on peptide sequences about 20 amino acids long have indicated that addition of trifluoroethanol or $(CF_3)_2CHOH$ to aqueous solutions does not induce helix formation in new regions of the peptides, but mainly stabilizes helical parts already present in equilibrium with more random structures (Dyson et al., 1988; Khan et al., 1990).

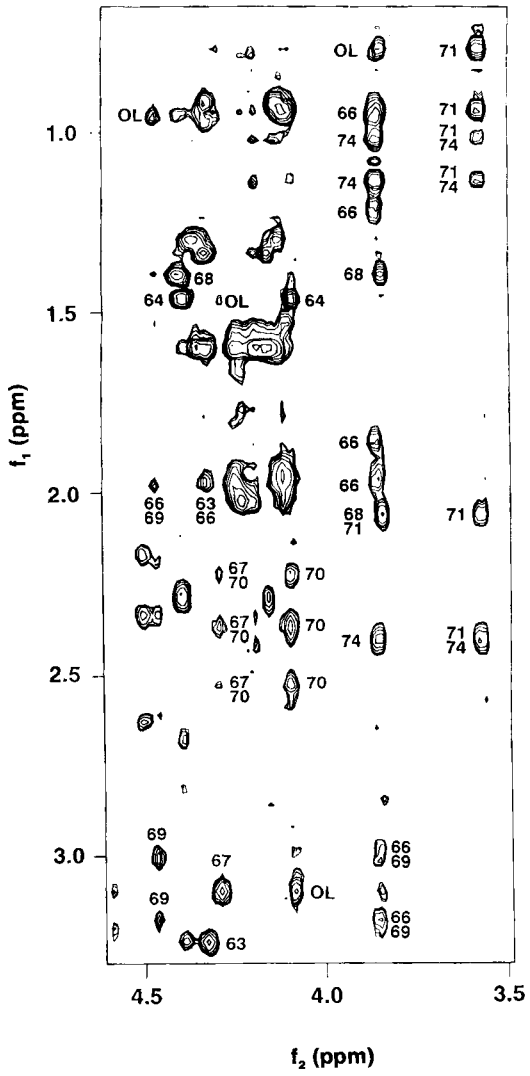

Fig. 7. Part of a NOESY proton NMR spectrum at 500 MHz of apoCII fragment 50–79 in 58% (by vol.) ^1H_2O, 35% $(CF_3)_2CHOH$, 7% ^2H_2O, 20 mM acetic acid at 25°C. The crosspeaks from residues involved in $i-i+3$ interactions are indicated by two residue numbers. Corresponding intraresidual peaks are labeled by one residue number. OL, $i-i+3$ crosspeaks, that could not be unambiguously assigned to particular residues due to overlap. The mixing time used was 200 ms.

The CD results, after addition of the $Ole_2GroPCho$ lipid vesicles, show that native apoCII has its secondary structure significantly affected by the vesicles (increased α-helical content), whereas the fragment remains unaffected. These results are in good agreement with previous observations that native apoCII associates with phospholipids (Morrisett et al., 1977) whereas a fragment composed of residues 56–79 does not interact with phospholipids (Sparrow and Gotto, 1980).

The CD spectra of both apoCII and the fragment in aqueous solution were found to be somewhat variable due to sample history, to concentration and to pH. Some of these effects are probably related to peptide self-association (Mantulin et al., 1980).

The aim of the present study was to directly determine the solution structure of a functional fragment of apoCII comprising residues 50–79. The fragment was found to be amenable to detailed ^{1}H-NMR studies after addition of 35% $(CF_3)_2CHOH$, which led to considerably increased resolution of the ^{1}H NMR spectra. It was evident that $(CF_3)_2CHOH$ prevented self-association of the fragment at the relatively high protein concentrations needed for NMR studies. Furthermore, this concentration of $(CF_3)_2CHOH$ gave a 2.5-fold increase in the α-helical content from about 20% to 50% of the peptide, as estimated from our CD studies. A straightforward explanation for the $(CF_3)_2CHOH$-induced increase in α-helix secondary structure is that $(CF_3)_2CHOH$ stabilizes α-helical structures present in aqueous solution in an equilibrium between helical and random conformations. Therefore, the relative mass of the helical conformation increases in the mixed solvent and becomes recognizable by NMR (Nelson and Kallenbach, 1986, 1989; Dyson et al., 1988; Khan et al., 1990).

Attempts to measure whether the apoCII fragment could activate lipoprotein lipase in the presence of $(CF_3)_2CHOH$ were made. The studies were impossible to carry out due to the strong sensitivity of lipoprotein lipase to the solvent. Complete inactivation of the enzyme itself was already obtained at 1% $(CF_3)_2CHOH$ (data not shown).

We were able to solve the sequential assignments of the proton resonances and to derive data for secondary structure, suggesting that about 60% of the residues (residues 55–64 and 66–77) in the sequence are in a relatively stable α-helical conformation. This is in contrast to the previously suggested Chou-Fasman predictions, with mainly β-sheet structure predicted for this part. The presently known sequences for apoCII from five animal species (summarized in Andersson et al., 1991) show that this part of the molecule is highly conserved and that there are no amino acid substitutions which would prevent formation of α-helical structure. Experiments with synthetic fragments of human apoCII showed that fragment 56–79 had full activity in the systems used (Kinnunen et al., 1977), whereas fragments 56–69 and 61–69 were inactive (Smith et al., 1980). Addition of residues at either end of these fragments (residues 51–69 or 61–79) restored some activity (Kinnunen et al., 1977; Smith et al., 1980). One suggested interpretation of the data was that, for stabilization of an important β turn comprising residues 61–65, a sufficient number of residues had to be present either on the amino-terminal side or on carboxy-terminal side of the turn (Smith et al., 1980; Smith and Pownall, 1984). Our data indicate that both of these segments on either side of Gly65 can attain an α-helical structure, but that Gly65 may not be part of a helix. The function of a glycine residue as an α-helix terminator has been previously pointed out (Chou and Fasman, 1974; Preveliege, 1989).

The α-helical structure seems to be particularly stable over residues 71–75. This is also in agreement with a theoretical hydrophobic-moment analysis which shows hydrophobic moments compatible with an α helix with amphipatic character, over residues 70–74 among the 30 residues comprising the carboxyl terminus of the apoCII (De Loof et al., 1987).

In conclusion, the results from our NMR studies on the 50–79 carboxy-terminal fragment of apoCII show fairly stable α helices for residues 55–64 and 66–77, i.e. involving about 18 of the 29 amino acid residues or 60% of the sequence. These results are in good agreement with the information from CD spectra that about 50% of the peptide displays α-helical secondary structure in an aqueous solution containing 35% $(CF_3)_2CHOH$.

ApoCII and its 50–79 fragment are examples of linear peptides which self-associate and do not adopt well-defined structures in aqueous solution. In more hydrophobic environments, more well-defined solution structures may become

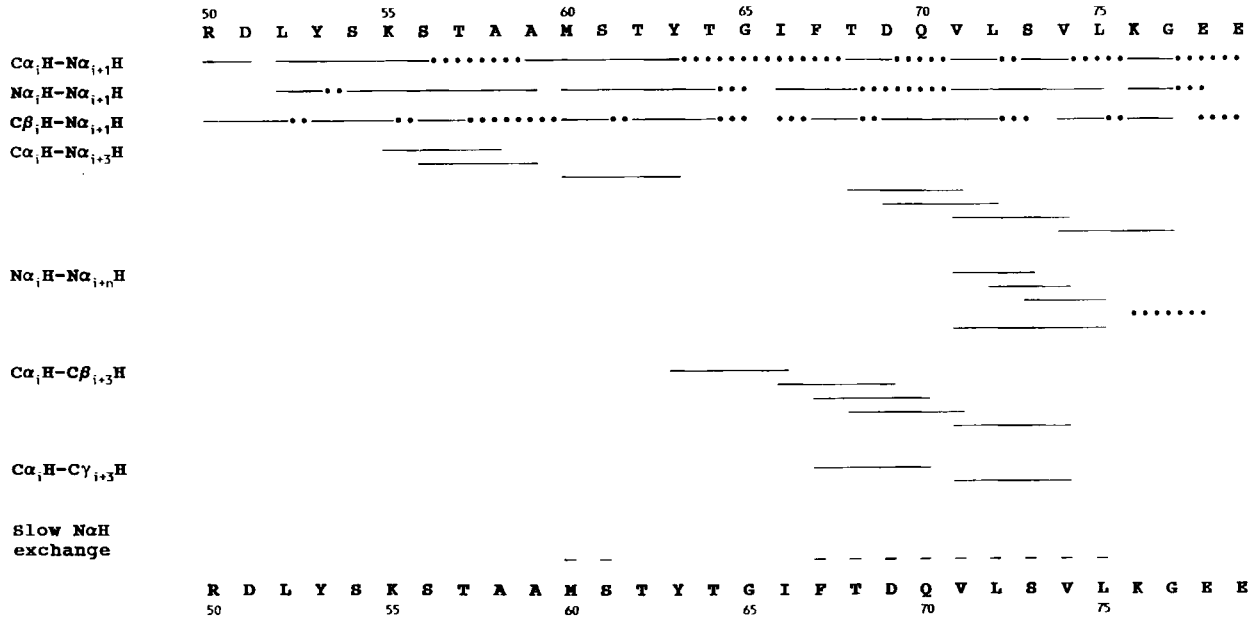

Fig. 8. Summary of observed sequential or medium range NOESY crosspeaks. Slowly exchanging NαH are also indicated. Dotted lines indicate steps which are uncertain due to spectral overlap. The assignments are indicated using one-letter codes for amino acids and residue numbers are as for intact apoCII.

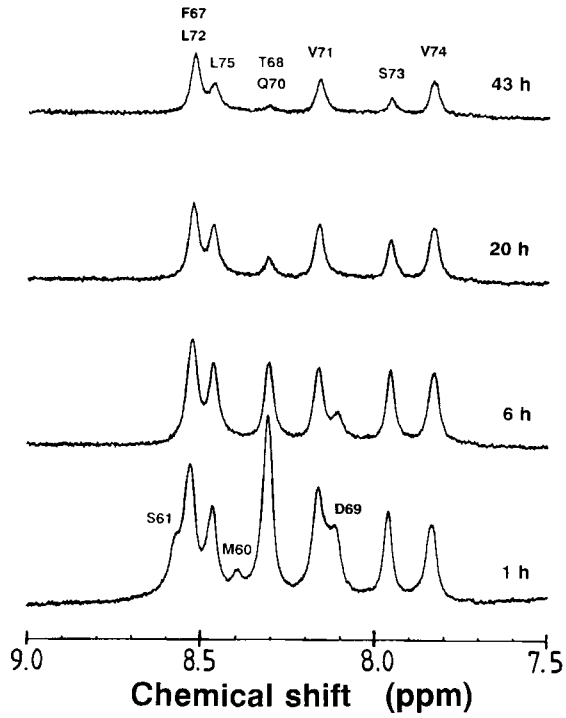

Fig. 9. ^{1}NMR spectra (500 MHz) of the apoCII fragment 50–79 showing the NαH resonances at different times after ^1H_2O to ^2H_2O exchange. The assignments of the NαH to the different residues are indicated in the figure, with the rapidly exchanging amide protons in the spectrum after 1 h and the slowly exchanging ones in the spectrum after 43 h.

stabilized, as exemplified by our studies in a mixed H_2O $(CF_3)_2CHOH$ solvent. This ability to form helical segments may be of importance for the interactions associated with the biological role of apoCII.

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