Heteronuclear NMR studies of human serum apolipoprotein A-I

Part I. Secondary structure in lipid-mimetic solution

Mark Okon[a,*], Philippe G. Frank[b], Yves L. Marcel[b], Robert J. Cushley[a]

[a] *Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*
[b] *Lipoproteins and Atherosclerosis Research Group, University of Ottawa Heart Institute, Ottawa, ON, Canada K1Y 4E9*

Received 13 December 2001; revised 5 March 2002; accepted 6 March 2002

First published online 26 March 2002

Edited by Thomas L. James

Abstract The apolipoprotein A-I (apoA-I) solution structure in the presence of sodium dodecyl sulfate (SDS) was determined by combination of chemical shift index and torsion angle likelihood obtained from shift and sequence similarity methods. ApoA-I in lipid-mimetic solution is composed of α-helices (residues 8–32, 45–64, 67–77, 82–86, 90–97, 100–118, 122–140, 146–162, 167–205, 210–216 and 221–239), with 2–5 residue irregular segments between helical repeats, and the irregular segment 78–81 within helical repeat 2. ApoA-I is a monomer in the SDS complex and no evidence of interhelical interactions is found. Comparison of the apoA-I and apoA-I(1–186) [Okon et al., FEBS Lett. 487 (2001) 390–396] solution structures revealed that apoA-I undergoes a conformational change around Pro121. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Protein structure; Apolipoprotein; Nuclear magnetic resonance; Chemical shift index; Torsion angle likelihood obtained from shift and sequence similarity; Sodium dodecyl sulfate

1. Introduction

Apolipoprotein A-I (apoA-I) is a single polypeptide of 243 amino acid residues, and the major constituent in high density lipoprotein (HDL), comprising approximately 70% of total protein. A key metabolic role of apoA-I is its ability to activate lecithin:cholesterol acyltransferase, an enzyme which transesterifies the *sn*-2 fatty acid from phosphatidylcholine to cholesterol to yield cholesteryl esters [1]. ApoA-I has also been suggested as a probable ligand for the HDL receptor, SR-BI, [2] and to play a central regulatory role in cholesterol efflux [3,4]. ApoA-I is hydrophobic, aggregates in aqueous solution, and has two 11 and eight 22 residue tandem repeats, which were proposed to form amphipathic helices with distinct hydrophilic and hydrophobic faces [5]. Based upon primary sequence analysis, and circular dichroism and infrared results, a secondary structure of apoA-I was predicted wherein the tandem repeats formed antiparallel helical regions linked by short, tight turns [6,7]. Adjacent helices were proposed to be close enough to form salt bridges [8]. Recently, the X-ray structure of lipid-free tetramers of the N-terminal deletion mutant apoA-I(44–243) has been reported by Borhani et al. [9].

In the present communication we report the solution secondary structure of isotopically labeled apoA-I determined by multidimensional heteronuclear nuclear magnetic resonance (NMR) in a lipid-mimetic environment of excess sodium dodecyl sulfate (SDS). The secondary structure of intact apoA-I is compared with our recently published solution secondary structure of apoA-I(1–186) in SDS [10].

2. Materials and methods

2.1. Sample production and purification

The [u-^{13}C, u-^{15}N]apoA-I sample, including the N-terminal extension Met-Arg-Gly-Ser-$(His)_6$-Met [11], was expressed in a bacterial system described earlier [12], using Martek 9-CN media containing 98% ^{13}C and 98% ^{15}N (Martek Biosciences, Columbia, MD, USA). [u-^{15}N] apoA-I was prepared in the same system using [u-^{15}N] Martek 9. ApoA-I plus leader had a molecular weight of 29 500. After purification on nitriloacetic acid agarose (NTA, from Qiagen, Valencia, CA, USA), the uniformly labeled protein (7.5 mg/l media) was dialyzed against 5 mM NH_4HCO_3, and lyophilized.

2.2. Circular dichroic measurements

Circular dichroic spectroscopy was performed using a Jasco J41A spectropolarimeter, as previously described [13]. The percentage of α-helix content was calculated from the molar ellipticity at 222 nm, using a mean residue weight of 115.2 for native apoA-I.

2.3. NMR spectroscopy and structure analysis

The samples used in the NMR studies were: 2 mM [u-^{15}N]apoA-I in 95% H_2O:5% D_2O solution, pH 6.5 (sample A) and 2 mM [u-^{13}C, u-^{15}N]apoA-I in 95% H_2O:5% D_2O solution (sample B). The temperatures of both samples and pH values of sample B were varied. The pH values were adjusted by adding small amounts of 0.1 N HCl or NaOH. All solutions contained SDS-d_{25}; protein:SDS ratio 1:140 mol/mol. The NMR spectra were recorded on a Bruker AMX-600 spectrometer operating at 600.13 MHz (^{1}H) and equipped with a *Z*-gradient triple-resonance probe. In all experiments quadrature detection in the indirectly detected dimensions was obtained using States-TPPI [14], modified to give additional suppression of axial peaks [15]. The following experiments, and their modifications to the published procedures, were applied to samples A and B (the sample conditions are denoted in parentheses).

A two-dimensional (2D) ^{1}H/^{15}N heteronuclear single quantum coherence (HSQC) spectrum with water suppression by gradient-tailored

*Corresponding author. Present address: University of British Columbia, Department of Chemistry, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1. Fax: (1)-604-822 5227.
E-mail address: mokon@chem.ubc.ca (M. Okon).

Abbreviations: Apo, apolipoprotein; HDL, high density lipoprotein; SDS, sodium dodecyl sulfate; NMR, nuclear magnetic resonance; TOCSY, total correlation spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy; HSQC, heteronuclear single quantum coherence; CSI, chemical shift index; TALOS, torsion angle likelihood obtained from shift and sequence similarity

0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(02)02600-5

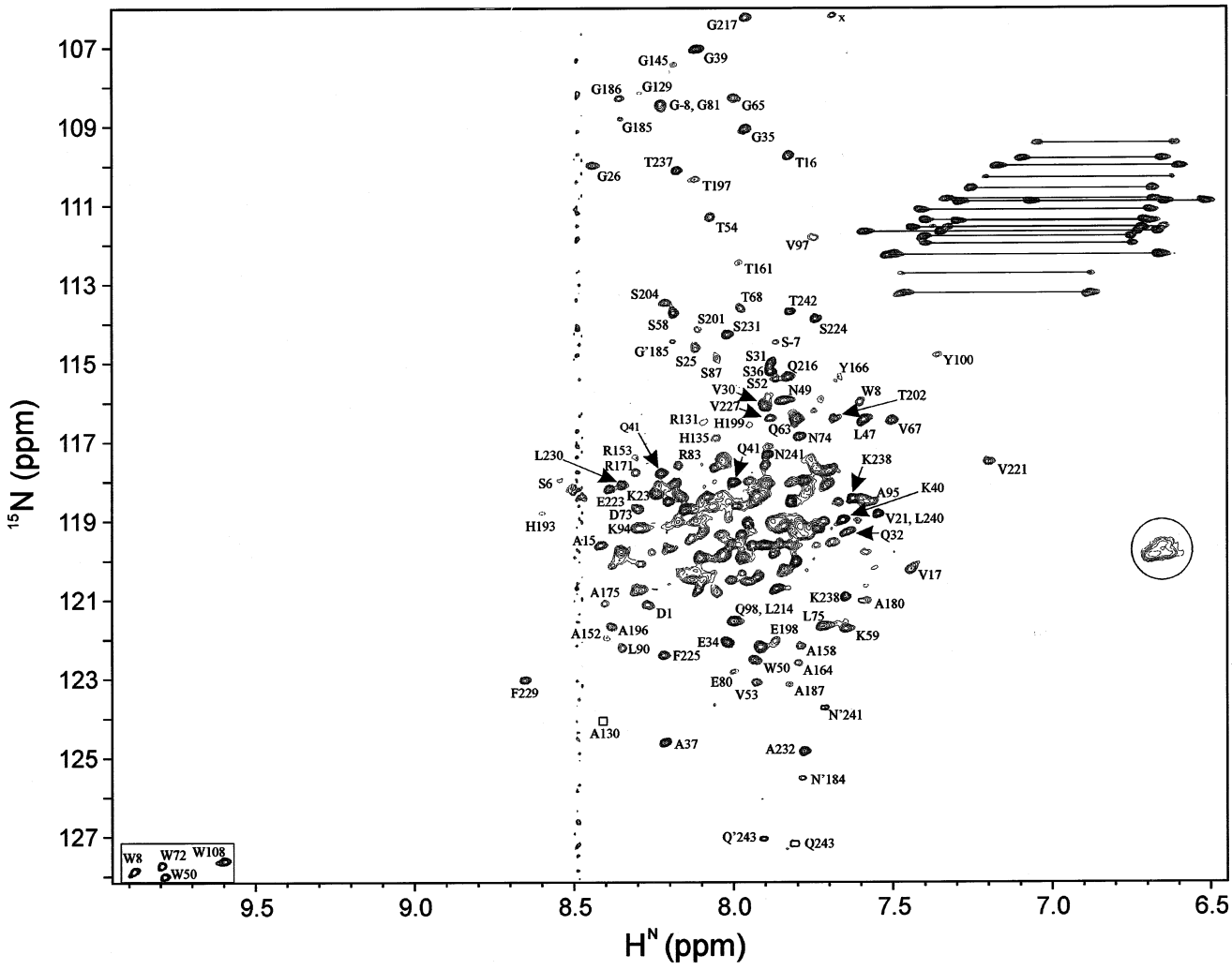

Fig. 1. 2D ^{1}H–^{15}N HSQC correlation spectrum of apoA-I. The cross-signals, mainly representing one amino acid, are labeled with their residue name and number. The signals from N1H of Trp side chains are in rectangle in the left bottom corner. Folded in ^{15}N dimension peaks from Arg side chains are in the circle. NH_2 signals from Asn and Gln side chains are connected by straight lines. Signals labeled N′184, N′241 and Q′243 are assigned to deamidated forms of apoA-I with N184 and N241 replaced by D184 and D241, respectively. Signals labeled S-7 and G-8 belong to the aminoterminal tag, and labeled 'x' to unassigned impurity. Vertical noise at 8.5 ppm arises from folded residual H_2O signal. Intensity of cross-peak from Ala 130 is lower than selected cutoff and its position is denoted by a small square.

excitation (WATERGATE) [16] of sample A was acquired at 49°C (64 transients) with 256×256 complex points and spectral widths of 3.095 ppm (H^N) and 24.00 ppm (^{15}N) with carrier positions at 8.3 and 116.6 ppm, respectively.

The 3D ^{15}N-edited total correlation spectroscopy (TOCSY)–HSQC spectrum [17] of sample A was acquired at 40 and 50°C and performed with the DIPSI-2rc isotropic mixing sequence [18] using a mixing time of 86 ms. The 3D ^{15}N-edited gradient-enhanced nuclear Overhauser enhancement spectroscopy (NOESY)–HSQC [19] spectrum was obtained for sample A at 40°C and recorded as described previously [10]. 3D ^{15}N-edited NOESY–HSQC spectra were obtained for sample A at 40, 50 and 57°C and for sample B (pH 6.0 and 7.0 at both 42 and 52°C, pH 8.0 at 42°C). The pulse sequence was based on a 3D ^{15}N-edited NOESY–HMQC pulse sequence [20] with the following modifications: (i) an HSQC step was inserted instead of HMQC, (ii) pulse field gradients were used to minimize artefacts [21], and (iii) the WATERGATE pulse sequence [16] was used to suppress the water signal. The 3D ^{15}N/^{15}N-edited NOESY–HSQC spectrum of sample A (42°C) was recorded as a variant of the XTONOX experiment [22], where the TOCSY evolution period was omitted.

The 3D spectra of sample B, HNCO, HNCA and HN(CO)CA [23] (pH 6.0 and 7.0 at both 42 and 52°C, pH 8.0 at 42°C), and the CBCA(CO)NH [24] and HN(COCA)HA [25] spectra (pH 7.0, 42°C), were recorded as described previously [10]. The 3D HNHA spectrum [26] of sample A (50°C) was acquired (48 transients) with 256 (H^N)×48 (^{1}H)×40 (^{15}N) complex points and spectral widths of 2.7 (H^N), 6.1 (^{1}H) and 24.0 (^{15}N) ppm.

The 4D ^{15}N/^{15}N-edited NOESY experiment was applied only to sample A. The pulse sequence for this experiment was based on a ^{13}C/^{13}C-edited NOESY experiment [27]. All NOESY spectra were recorded with a mixing time of 150 ms.

The numbers of scans were eight to 96 for 3D and two for 4D experiments. Proton chemical shifts were referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) directly, and ^{13}C and ^{15}N chemical shifts were indirectly referenced to DSS [28]. Data were processed and analyzed on an SGI O2 workstation using nmrPipe/nmrDraw [29] and NMRView3 [30]. ApoA-I spectra were assigned in the same way as we used to assign apoA-I(1–186) spectra [10]. The apoA-I secondary structure was determined from the chemical shift values using the chemical shift index (CSI) method [28,31–33] and the torsion angle likelihood obtained from shift and sequence similarity (TALOS) program [34].

To compare apoA-I and apoA-I(1–186) at the same conditions (1 mg/ml, pH 6.4, 39°C, protein:SDS ratio 1:140 mol/mol), three additional 3D apoA-I spectra – (HB)CBCA(CO)HN, HNCACB [35,36] and HNCO – were recorded on a Varian Unity-Inova NMR-spectrometer operating at 599.76 MHz (^{1}H) and equipped with an *X*-, *Y*- and *Z*-gradient triple-resonance probe.

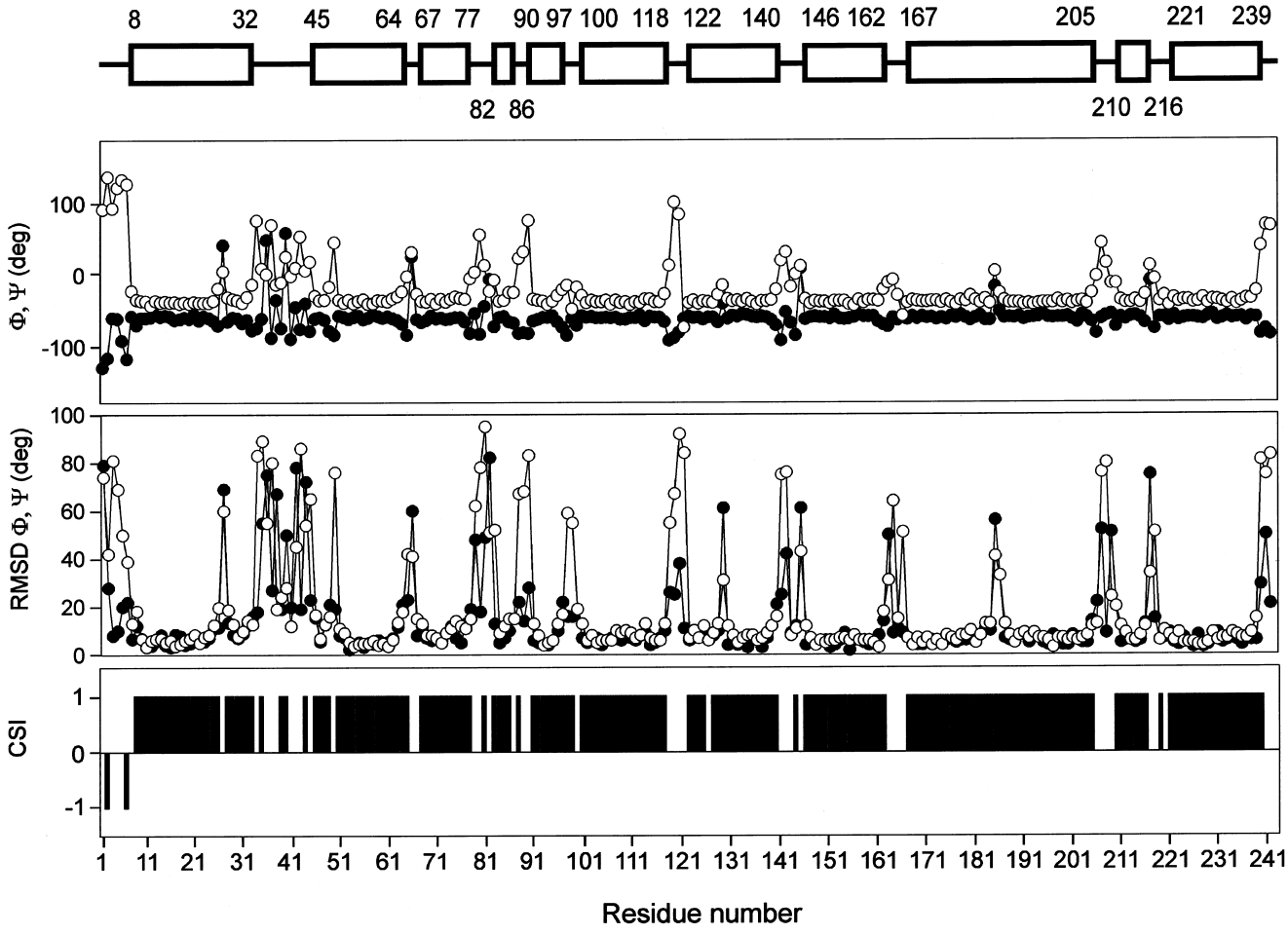

Fig. 2. Summary of structural information obtained from NMR spectra at pH 7.0, 42°C. From bottom to top: consensus of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and $^{13}C'$ CSI values; Φ (filled circles) and Ψ (open circles) angle values predicted by TALOS; apoA-I solution secondary structure (α-helices are depicted by rectangles and irregular regions by lines). The average backbone torsion angles among all well-defined α-helices (RMSD $\leq 8°$) are: $\Phi_{aver} = -63.2 \pm 2.0$ and $\Psi_{aver} = -41.9 \pm 2.1$ degrees.

3. Results and discussion

3.1. Global characteristics of apoA-I NMR spectra in SDS solution and tertiary organization of apoA-I

The salient feature of apoA-I NMR spectra in solution is the small chemical shift dispersion. The majority of the H^N signals are found in the narrow range between 8.5 and 7.5 ppm (Fig. 1). The chemical shift range for signals from apoA-I amino acid side chains is also quite narrow. For example, 37 $C^{\delta}H_3$ Leu and 13 $C^{\gamma}H_3$ Val signals are all distributed in a narrow range of 0.8–1.1 ppm, indicating the absence of any possible ring current shifts that could be induced by long range interhelical hydrophobic interactions between 50 aliphatic and 22 aromatic side chains in the tertiary

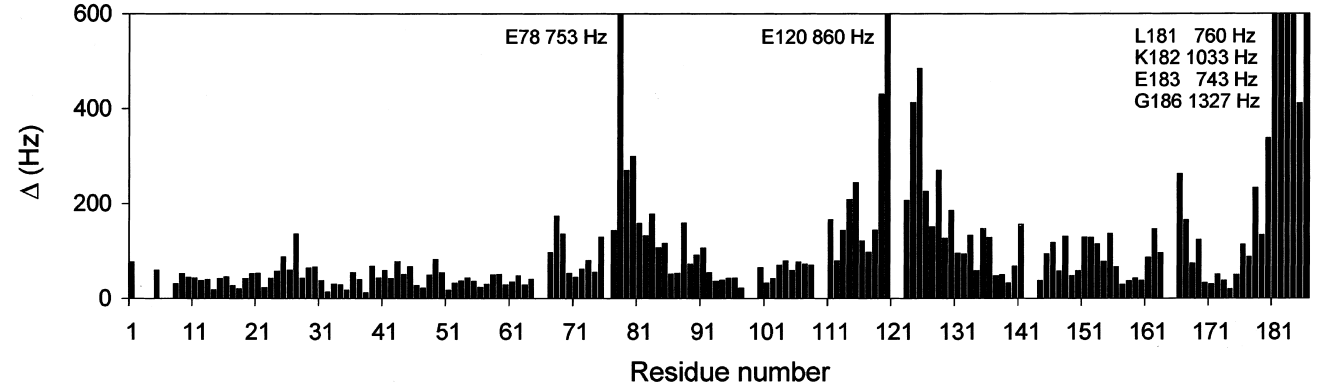

Fig. 3. Chemical shift perturbations (Δ) defined as sum of the absolute values of the backbone H^N, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$ chemical shift differences due to different lengths of apoA-I (chemical shifts of apoA-I(1–186) and apoA-I taken under the same conditions (1 mg/ml, pH 6.4, 39°C, protein:SDS ratio 1:140 mol/mol) are compared).

apoA-I structure. The most likely explanation is that lipid-bound apoA-I is in a 'molten globular-like' state with the α-helices being relatively weakly stabilized by tertiary interactions, as was suggested for lipid-free apoA-I structure [37].

3.2. Secondary structure of apoA-I in SDS solution

Both CSI and TALOS predict a predominantly α-helical structure for the protein in a lipid environment (Fig. 2). The results are almost identical to those we determined for apoA-I(1–186) in SDS [10]. CSI values of zero are found for 2–5 residues between helical repeats and are accompanied by deviation from the ideal α-helical values of TALOS-predicted torsion angles with increased root mean square deviations (RMSD; Fig. 2). These regions we denote as irregular structures, i.e. as regions with no well-defined structure. The irregular structures occur between the helical repeats predicted by Segrest and coworkers [5]. We also calculated apoA-I structures at pH 6.0, 42°C and at pH 7.0, 52°C. Although the CSI values remain unchanged with pH and temperature, TALOS predicts that the irregular regions become 'more helical' at pH 6.0, especially regions 78–81, 87–89 and 208–209.

Indication that spontaneous deamidation of apoA-I takes place at Asn184 and Asn241 positions was indicated from comparison of HNCA, HN(CO)CA and CBCA(CO)NH spectra of apoA-I. The deamidation is accompanied by perturbations of backbone chemical shifts of 5–10 residues situated (in the apoA-I secondary structure) around deamidated residues.

3.3. Local characteristics of apoA-I NMR spectra and comparison of apoA-I and apoA-I(1–186) structures

Analysis of the apoA-I NMR spectra shows that resonance lines from residues constituting helical repeats 4 and 5 (residues 100–140) have smaller intensities then the lines from the rest of the protein. Such behavior is usually the characteristic of unstable structure with intermediate exchange rates between existing conformers, and we would expect to observe conformational and/or spectral changes from the region when conditions vary.

Indeed, comparing apoA-I and apoA-I(1–186) [10] we find that the difference between the two structures is in the region around Pro121, which is the midpoint between helices 4 and 5. The strong chemical shift perturbations in the region reflect this fact (Fig. 3). It can be seen that the perturbations monotonically decrease to either side of Pro121. The $^{13}C^{\alpha}$ chemical shift of Glu120 undergoes an especially strong perturbation, 3.9 ppm upfield in apoA-I compared with apoA-I(1–186). Such a large shift, together with upfield $^{13}C^{\alpha}$ shift of 1.5 ppm for Val119, indicates a more extended structure around Pro121 in apoA-I. It is interesting to note that TALOS predicts a Val119 conformational change from helical (for apoA-I(1–186)) to extended (for apoA-I at 52°C). The chemical shift perturbations for residues near Glu78 in the sequence (Fig. 3) do not result in any prediction of a structure change for the region and seemingly reflect only the unstable nature of the region.

Apparently, the structure of apoA-I(1–186) is more 'rigid' in the region around Pro121. The result is consistent with the data obtained by the limited proteolysis of lipid-free apoA-I and apoA-I(1–186). The minor cleavage sites in apoA-I, including Leu122, were not found in apoA(1–186) [38]. The NMR evidence strongly suggests apoA-I undergoes a conformational change around the Pro121 position as conditions vary. Existence of a mobile domain composed of residues 99–143 was proposed from investigation of the immunoreactivity of a series of epitopes distributed along the sequence in lipid-free and lipid-bound forms of apoA-I [39].

Our NMR-derived apoA-I structure in lipid-mimetic solution closely resembles the crystal structure except for the irregular segments between helical repeats. We also see no evidence showing eight amino acids in the last 22-mer repeat of apoA-I (residues 220–227) in an extended, non-helical conformation as indicated by the crystal structure [9]. On the contrary, NMR gives a very well-defined helix for residues 221–227.

Acknowledgements: This work was supported by grants from the Natural Science and Engineering Research Council of Canada (NSERC) to R.J.C., and a Medical Research Council group grant (GR-11471) to Y.L.M. P.G.F. was supported by a scholarship from the Heart and Stroke Foundation of Canada. The NMR facility was financed in part by NSERC. We thank Vivian Franklin for expert technical assistance with apoA-I expression. We also thank Dr. Lawrence McIntosh (University of British Columbia) for the use of Varian NMR-spectrometer.

References

[1] Fielding, C.J., Shore, V.G. and Fielding, P.E. (1972) Biochem. Biophys. Res. Commun. 46, 1493–1498.
[2] Acton, S., Rigotti, A., Landschulz, K.T., Xu, S., Hobbs, H.H. and Krieger, M. (1996) Science 271, 518–520.
[3] Castro, G.R. and Fielding, C.J. (1988) Biochemistry 27, 25–29.
[4] Zhao, Y., Sparks, D.L. and Marcel, Y.L. (1996) J. Biol. Chem. 271, 25145–25151.
[5] Segrest, J.P., Jones, M.K., De Loof, H., Brouillette, C.G., Venkatachalapathi, Y.V. and Anantharamaiah, G.M. (1992) J. Lipid Res. 33, 141–166.
[6] Edelstein, C., Kezdy, F.J., Scanu, A.M. and Shen, B.W. (1979) J. Lipid Res. 20, 143–153.
[7] Nolte, R.T. and Atkinson, D. (1992) Biophys. J. 63, 1221–1239.
[8] Lins, L., Brasseur, R., De Pauw, M., Van Biervliet, J.P., Ruysschaert, J-M., Rosseneu, M. and Vanloo, B. (1995) Biochim. Biophys. Acta 1258, 10–18.
[9] Borhani, D.W., Rogers, D.P., Engler, J.A. and Brouillette, C.G. (1997) Proc. Natl. Acad. Sci. USA 94, 12291–12296.
[10] Okon, M., Frank, P.G., Marcel, Y.L. and Cushley, R.J. (2001) FEBS Lett. 487, 390–396.
[11] Bergeron, J., Frank, P.G., Emmanuel, F., Latta, M., Zhao, Y., Sparks, D.L., Rassart, E., Denefle, P. and Marcel, Y.L. (1997) Biochim. Biophys. Acta 1344, 139–152.
[12] Burgess, J.W., Frank, P.G., Franklin, V., Liang, P., McManus, D.C., Desforges, M., Rassart, E. and Marcel, Y.L. (1999) Biochemistry 38, 14524–14533.
[13] Frank, P.G., N'Guyen, D., Franklin, V., Neville, T., Desforges, M., Rassart, E., Sparks, D.L. and Marcel, Y.L. (1998) Biochemistry 37, 13902–13909.
[14] Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989) J. Magn. Reson. 85, 393–399.
[15] Hammarström, A. and Otting, G. (1994) J. Magn. Reson. Ser. A 109, 246–249.
[16] Sklenar, V., Piotto, M., Leppik, R. and Saudek, V. (1993) J. Magn. Reson. Ser. A 102, 241–245.
[17] Marion, D., Driscoll, P.C., Kay, L.E., Wingfield, P.T., Bax, A., Gronenborn, A.M. and Clore, G.M. (1989) Biochemistry 28, 6150–6156.
[18] Cavanagh, J. and Rance, M. (1992) J. Magn. Reson. 96, 670–678.
[19] Zhang, O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) J. Biomol. NMR 4, 845–858.
[20] Marion, D., Kay, L.E., Sparks, S.W., Torchia, D.A. and Bax, A. (1989) J. Am. Chem. Soc. 111, 1515–1517.
[21] Bax, A. and Pochapsky, S. (1992) J. Magn. Reson. 99, 638–643.
[22] Ernst, R.R., Boentges, S. and Schmidt, M. (1996) in: Encyclopedia of Nuclear Magnetic Resonance (Grant, D.M. and Harris, R.K., Eds.), Vol. 5, pp. 3129–3133, Wiley, New York.
[23] Grzesiek, S. and Bax, A. (1992) J. Magn. Reson. 96, 432–440.

[24] Grzesiek, S. and Bax, A. (1992) J. Am. Chem. Soc. 114, 6291–6293.
[25] Olejniczak, E.T., Xu, R.X., Petros, A.M. and Fesik, S. (1992) J. Magn. Reson. 100, 444–450.
[26] Vuister, G.W. and Bax, A. (1993) J. Am. Chem. Soc. 115, 7772–7777.
[27] Clore, G.M., Kay, L.E., Bax, A. and Gronenborn, A.M. (1991) Biochemistry 30, 12–18.
[28] Wishart, D.S. and Nip, A.M. (1998) Biochem. Cell Biol. 76, 153–163.
[29] Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) J. Biomol. NMR 6, 277–293.
[30] Johnson, B.A. and Blevins, R.A. (1994) J. Biomol. NMR 4, 603–614.
[31] Wishart, D.S., Sykes, B.D. and Richards, F.M. (1991) J. Mol. Biol. 222, 311–333.
[32] Wishart, D.S., Sykes, B.D. and Richards, F.M. (1992) Biochemistry 31, 1647–1651.
[33] Wishart, D.S. and Sykes, B.D. (1994) J. Biomol. NMR 4, 171–180.
[34] Cornilescu, G., Delaglio, F. and Bax, A. (1999) J. Biomol. NMR 13, 289–302.
[35] Muhandiran, D.R. and Kay, L.E. (1994) J. Magn. Reson. B103, 203–216.
[36] Kay, L.E., Keiter, P. and Saarinen, T. (1992) J. Am. Chem. Soc. 114, 10663–10665.
[37] Gursky, O. and Atkinson, D. (1996) Proc. Natl. Acad. Sci. USA 93, 2991–2995.
[38] Rogers, D.P., Roberts, L.M., Lebowitz, J., Engler, J.A. and Brouillette, C.G. (1998) Biochemistry 37, 945–955.
[39] Calabresi, L., Meng, Q.H., Castro, G.R. and Marcel, Y.L. (1993) Biochemistry 32, 6477–6484.