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Lipid binding ability of human apolipoprotein E N-terminal domain isoforms: correlation with protein stability?☆

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Abstract

Human apolipoprotein (apo) E exists as one of three major isoforms, E2, E3 or E4. Individuals carrying the ε4 allele have an increased risk of heart disease and premature onset of Alzheimer's disease. To investigate the molecular basis for this phenomenon, the N-terminal domain of apoE3, apoE2 and apoE4 were expressed in bacteria, isolated and employed in lipid binding and stability studies. Far UV circular dichroism spectroscopy in buffer at pH 7 revealed a similar amount of α-helix secondary structure for the three isoforms. By contrast, differences were noted in apoE-NT isoform-specific transformation of bilayer vesicles of dimyristoylphosphatidylglycerol (DMPG) into discoidal complexes. ApoE4-NT induced transformation was most rapid, followed by apoE3-NT and apoE2-NT. To determine if differences in the rate of apoE-NT induced DMPG vesicle transformation is due to isoform-specific differences in helix bundle stability, guanidine HCl denaturation studies were conducted. The results revealed that apoE2-NT was the most stable, followed by apoE3-NT and apoE4-NT, establishing an inverse correlation between helix bundle stability and DMPG vesicle transformation rate at pH 7. When the zwitterionic dimyristoylphosphatidylcholine (DMPC) was employed as the model lipid surface, interaction of apoE-NT isoforms with the lipid substrate was slow. However, upon lowering the pH from 7 to 3, a dramatic increase in the rate of DMPC vesicle transformation rate was observed for each isoform. To evaluate if the increased DMPC vesicle transformation rates observed at low pH is due to pH-dependent alterations in helix bundle stability, guanidine HCl denaturation studies were performed. ApoE2-NT and apoE3-NT displayed increased resistance to denaturation as a function of decreasing pH, while apoE4-NT showed no change in stability. Studies with the fluorescent probe, 8-anilino-1-naphthalene sulfonic acid, indicated an increase in apoE hydrophobic surface exposure upon decreasing the pH to 3.0. Taken together, the data indicate that changes in the stability of secondary structure elements in apoE-NT isoforms are not responsible for

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pH-induced increases in lipid binding activity. It is likely that pH-induced disruption of inter-helical tertiary contacts may promote helix bundle conformational changes that present the hydrophobic interior of the protein to potential lipid surface binding sites.

Keywords: Apolipoprotein E; Isoforms; Protein stability; Tertiary structure; Lipid binding

1. Introduction

Exchangeable apolipoproteins play a critical role in plasma lipoprotein metabolism through their function(s) as: (a) structural components of lipoproteins; (b) activators of lipid metabolic enzymes and proteins; or (c) ligands for cell surface receptors [1,2]. As many as 11 different exchangeable apolipoproteins have been identified in human plasma, characterized by a common structural motif: the amphipathic α-helix [3]. These helices possess well-defined polar and non-polar faces that interact at the lipoprotein surface with the aqueous and hydrophobic milieu, respectively. Employing gene disruption techniques, it has been shown that a deficiency in apolipoprotein (apo) E results in massive accumulation of remnant lipoproteins, leading to severe hypercholesterolemia and premature atherosclerosis [4,5]. On the other hand, transgenic mice over expressing apoE manifest decreased plasma cholesterol levels on a chow diet and a marked resistance to diet-induced hypercholesterolemia. These studies document the direct relevance of apoE in modulating plasma lipoprotein metabolism, a property attributed to its ability to act as a ligand for the low density lipoprotein (LDL) receptor.

In humans, apoE is a 34 kDa protein that exists as one of three predominant isoforms differing in amino acids at two positions [6]. ApoE3, the most abundant isoform, contains a single cysteine at position 112 and an arginine at position 158. ApoE4 has arginines at position 112 and 158 while apoE2 possesses cysteines at both positions. ApoE is comprised of two independently folded domains that can be isolated following proteolytic cleavage at a loop region linking the two domains [7,8]. The 22 kDa N-terminal (NT) domain (residues 1–191) adopts a water soluble, monomeric, globular conformation that is resistant to denaturation. It houses high affinity binding sites for the LDL receptor family of proteins, a function that is retained even in the isolated 22 kDa NT domain. By contrast, the 10 kDa C-terminal domain (residues 216–299) is less stable to denaturation, has a high lipid binding affinity and is responsible for apoE self association in the absence of lipid. Importantly, the apoE NT domain must be lipid-bound in order to elicit receptor-binding activity. This domain associates poorly with lipoproteins upon incubation with plasma [9,10], suggesting that either the lipid binding affinity of this domain is weak relative to other apolipoproteins, or the C-terminus is required to initiate association with spherical lipoprotein particles.

In 1991, Wilson et al. [13] reported the X-ray crystal structure of the 22 kDa N-terminal domain of human apoE3 in the lipid-free state. This structure is comprised of a bundle of four elongated amphipathic α-helices organized in an up-down topology, Fig. 1. The LDL receptor-binding sites are controlled by critical basic residues confined to helix 4, which are required to be presented in an appropriate conformation to elicit receptor recognition. The interior of the bundle contains several leucine residues that align to form a leucine zipper-like motif. In addition, inter- and intra-helical salt bridge interactions stabilize the protein structure. These factors probably contribute to the unusually high stability of this domain, compared to other exchangeable apolipoproteins [8]. It has been proposed that the four-helix bundle NT domain undergoes an opening about putative hinge loops, thereby facilitating interaction of the hydrophobic interior with the lipid surface [6,11,12]. X-Ray structure determination and site directed mutagenesis studies of the NT domain of apoE isoforms have improved our understanding of the structural basis of isoform-specific differences in

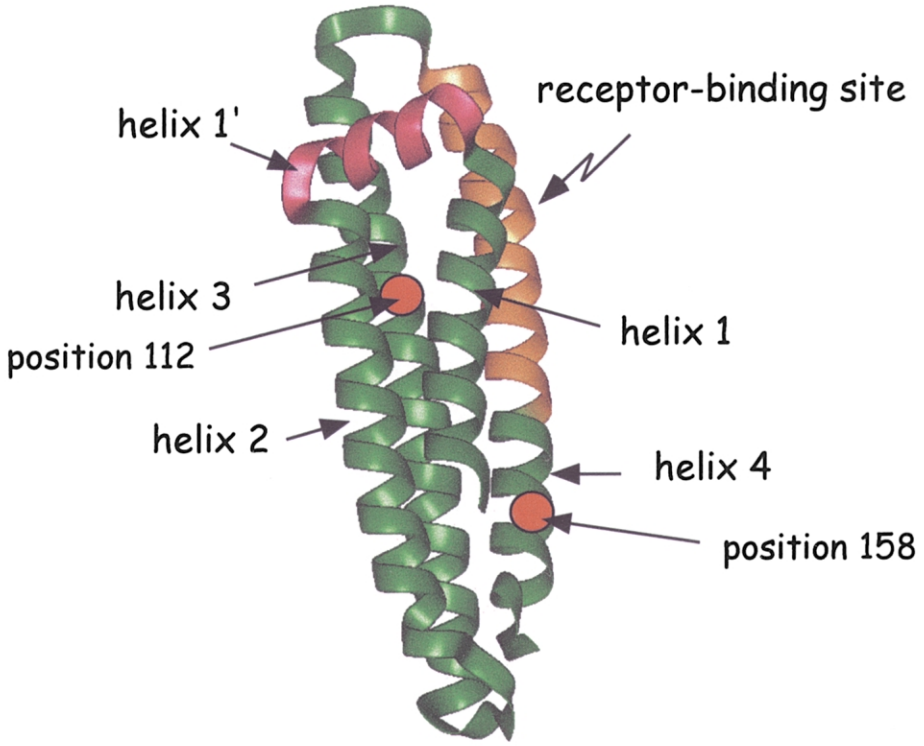

Fig. 1. Ribbon diagram of apoE NT helix bundle. Highlighted are positions 112 and 158 (red circles), which are occupied by Arg or Cys residues in apoE2, E3 and E4 isoforms. The receptor-binding domain (gold) is located in helix 4 (residues 130–150). A short helix, helix 1' (pink) links helices 1 and 2.

apoE-mediated lipoprotein metabolism. For example, apoE2 possesses dramatically reduced affinity for the LDL receptor and is associated with type III hyperlipoproteinemia [14,15], a property that arises from an altered salt bridge interaction, which results in relocation of the Arg^{150} side chain outside of the receptor recognition region [16]. By the same token, it has been shown that the binding preference of the E4 isoform for the larger very low density lipoprotein (VLDL), which is correlated with increased plasma cholesterol and LDL levels [17,18], is influenced by a specific interdomain salt bridge interaction between Arg^{61} and Glu^{255} [19–21]. Moreover, apoE4-carrying individuals are predisposed to develop Alzheimer's disease [22].

In studies of stability properties of human apoE, Morrow et al. [23] attributed the isoform-specific differences in functional ability to the N-terminal domain. These authors found that the order of stability of the NT isoforms was as follows: apoE2 > apoE3 > apoE4. In an independent study, Clément-Collin et al. [24] observed that a combination of moderately low pH and *n*-propanol induces apoE NT conformational changes that are reminiscent of those postulated to occur upon lipid binding. Based on these reports, we evaluated the effect of pH on NT lipid-binding activity of apoE3 [25] and demonstrated that the ability of apoE3 NT domain to bind lipid increases as a function of decreasing pH. In the present report, we extend these studies to the different isoforms of apoE and evaluate the hypothesis that increased lipid binding activity at low pH is related to pH-dependent changes in protein stability.

2. Experimental

2.1. Bacterial expression and purification of apoE isoforms

Recombinant apoE2, E3 and E4 NT domains (residues 1–183) were over expressed in *E. coli* BL21(DE3) harboring a pET22b vector (Novagen), with the respective gene cloned immediately downstream to the vector encoded pelB leader sequence [26]. The proteins were isolated from the culture medium and purified by affinity chromatography on a heparin-Sepharose CL-6B (Pharmacia Biotech), and the purity assessed by SDS-PAGE and analytical HPLC.

2.2. Lipid binding studies

Bilayer vesicles of dimyristoylphosphatidylglycerol (DMPG) or dimyristoylphosphatidylcholine (DMPC) were prepared by extrusion through 200-nm filters as described by Weers et al. [27]. Protein was dissolved in 20 mM Tris HCl, pH 7.2, 150 mM NaCl, 0.5 mM EDTA or 50 mM citric acid of a given pH. Unless otherwise indicated, 400 μg phospholipid and 100 μg apolipoprotein were mixed in a thermostated cuvette at 23 °C (DMPG) or 24 °C (DMPC). Sample light scattering was measured on a Perkin–Elmer LS 50B luminescence spectrometer, with the excitation and emission monochromators set at 567 nm (DMPG) or 625 nm (DMPC), using a slit width of 3 nm. The size of the disc complexes generated upon incubation of apoE NT isoforms with DMPC was determined by native gradient gel electrophoresis and comparison of particle migration with standards of known Stokes diameter (bovine serum albumin, 71 Å; lactate dehydrogenase, 82 Å; catalase, 104 Å; horse ferritin, 122 Å; and thyroglobulin, 170 Å).

2.3. Circular dichroism spectroscopy

Far UV circular dichroism (CD) spectra were obtained using a Jasco J-720 spectropolarimeter using Jasco J-700 Hardware Manager Software version 1.10.00 on a Pentium computer running Windows 98. The spectropolarimeter was routinely calibrated using a 0.06% (w/v) solution of ammonium d-10 camphor sulphonate. The sample chamber was maintained at a constant 25 °C using a Lauda RM6 refrigerated recirculating water bath. Protein concentration ranged from 0.1 to 0.6 mg/ml. The concentrations of ApoE NT isoform stock solutions were determined hydrodynamically by fringe count in a Beckman XLI analytical ultracentrifuge according to Babul and Stellwagen [28]. The pH of the solution was varied as described above. For guanidine hydrochloride (GdnHCl) denaturation experiments, samples were incubated over night at given denaturant concentration in order to attain equilibrium. Baseline correction, noise reduction and ellipticity calculations for the scans were done using Jasco J-700 for Windows Standard Analysis Software version 1.20.00. Molar ellipticity ([θ]) was calculated as:

$$[\theta] = (\mathrm{MRW})(\theta_{222})/10lc$$

where MRW is the mean residue weight (taken to be 115.5, 115.8 and 116.1, for Apo E2, E3 and E4 NT domains, respectively). θ_{222} is the measured ellipticity at 222 nm in millidegrees, l is the cuvette path length in centimeters and c is the protein concentration in milligrams per milliliter. For every scan the ellipticity values from 240 nm to the scan minimum wavelength were analyzed using the Contin deconvolution program of Provencher and Glöckner [29].

2.4. Fluorescence studies

Fluorescence spectra were obtained using a Perkin Elmer LS 50B luminescence spectrometer. Incubations were carried out in 1 ml buffer (100 mM sodium citrate) containing 5 μM apoE NT and 250 μM 8-anilino-1-naphthalene sulfonate (ANS) at the pH indicated. At pH 7.0, 100 mM sodium phosphate was used as buffer. Samples were excited at 395 nm (slit width 3 nm) and emission monitored between 400 and 600 nm (slit width 3 nm). The emission intensity at 472 nm was used as a measure of ANS binding. Values are the average of three emission scans.

3. Results

Far UV CD spectra of the NT domain of human apoE2, apoE3 and apoE4 at pH 7.0 revealed that

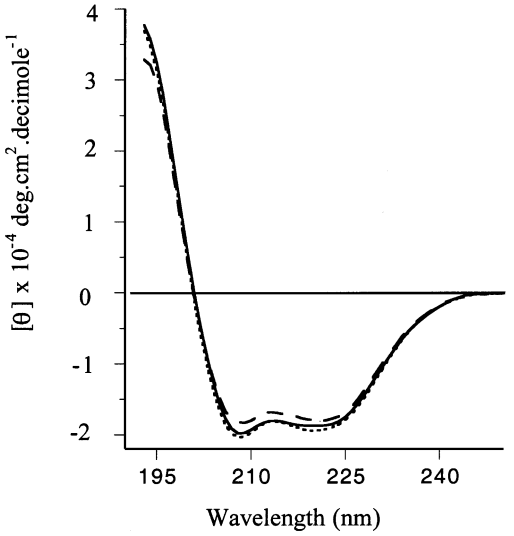

Fig. 2. Far UV CD spectra of apoE-NT isoforms. Spectra were obtained in 50 mM sodium phosphate, pH 7.0. ApoE2-NT (dashed line), apoE3-NT (solid line) and apoE4-NT (dotted line).

the recombinant proteins possess a similar content of α-helical secondary structure (Fig. 2). For each isoform the spectra reveal dual minima of ellipticity at 208 and 222 nm, and computer based secondary structure analysis (Table 1) indicated a high percentage of α-helical content, consistent with X-ray crystallography data of the isoforms [13,16,19].

Table 1
Provencher–Glöckner analysis of secondary structure conformers of ApoE NT isoforms[a]

	% Conformer			
	α-helix	β-sheet	β-turn	Remainder
ApoE2 NT	56	11	0	33
ApoE3 NT	63	17	6	14
ApoE4 NT	60	5	15	20

[a] Far UV C.D. ellipticity values were analyzed using the Contin deconvolution program of Provencher and Glöckner [29].

Exchangeable apolipoproteins are characterized by their ability to transform large phospholipid bilayer vesicles into smaller discoidal complexes, a property that is a measure of their lipid-binding capability. The ability of the NT domain of apoE isoforms to induce this transformation was examined by monitoring the decrease in light scattering that accompanies this process at pH 7.2 (Fig. 3). In control incubations lacking apolipoprotein, no changes in DMPG vesicle light scattering intensity

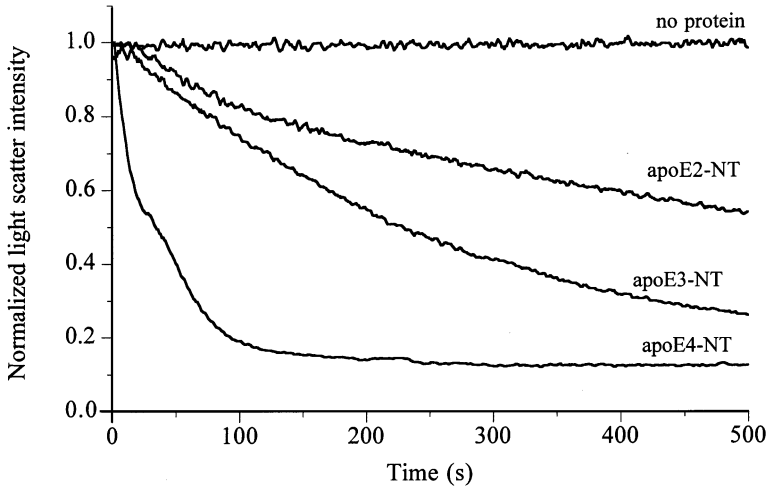

Fig. 3. Effect of apoE-NT isoforms on light scattering intensity of DMPG vesicles. One hundred micrograms of a given apoE NT isoform was incubated with 400 μg DMPG vesicles at 23 °C. The transformation of vesicles into discoidal complexes was monitored by light scatter intensity (right angle), plotted as a function of time. The excitation and emission monochromators were set at 567 nm and the slit width at 3 nm.

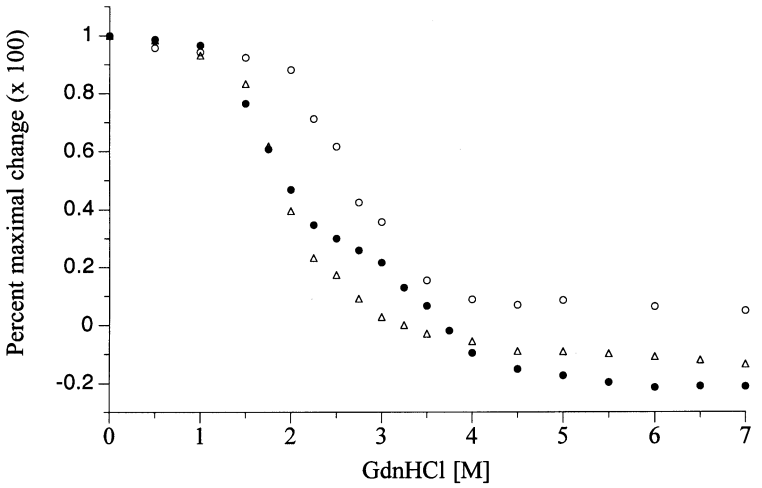

Fig. 4. GdnHCl-induced unfolding of apoE-NT isoforms. GdnHCl was added to a solution of a given apoE NT isoform, incubated for 24 h and, at each concentration, the molar ellipticity at 222 nm was determined. The data were normalized and plotted as percent of maximum change. ApoE2-NT (open circles), apoE3-NT (closed circles) and apoE4-NT (open triangles).

were observed. In contrast, addition of apoE NT resulted in rapid decrease of light scatter intensity, indicating formation of the much smaller protein lipid complexes. The vesicular transformation ability followed the order apoE4 > E3 > E2 NT domains.

As lipid binding involves 'opening' of the apoE NT domain helix bundle structure to facilitate a binding interaction of its hydrophobic interior with the lipid surface, we postulated that isoform-specific differences observed in DMPG vesicle transformation are due to a differential ability to undergo an opening. To assess their intrinsic ability to open, we employed denaturant-induced unfolding at neutral pH as a measure of the relative helix-bundle stability of the different apoE-NT isoforms (Fig. 4). The molar concentration of GdnHCl required to elicit a 50% decrease in ellipticity at 222 nm ($[GdnHCl]_{1/2}$) was calculated for each isoform. The denaturation midpoints were observed to be 2.7, 2.4 and 2.0 M for apoE2, E3 and E4 NT domains, respectively. Thus, apoE NT domain protein stability follows the order E2 > E3 > E4, in agreement with previous results [23]. It appears that apoE-NT induced DMPG vesicle transformation rate among the different isoforms is correlated with decreased resistance to GdnHCl denaturation. This suggests that progressive substitution of Arg at positions 112 and 158 has an effect on apoE NT domain stability and its functional capability at pH 7.2. Since the isoforms vary in their salt bridge interactions, we proceeded to perturb these electrostatic interactions by lowering the solution pH. We postulated that progressive protonation of acidic amino acid side chains would disrupt inter- and intra-helical salt bridges, which may be reflected in the resistance to GdnHCl-induced denaturation and in the lipid-binding abilities of the isoforms.

Because DMPG solutions form a precipitate upon addition of apoE NT domain under acidic conditions, we employed the zwitterionic phospholipid, DMPC for these studies. The interaction of apoE3-NT with DMPC vesicles is weak, as samples of DMPC vesicle populations do not transform well at the lipid transition temperature [25]. Based on previous work it is known that apoE3 NT domain-dependent DMPC vesicle transformation is dramatically increased upon lowering the solution pH to 3.0 [25]. We had previously demonstrated that apoE3-NT retains its secondary structure content at this pH [26], and suggested that loss of tertiary structure in apoE3 NT domain facilitates DMPC vesicle transformation [25]. We extended these studies and examined the relative ability of apoE2, E3 and E4 NT domain isoforms to trans-

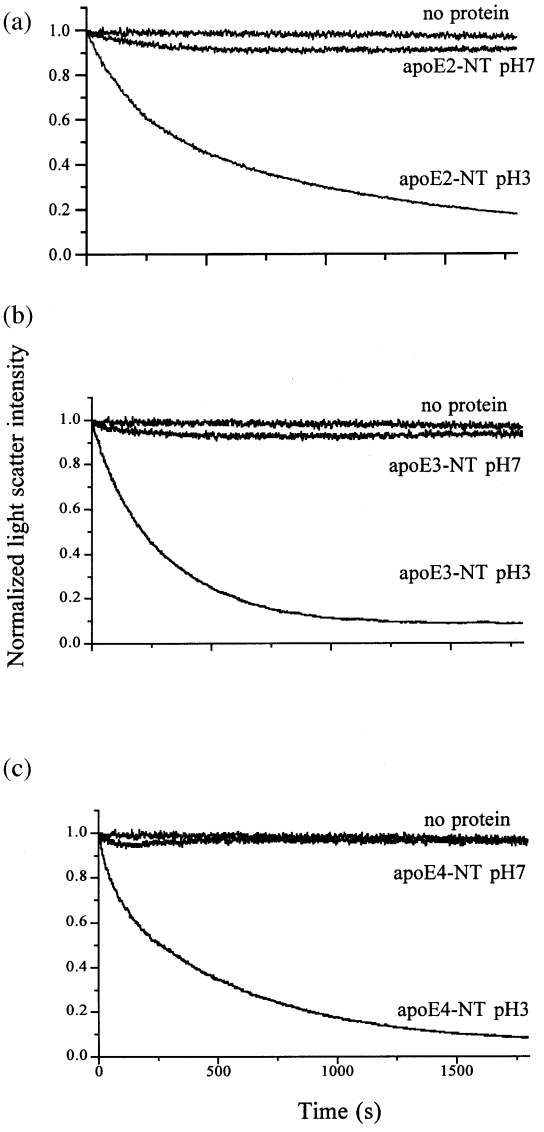

Fig. 5. Effect of solution pH on apoE-NT isoform-dependent transformation of DMPC vesicles into discoidal complexes. Panel (a) apoE2-NT (100 μg of protein) was incubated in the presence of 500 μg DMPC vesicles at 24 °C at the indicated pH. Sample right angle light scatter intensity is plotted as a function of time. Panel (b) apoE3-NT; Panel (c) apoE4-NT.

form DMPC bilayer vesicles to discoidal particles as a function of pH (Fig. 5). In each case, transformation rates were slow at neutral pH under these conditions. At pH 3.0, however, each isoform displayed a dramatic increase in its ability to induce DMPC vesicle transformation into discoidal particles. We did not observe significant isoform-specific differences in the binding interaction at low pH and the particles generated with each isoform had a diameter in the range of 150 Å.

We hypothesized that the strong increase in vesicle transformation rate at pH 3 observed for each of the domain isoforms may be due to a change in protein stability at pH 3. To test this, GdnHCl denaturation studies were conducted as a function of pH (Fig. 6). Based on the midpoint of GdnHCl-induced denaturation, the stability of apoE2 NT domain increased from 2.7 to 3.5 M GdnHCl upon lowering the sample pH from 7 to 3. Likewise, apoE3 NT responded to the lower sample pH by an increase in the midpoint of GdnHCl denaturation from 2.4 to 3 M. In case of the apoE4 NT domain, a decrease in solution pH did not lead to significant differences in stability, and remained at 2 M GdnHCl. Thus, comparison of the stability properties between the isoforms demonstrated that the E4 NT domain was the least stable, followed by E3 and E2 NT domains at pH 7. Lowering the solution pH to 3 did not result in a change in the relative order of the differences in stability, but differences between the isoforms were more pronounced. Therefore, the results observed in the DMPC vesicle transformation experiments at low pH are not due to pH-induced alteration in secondary structure stability.

Subsequently, we hypothesized that pH-dependent exposure of the hydrophobic interior of the apoE-NT helix bundle in the absence of major changes in secondary structure may enhance attraction to lipid surfaces, as seen by an increased ability to transform DMPC vesicles into discoidal complexes. To detect subtle changes in apoE3 NT tertiary structure as a function of pH, we performed dye binding experiments with the extrinsic fluorescent probe, ANS. Whereas ANS displays low fluorescence in buffer alone over the range of pH values employed, its fluorescence is dramatically enhanced (together with a large blue shift in wavelength of maximal fluorescence emission, λ_{max}) upon interaction with hydrophobic sites on proteins. At pH 7 apoE-NT isoforms elicited a small increase in ANS fluorescence, indicating the existence of few solvent exposed hydrophobic

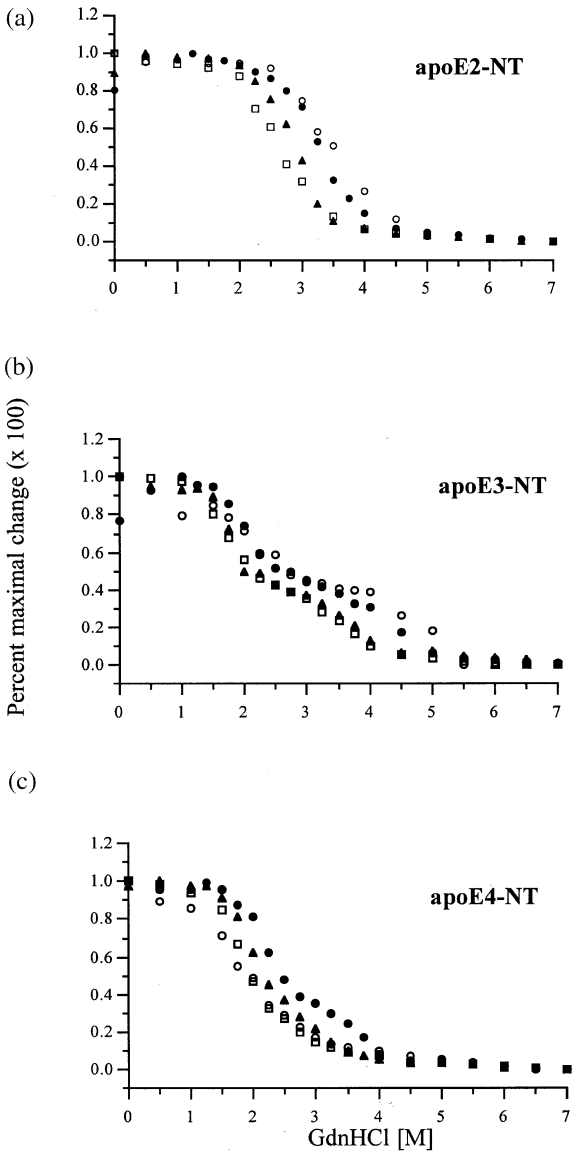

Fig. 6. Effect of solution pH on GdnHCl-induced denaturation of apoE-NT isoforms. GdnHCl was added to a solution of a given apoE-NT isoform at pH 3 (open circle), pH 4 (closed circle), pH 6 (closed triangle) and pH 7 (open square). The molar ellipticity at 222 nm was determined at each concentration of GdnHCl. The data were normalized and plotted as percent of maximum. Panel (a) apoE2-NT; Panel (b) apoE3-NT; Panel (c) apoE4-NT.

pockets in the globular four-helix bundle (Fig. 7). By contrast, each of the isoforms induced >10-fold increase in ANS fluorescence intensity when the solvent pH was decreased to pH 3.0. The increases in ANS binding correlate with the observed enhancement of apoE NT-induced DMPC vesicle transformation activity, but did not reveal significant isoform-specific differences.

4. Discussion

A common feature of exchangeable apolipoproteins is their ability to exist in a dual state: a lipid-free helix bundle state and a lipid-bound open state. The helix bundle organization of these proteins offers a unique way to sequester the hydrophobic protein interior in a lipid free state until a metabolic need arises. Appearance of hydrophobic patches on metabolizing lipoproteins leads to recruitment of apolipoproteins to the particle surface. In the case of apoE, the CT domain, with its

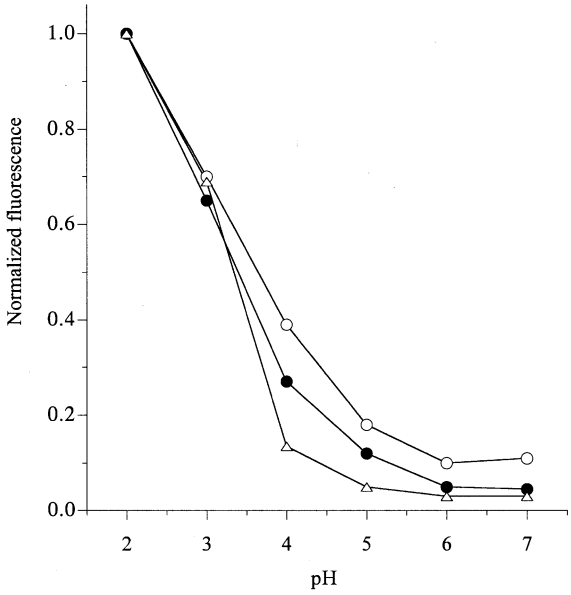

Fig. 7. ANS binding to apoE NT isoforms as a function of pH. Excess ANS was added to the samples at the pH indicated. Samples were excited at 395 nm (slit width 3 nm) and emission monitored at 472 nm. ApoE2 NT (open circle), apoE3 NT (closed circle) and apoE4 NT (open triangle). Fluorescence values reported were normalized to the maximum intensity observed for each isoform.

higher lipid-binding affinity, is envisaged to interact with the hydrophobic surface as the NT domain has a weak lipid binding affinity. However, the NT domain bearing the binding sites for the LDL receptor family of proteins is required to be in an open, lipid bound conformation in order to elicit receptor-recognition and binding. We postulated that the conformational flexibility of the NT domain is a key element in regulating the receptor-binding capability and therefore the cellular uptake and clearance of lipoprotein particles. Key issues that remain unanswered are: what triggers the opening of the NT 4-helix bundle? Are there isoform-specific differences in the lipid-binding interaction? The latter question is of particular interest because the main apoE isoforms E2, E3 and E4 are associated with cardiovascular and Alzheimer's diseases [22,30,31].

In an attempt to resolve this issue, we utilized isolated fragments of apoE isoform encompassing the entire four-helix bundle. This isolated domain is an independently folded structural and functional unit, the crystal structure of which is known for all the three isoforms [13,16,19]. It should be noted that amino acid differences between the isoforms are confined to the NT domain, and with the exception of inter-domain interactions between the NT and CT segments of the protein, functional differences between the isoforms can be attributed to the behavior of the NT domains. The X-ray structure reveals that the helix bundle interior is comprised largely of hydrophobic residues that are sequestered from the aqueous milieu in the lipid-free state. Far UV CD analysis of the isoforms revealed the presence of approximately 60% α-helix content, in agreement with earlier studies [8,12,23,26]. Two distinct features of the protein interior are a leucine zipper motif and several inter- and intra-helical salt bridge interactions that contribute to the tertiary contacts in the helix bundle interior. Interestingly, the stability of the NT and CT domains correlate inversely with their lipid-binding affinities. The CT domain, with a high lipid affinity has a low stability as measured by its low resistance to denaturant-induced unfolding, requiring approximately 0.9 M GdnHCl to undergo a 50% loss in molar ellipticity at 222 nm [7,8]. On the other hand, the NT domain, with its weak lipid affinity elicits a greater resistance to unfolding, requiring approximately a three-fold higher concentration of GdnHCl to elicit a similar loss in ellipticity. While the mid-point of denaturation of the CT domain is consistent with other exchangeable apolipoproteins, 0.3–1.1 M [32–35], that of the NT domain is well beyond this range.

GdnHCl denaturation studies showed that the stability of the NT domain of apoE2 > E3 > E4 (this study and Morrow et al. [23]), while the ability of apoE NT isoforms to transform DMPG vesicles to discoidal complexes follows the order, E4 > E3 > E2. In an attempt to address the apparent inverse correlation between stability, as reflected by resistance to GdnHCl-induced unfolding, and lipid binding capability, we investigated the effect of lowering the pH on the lipid binding properties of apoE NT domain isoforms. Our rationale was that the progressive protonation of the carboxylate side chains would annul the salt bridge interactions, thereby weakening the helix bundle stability and leading to increased lipid binding activity. Indeed, a dramatic increase in the ability to transform DMPC vesicles to discoidal complexes was noted upon lowering the pH to 3 in all the isoforms, as was observed earlier for apolipophorin III from *Manduca sexta* [36] and *Locusta migratoria* [37]. However, the stability parameter of the isoforms at the lower pH was paradoxical. While apoE4 did not show any alteration in its resistance to GdnHCl-induced unfolding, apoE2 and E3 showed an increased resistance to unfolding. It may be concluded that correlating the stability of the protein in terms of GdnHCl-induced loss in molar ellipticity with lipid binding ability, may not be appropriate as absorbance at 222 nm is a mere reflection of the overall α-helicity and not an accurate reflection of tertiary contacts in the protein.

In order to qualitatively assess the status of tertiary contacts that maintain the helix bundle state, we employed ANS fluorescence as an indicator of relative exposure of hydrophobic sites in the protein. Each isoform showed an increase in ANS fluorescence under acidic conditions, indicating exposure of hydrophobic sites on apoE-NT. This may facilitate interaction with the lipid surface and may explain the observed dramatic

Photo taken in 1995 of John Edsall, Ephraim Katzir and Cyril Kay in front of Athabasca Falls in Jasper National Park, Alberta, following a protein symposium held in honor of Dr. Kay.

enhancement in lipid binding at low pH. The increase in hydrophobic surface can be a result of a looser tertiary structure, such as that of a molten globule [36]. However, this may also be a direct result of protonation of Asp and Glu side chains. The apparent lack of difference in lipid binding activity between isoforms at pH 3 can be a result of a major change in tertiary structure or protonation of Asp and Glu residues that has a greater effect on apoE lipid binding compared to the Arg and Cys mutations in the isoforms.

Taken together the data provide insight into the structural basis of isoform-specific differences in aopE physiology. Whereas secondary structure content is critical for helix bundle structural integrity, inter-helical tertiary interactions appear to regulate lipid binding-induced conformational changes and hence, may be responsible for pathological consequences associated with apoE4. Further work is required to fully define the molecular basis of apoE structural adaptability and its role in lipid binding, receptor binding, cardiovascular disease and Alzheimer's disease.

Acknowledgments

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Appendix A: A personal note

One of us (C.M. Kay) had the good fortune to select John Edsall, one of the fathers of modern protein science as his Ph.D. supervisor at Harvard during the period 1952–1956.

He was an incredible teacher—friendly, inspiring, patient, thorough and always stimulating. Among my strongest recollections of that period with John was his astounding memory and his love of fundamental science. Suggestions of articles to read were always supported with volume and page numbers from his head. He was always accessible—no question was too trivial—and

whenever I had a problem, he would stride to the blackboard where he derived and graphed the equations for me in detail.

I also recall that this was a time when John was willing to speak out on issues in which science impinges on other sectors of society. For example, during the McCarthy era of the 1950s, the US Public Health Service was revoking the research grants of investigators because of unevaluated adverse information in their security files. The investigators were not told what was going on or given an opportunity to answer the alleged charges which were, in any case, irrelevant to the criteria for awarding grants for unclassified research. John Edsall took the courageous and leadership stance of writing an article in *Science* in which he resolved neither to ask for, nor accept funds from any government agency that denied support to others for unclassified research for reasons unconnected with scientific competence or personal integrity. Subsequently, and certainly in part because of John's leadership, the Eisenhower administration called on all government agencies to stop the abuses and to follow accepted procedures.

Another attribute which John already exhibited when I was a student and which made a lasting impression on me was the fact that he was one of the few individuals who took the time to reflect on the broader significance of what had been discovered in protein chemistry and biochemistry, as well as documenting what are often the tortuous routes by which these discoveries are made. As an example of one historical-related activity, John Edsall served as chairman of a committee working to save the unpublished correspondence and archival papers of important workers in the fields of biochemistry and molecular biology. The principal result was a monograph listing and describing some 600 collections that provide us with a better perspective of what actually went on in the course of the development of these disciplines than is possible to ascertain from the published record alone. Fortunately for us, John Edsall, recognizing that science is above all a world of ideas in motion, through such historical writings, has documented the tracks of discovery, with all their flaws and blemishes, so that they are not lost to view, and we can learn from them.

Since leaving Harvard in 1956, I have kept in touch with John. We generally met at least once a year in Cambridge for dinner and I have had the pleasure to host him at Alberta on four occasions including two trips to the Rockies. Our conversations were always stimulating and all-encompassing. I consider myself most fortunate to have had a long-lasting friendship with such a great human being in whom science, humanism and society have always been inseparable.

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