Lipid binding properties of the Tangier apolipoprotein A-I and its isoproteins

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Abstract The apolipoprotein A-I was isolated from the plasma of normal individuals and of three homozygous patients with Tangier disease by immunoprecipitation. The apoA-I isoforms were further fractionated by isofocusing on polyacrylamide gels. The physicochemical behavior of normal and Tangier apoA-I and of the isoproteins-2 and -4 was studied by monitoring the tryptophanyl fluorescence emission as a function of temperature, pH, and under exposure to guanidinium (guanidine) hydrochloride (GdmCl). Lipid-apoprotein complexes were generated by incubation with dimyristoylphosphatidylcholine and isolated by density gradient ultracentrifugation. Our results show that normal apoA-I and its isoprotein-4 associate with lipids to yield a complex containing 150–200 mol lecithin/mol apoA-I. The isoprotein-2 of normal apoA-I and the isoprotein-4 of Tangier apoA-I generate lipid-rich complexes with lecithin, while the isoprotein-2 of Tangier apoA-I shows only a limited association with lipids. ApoA-I normal and Tangier and their isoproteins-4 undergo a structural transition around 45°C, which is not observed in the lecithin-apoA-I complexes. This transition is accompanied by an increased exposure of the tryptophanyl residues to the solvent. This transition was observed for the isoprotein-2 of apoA-I Tangier both in its lipid-free form and in the presence of lecithin. The pH denaturation of apoA-I and of the isoprotein-4 between pH 9 and 13 and between pH 7 and 2 is accompanied by a similar conformational transition. The transition occurs around pH 10.8 for the native apoproteins and is shifted towards respectively higher and lower pH's as result of the protective action of lipid binding on the protein conformation. Such an effect was not observed with the isoprotein-2 of apoA-I Tangier which is denatured at lower pH's both in its native form and in a lipid-protein mixture. Finally the denaturation of apoA-I by GdmCl indicates that apoA-I normal and Tangier undergo structural changes around 1 M GdmCl, whereas the apoA-I-Tangier-lecithin complex is more susceptible to denaturation than the complex with apoA-I normal. These data suggest that the apoA-I normal and Tangier and their isoproteins-4 are able to associate with lipids although the association between apoA-I Tangier with lecithin is weaker than that of apoA-I normal. The isoprotein-2 of normal apoA-I associates to a greater extent with lipids than the isoprotein-2 of Tangier apoA-I, whose structure differs from that of the isoprotein-4. These properties probably account for the presence of lipid-free apoA-I in the plasma of patients with Tangier disease.—**Rosseneu, M., G. Assmann, M. J. Taveirne, and G. Schmitz.** Lipid binding properties of the Tangier apolipoprotein A-I and its isoproteins. *J. Lipid Res.* 1984. **25:** 111–120.

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Tangier disease is a rare hereditary disease characterized by an absence of normal HDL in the plasma of the patients (1–4). Several hypotheses have been postulated to account for the defect, among which is a deficient association of the Tangier apoA-I protein with lipids. A recent report by Kay et al. (5) suggests that Tangier apoA-I differs in its amino acid composition from normal apoA-I. Zannis et al. (6, 7) have recently demonstrated that the distribution of the apoA-I isoproteins in plasma is different in Tangier patients compared to normal individuals. They have postulated that the abnormality could be due to a defect in the conversion of the major intestinal or hepatic apoA-I-isoprotein (isoprotein-2) to the major plasma apoA-I-isoprotein (isoprotein-4). We have studied the physicochemical behavior and lipid-binding properties of the major apoA-I isoproteins (2 and 4) isolated from Tangier plasma in comparison to the major isoprotein (4) of normal apoA-I. Our results represent the first report of significant differences in the lipid-binding properties of normal apoA-I and Tangier apoA-I and support the hypothesis of an isoprotein abnormality leading to a dysfunction of apoA-I in Tangier disease.

MATERIALS AND METHODS

Isolation and purification of Tangier and normal A-I

For the preparation of apoA-I, either 50 μl of normal serum or 5000 μl of Tangier serum were obtained under sterile conditions, mixed with 700 μl of a commercially available apoA-I antibody (Behring, Charge-No.: 3540) and incubated for 24 hr at room temperature (8, 9). The immunoprecipitation conditions were optimized according to the Heidelberg plot (9) and the supernatant was

Abbreviations: HDL, high density lipoprotein; DMPC, dimyristoylphosphatidylcholine; GdmCl, guanidinium (guanidine) hydrochloride.

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free of residual apoA-I as monitored by rate nephelometry and electroimmunoassay (9). After incubation, the mixtures were centrifuged for 10 min at 4500 rpm in conical glass tubes. The precipitates were washed twice with 1 ml of saline by vortexing and by subsequent centrifugation. The lipid contents of the whole immunoprecipitate and of the fractions separated by isofocusing were analyzed by high performance thin-layer chromatography. Based on the protein content, the individual isoforms contained less than 2% of the lipids of the whole immunoprecipitate. For preparation of the whole normal and Tangier apoA-I, the samples were processed by one-dimensional SDS gel electrophoresis according to the method of Neville (10).

For isolation of the individual isoforms of normal and Tangier apoA-I, the precipitate was resuspended in 100 μl of distilled water and 25-μl aliquots of this suspension were dissolved in 70 μl of Tris-HCl, 0.05 M, pH 8.6 buffer, containing 2% ampholytes (LKB pH 3–7), 1% decylsulfate (Eastman-Kodak), and 5 μl of beta-mercaptoethanol in 6 M freshly deionized urea (Merck mixed-bed resin). The samples were incubated in the buffer for 2–3 hr prior to application on 2 mm vertical isoelectric focusing polyacrylamide gels (pH 3–7). Isoelectric focusing was performed in a Bio-Rad Model 220 flat bed electrophoresis cell. Polyacrylamide gel preparation and selection of buffers was according to previously described methods (11). After isoelectric focusing, one of the sample tracks was sliced and stained with Coomassie blue in order to visualize the apoprotein bands. The polymorphic forms of apoA-I were then sliced from the gels to match the stained bands.

The major isoproteins were named after the classification of Zannis et al. (7) as isoproteins-2 and -4. In the plasma of normal individuals the isoprotein-4 represents about 75% of the total apoA-I concentration, whereas the isoproteins 2 and 4 account for, respectively, 50 and 47% in the plasma of the Tangier patients.

The gels were washed in 0.05 M Tris-HCl buffer, pH 8.6, containing 6 M urea and the protein was eluted from the gel by pressing through Seraclear® tubes (Technicon) into glass tubes. The extracted apoproteins were dialyzed against 0.001 M ammonium bicarbonate, 0.01% NaN_3, pH 8.6.

Protein was measured by the method of Lowry et al. (12) prior to lyophilization in conical glass tubes. The purity of the apoA-I recovered after isofocusing was monitored by two-dimensional SDS polyacrylamide gel electrophoresis with silver staining (13). Electrophoretic transfer of the apoproteins from the isofocusing and SDS gels to nitrocellulose and subsequent reaction with monospecific antibodies against apoA-I, apoA-II, apoA-IV, and albumin was also performed. The visualization of the bands with fluorescent-labeled protein A was done according to the method of Burnette (14).

Fluorescence measurements

The tryptophanyl fluorescence emission in both normal and Tangier apoA-I was measured on a Aminco-Bowman SPF 500 spectrofluorimeter. Spectra were recorded by means of a Hewlett-Packard recorder 7225 A and could be stored on tapes in a Hewlett-Packard 9815 A calculator. The software for the calculator enabled calculation of the maximum wavelength of the peak, blank subtraction, and correction for dilution. Energy-corrected spectra were calculated using an Aminco-Bowman program, to correct for the response of the instrument as a function of the wavelength. The excitation wavelength was set at 280 nm and the intensity emission was recorded both at 330 nm and at the maximal wavelength of the peak. Scans were performed between 250 and 490 nm with a slit width of 8 nm. The protein concentration was around 20 μg/ml so that absorption and scattering effects were negligible.

The kinetics of denaturation with GdmCl were followed by recording the emission intensity at 330 nm as a function of time for about 1 hr (15).

In all of the temperature studies, solutions were allowed to reach equilibrium as the measurements were performed 15 min after temperature adjustment. The temperature was measured inside the cuvette containing the blank solution by using an electrical thermometer equipped with a microsonde with a sensitivity of 0.1°C.

The pH was measured on a Radiometer model 20 C pH meter calibrated with buffers. pH titrations were made by adding small aliquots of concentrated acid or base, using a micrometer-driven Agla syringe. Corrections were made for dilution.

Preparation of the complexes and denaturation with GdmCl

The complexes between normal and Tangier apoA-I were generated with dimyristoylphosphatidylcholine and phospholipid-cholesterol vesicles. (16, 17). The unilamellar vesicles, prepared by sonication, were incubated with the apoproteins at 24°C for 3 hr at lipid:protein ratios of 150 and 200 mol phospholipid/mol apoA-I for the vesicles containing 0 and 10 mol % cholesterol, respectively (16). These complexes were separated from the free apoproteins and lipids by gradient ultracentrifugation carried out in a NaBr gradient spanning the densities 1.08–1.16 g/ml. The gradient was prepared by use of an Auto-Densiflow (Büchler Instruments) and spun in a SW 50 1 rotor in a Beckman L5-65 preparative ultracentrifuge for 60 hr. The gradient was eluted by means of the same system and collected in 0.2-ml fractions in a Gilson fraction collector. The densities were derived from refractive index measurements on a Abbe refractometer.

The denaturation of apoA-I and apoA-I-lipid complexes was followed at GdmCl concentrations between 0

and 5 M by mixing the apoprotein with the corresponding amount of GdmCl dissolved in the same buffer. All experiments were conducted in Tris-HCL buffer, pH 8.1, 0.01 M Tris, 0.15 M NaCl. At concentrations higher than 5 μg/ml the protein was assayed by immunonephelometry (18) as Tangier apoA-I reacts to antisera against normal apoA-I (8). As observed with normal apoA-I (18), there was a good agreement between the concentrations determined by the method of Lowry et al. (12) and by the immunonephelometric assay. At lower concentrations, the protein content was estimated from the tryptophanyl emission at 333 nm after calibration with an apoA-I standard assayed by immunonephelometry. Phosphorus was assayed by an enzymatic technique using a kit of Biomerieux (France).

RESULTS

Isolation of the complexes of normal and Tangier apoA-I with lipids

The fractionation patterns of the mixtures consisting of lecithin and two of the isoproteins of apoA-I (N2 and N4) and the two major isoproteins of the Tangier apoA-I (T4 and T2), are shown in **Fig. 1A–D.** The density gradient profiles revealed the presence of a lipid-protein complex at the top and free protein at the bottom of the tubes. The presence of protein was detected by measuring the tryptophanyl emission at 330 nm. The protein and phospholipid concentrations together with the lipid-protein ratio throughout the peaks are also plotted on Fig. 1.

Ultracentrifugation resolved two lipid-protein complexes with a different lipid to protein ratio (**Table 1**). A lipid-rich complex, containing 240 mol phospholipid/mol protein is generated with the isoprotein-4 of normal apoA-I, a ratio similar to that reported for the whole apoA-I (16). The complexes generated with lecithin and the isoproteins-2 of normal and Tangier apoA-I or the isoprotein-4 of Tangier apoA-I contain approximately 550 mol of phosphatidylcholine/mol protein. The isoproteins-2 of normal and Tangier apoA-I associate with lecithin to form complexes with a similar composition (Table 1). The percentage of the isoprotein-2 of Tangier apoA-I incorporated into the complex is, however, significantly lower than that of the isoprotein-2 of normal apoA-I.

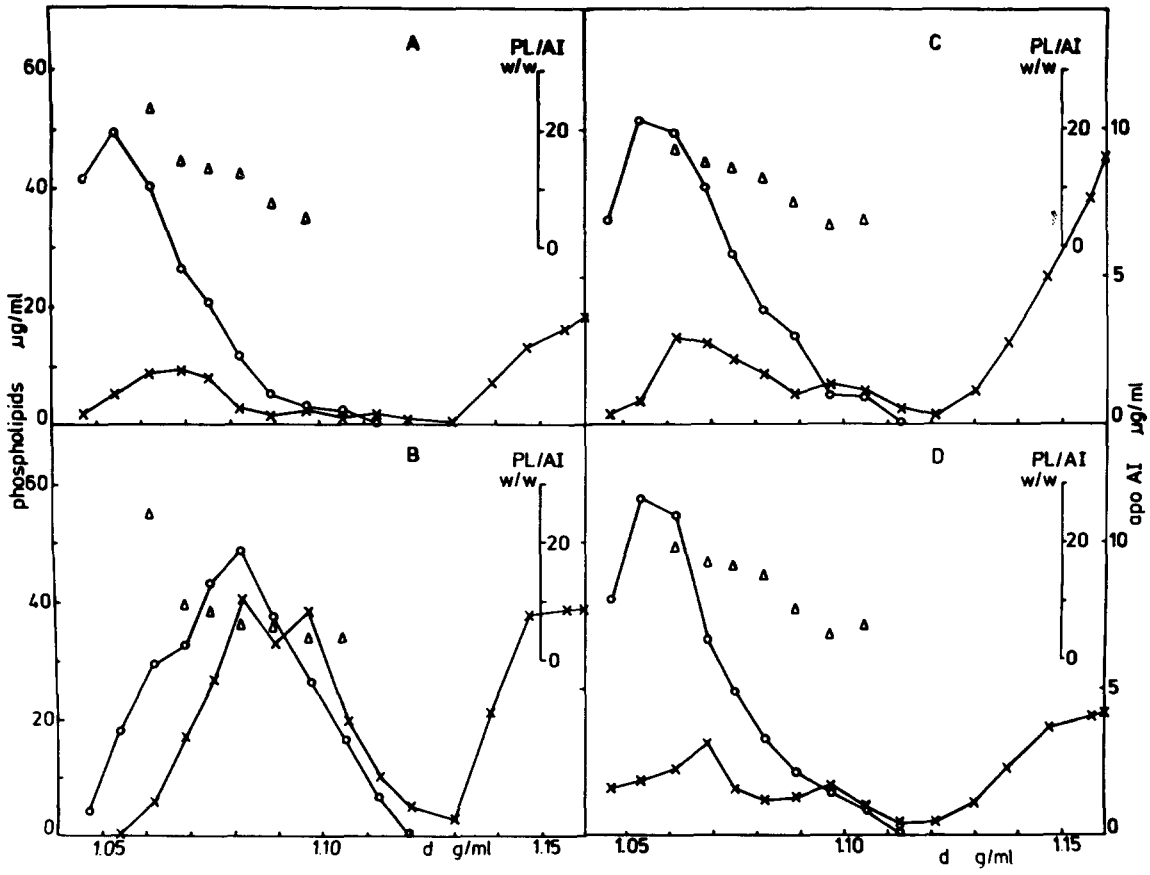

Fig. 1. Density gradient ultracentrifugation of DMPC-apoA-I mixtures. DMPC vesicles (100 μg) were incubated with the apoprotein (20–30 μg) at 25°C for 3 hr. The DMPC (O——O) and apoprotein (×——×) concentrations and the lipid/apoprotein (W/W) ratio (Δ) are plotted against density. Elution patterns are from: A, DMPC-normal apoA-I isoprotein-2; B, DMPC-normal apoA-I isoprotein-4 (N4); C, DMPC-Tangier apoA-I isoprotein-2 (T2); D, DMPC-Tangier apoA-I isoprotein-4 (T4).

TABLE 1. Characterization of the complexes of normal (N) and Tangier (T) apoA-I and DMPC vesicles

Original Mixture		Complex Isolated by Ultracentrifugation		
Apoprotein	mol DMPC/ mol ApoA-I	Density g/ml	mol DMPC/ mol ApoA-I	% ApoA-I in Complex
ApoA-I-N	160	1.086	213	82
		1.105	150	
ApoA-I-N-4	160	1.086	242	61
		1.101	155	
ApoA-I-N-2	160	1.072	568	50
ApoA-I-T-4	160	1.075	568	58
		1.100	206	
ApoA-I-T-2	160	1.075	568	30
		1.100	159	

Thermal stability of Tangier apoA-I, its isoproteins, and the lipid-protein complexes

The structural stability of normal and Tangier apoA-I towards thermal denaturation was investigated by monitoring both the maximal wavelength and the intensity of the tryptophanyl fluorescence emission as a function of temperature. **Fig. 2A–D** represents the maximal wavelength of the tryptophanyl emission in normal apoA-I, its major isoprotein N4, and the two major isoproteins of Tangier apoA-I, T4 and T2, respectively. Heating and cooling isotherms are represented on each figure for both the A-I apoprotein and the protein reassembled with phospholipids. The thermal denaturation is characterized by a red shift of the fluorescence from 333 nm at 20°C to around 340 nm at 60°C for the normal apoA-I and from 333 to 336 nm for the isoproteins N4 and T4. The mid-point of the transition lies around 45–50°C for the different isoproteins upon heating (**Table 2**). The transition is reversible upon cooling, however the mid-point temperature was consistently lower by 3–5°C, indicating some kind of hysteresis. The red shift of the tryptophanyl fluorescence during denaturation arises from an increased exposure of the tryptophanyl residues to the solvent in analogy with the effect of GdmCl (15). In contrast with the behavior of the A-I apoproteins and isoproteins, their complexes with DMPC do not exhibit any thermal transition around 40°C as no red shift could be detected in the tryptophanyl emission during either heating or cooling.

The maximal wavelength of the corrected spectra for the tryptophanyl emission of the complexes is around

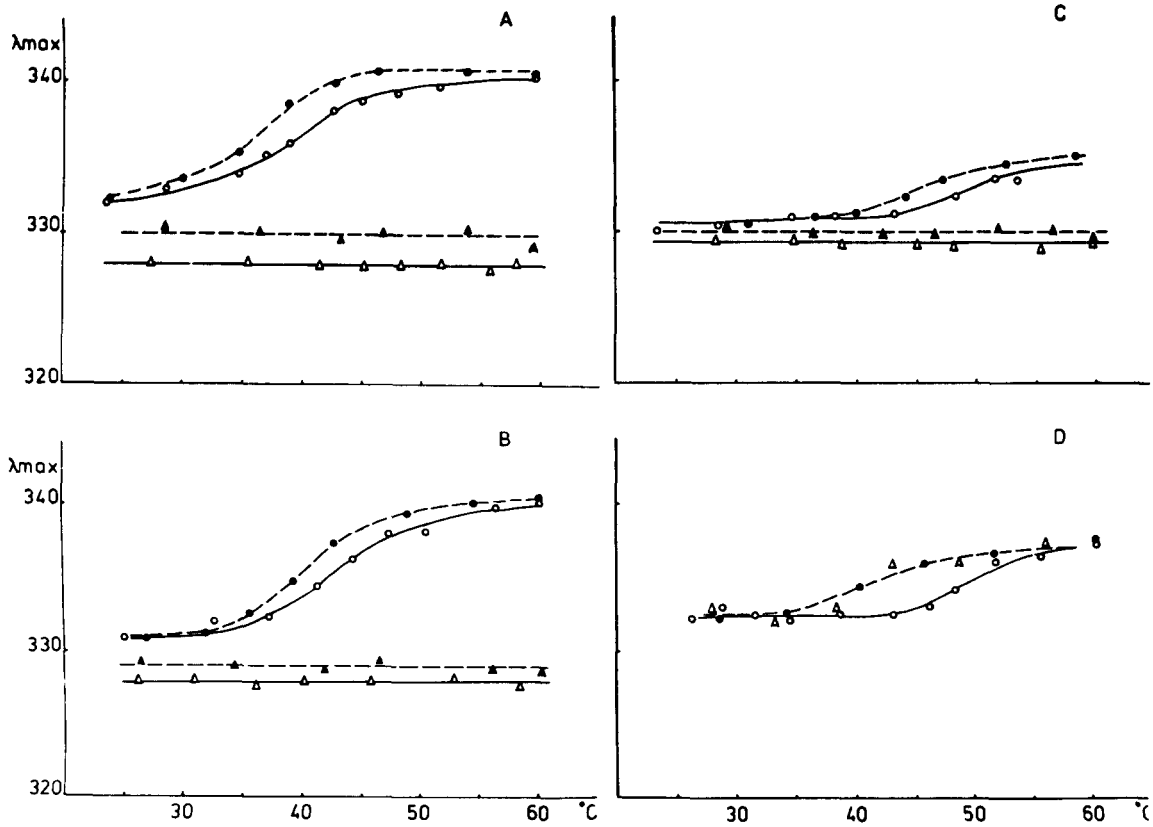

Fig. 2. Effect of temperature on the tryptophanyl fluorescence maximal wavelength for native apoA-I (O) and the apoA-I-DMPC complexes (Δ). (O——O), Increasing temperature; (●--●), reversal; A, normal apoA-I; B, normal apoA-I isoprotein-4; C, Tangier apoA-I isoprotein-4; D, Tangier apoA-I isoprotein-2.

TABLE 2. Temperature denaturation of normal and Tangier apoA-I

	Temperature Range (°C)	λ max. (nm) (20°C)	λ max. (nm) (60°C)	Δλ (nm)	$T_{t\text{-}c}$ (λ)	$T_{t\text{-}c}$ (I)
ApoA-I-N	20–60	333	341	9	48.0	44.5
ApoA-I-N + DMPC	20–60	328	328	0		
ApoA-I-N4	20–60	333	336.5	4.5	50.2	44.5
ApoA-I-N4 + DMPC	20–60	329	329	0		
ApoA-I-T4	20–60	332	336.0	4	48.8	43.5
ApoA-I-T4 + DMPC	20–60	326.5	326.5	0		
ApoA-I-T2	20–60	332	337	5	49.0	43.5
ApoA-I-T2 + DMPC	20–60	332	337	5	49.0	43.5

328 nm compared to 333 nm for the lipid-free protein, in agreement with the report by Matz and Jonas (19). The Tangier isoprotein T2-DMPC mixture behaves quite differently from the isoprotein-lipid complexes described above as the thermal transition was still observed in the presence of lipids (Fig. 2D). The isotherms for the heating and cooling of the lipid-isoprotein T2 mixture coincide with those measured for the cooling of the native isoprotein T2. The temperature dependence of the fluorescence intensity is plotted on **Fig. 3A–D,** revealing a transition in the same range as observed for the fluorescence maximal wavelength. In agreement with the results of Fig. 2A–D, this transition was detected for normal apoA-I, the isoproteins N4 and T4, while the respective complexes with DMPC showed no such transitions around 45°C. For the Tangier isoprotein T2, a transition was

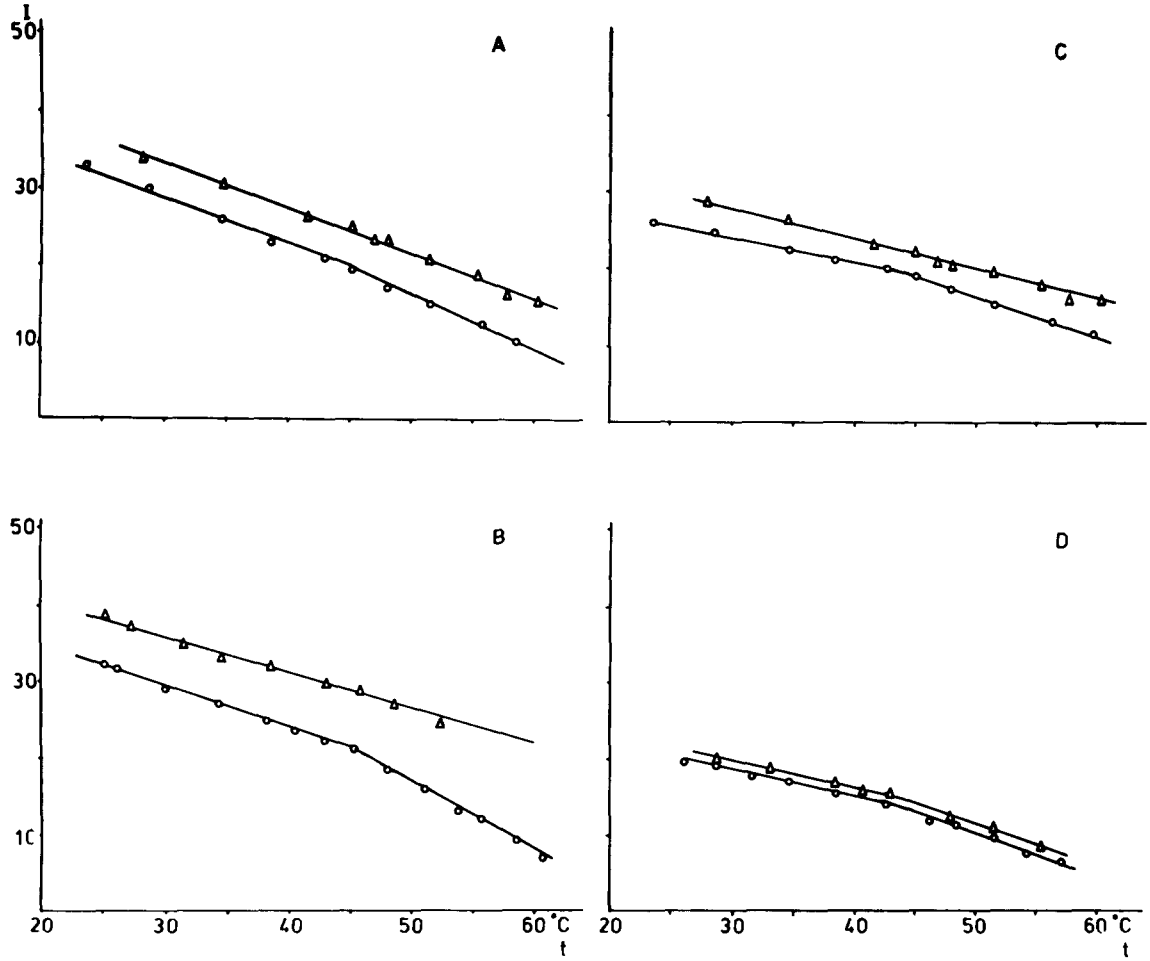

Fig. 3. Effect of increasing temperature on the fluorescence intensity for native apoA-I (○) and the apoA-I-DMPC-complexes (△). Legend as in Fig. 2.

observed both for the native isoprotein and the isoprotein-DMPC mixture, suggesting that no association takes places between this isoprotein and the phospholipid.

pH stability

The stability of normal and Tangier apoA-I and their isoproteins was followed between pH 7.4 and 12.5 for both the apoproteins and their complexes with DMPC (**Fig. 4A–D**).

The tryptophanyl emission peak in normal apoA-I and in the isoproteins N4 and T4 is shifted from 333 nm at neutral pH to 349 nm around pH 12, with a mid-transition point at pH 11.0 for normal apoA-I, compared to pH 11.0 and 10.7 for the N4 and T4 isoproteins, respectively. The peak value at 348 nm is similar to those measured for short peptides in aqueous solutions (20) and indicates that the tryptophanyl residues are exposed to the solvent at high pH. The fluorescence intensity is quenched above pH 9 with a maximal effect between pH 10.6 and 11.8.

The association of the apoproteins with phospholipids protects the protein against denaturation, as the mid-points of the denaturation curves are shifted towards higher pH's (Fig. 4). For the complexes with DMPC, normal apoA-I, and the isoproteins N4 and T4, the pH at the mid-transition lies, respectively, above 12.5, at pH 11.4, and at pH 11.6 (**Table 3**). The amplitude of the red shift for the tryptophanyl emission is less pronounced for the whole apoprotein-DMPC complex than for the complexes formed between DMPC and the isoproteins N4 and T4. The behavior of the T2 isoprotein indicates a higher susceptibility towards pH denaturation as the transition range was located between pH 9.2 and 10.6 with a mid-transition lying around pH 9.8. The maximal wavelength was only shifted from 333 nm to 342 nm compared to 349 nm for the isoproteins N4 and T4, indicating a more restricted conformational change. The denaturation pattern of the isoprotein T2-DMPC mixture was not significantly different from that of the isoprotein, in agreement with the results of the temperature stability experiments.

At acidic pH (**Fig. 5A–B**), similar modifications of the tryptophanyl peak were observed between pH 7.0 and 2.0, indicating an unfolding of the peptide chain to a more open, flexible form. In agreement with Gwynne, Brewer, and Edelhoch (21) we observed a shift of the emission peak from 333 to 338 nm, suggesting that the apoA-I structure is less disorganized at pH 2.0 than at pH 12.0. The structure of the apoA-I-N4-DMPC and

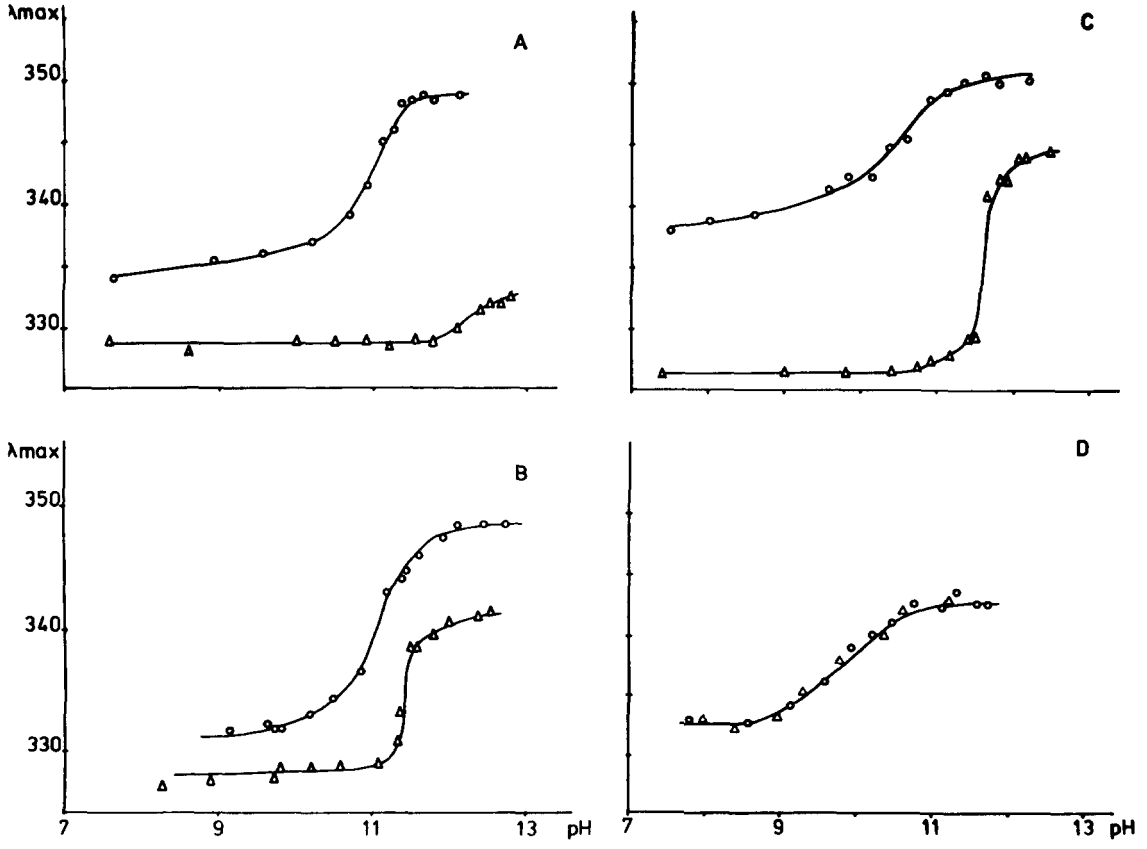

Fig. 4. Effect of basic pH on the fluorescence maximal wavelength for native apoA-I (○) and the apoA-I-DMPC complexes (△). Legend as in Fig. 2.

TABLE 3. pH Denaturation of normal and Tangier apoA-I

	pH Range	λ max (nm) (pH 8)	λ max (nm) (pH 12 / pH 2)	Δλ (nm)	pH_t
ApoA-I-N	8–12	332.5	349.0	16.5	10.95
ApoA-I-N + DMPC	8–12	329.0	332.5	3.5	>12
ApoA-I-N4	8–12	331.0	349	18	11.04
ApoA-I-N4 + DMPC	8–12	328.0	341.5	13.5	11.4
ApoA-I-T4	8–12	334.0	351.0	17	10.74
ApoA-I-T4 + DMPC	8–12	326.5	344.5	18	11.6
ApoA-I-T2	8–12	332.5	342.5	10	9.84
ApoA-I-T2 + DMPC	8–12	332.5	342.5	10	9.84
ApoA-I-N	8–2	332.5	339.0	6.5	3.0
ApoA-I-N + DMPC	8–2	328.0	328.0	0	
ApoA-I-T	8–2	332.0	337.5	5.5	2.7
ApoA-I-T + DMPC	8–2	327.5	327.5	0	

T4-DMPC complexes is more stable than that of the native protein as no red shift could be observed even at pH 2.0.

Stability in GdmCl

Most plasma proteins can be unfolded to random-coil polypeptides in concentrated solutions of GdmCl (20).

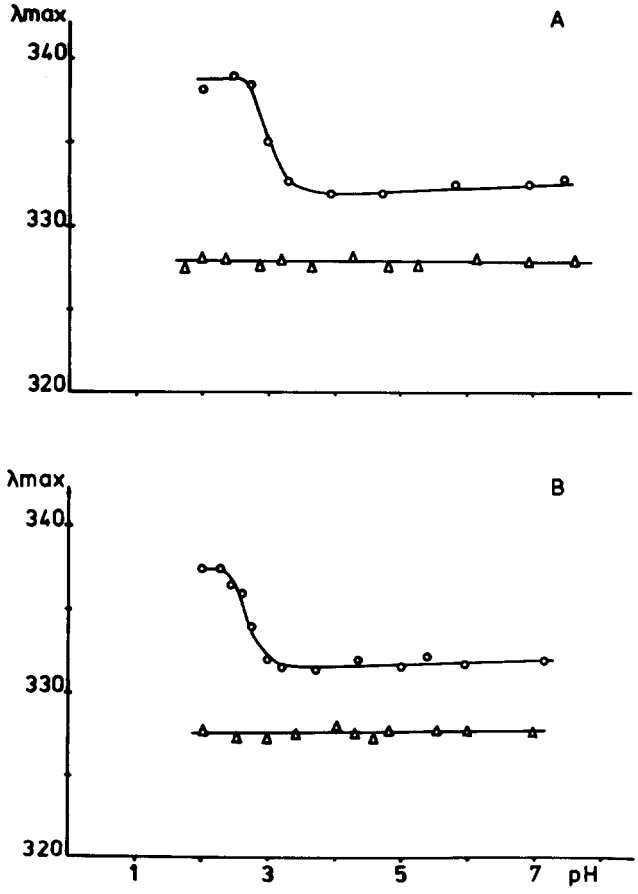

Fig. 5. Effect of acidic pH on the fluorescence maximal wavelength for native apoA-I (O) and the apoA-I-DMPC complex (Δ). A, normal apoA-I; B, Tangier apoA-I.

The stability of different proteins can be evaluated by their resistance to GmdCl denaturation. For this purpose we compared the behavior of normal and Tangier apoA-I, their isoproteins, and their phospholipid complexes during the exposure to GdmCl at concentrations between 0 and 6 M.

Exposure of normal and Tangier apoA-I to 5 M GdmCl strongly modifies the tryptophanyl fluorescence emission spectrum. The maximal wavelength is shifted from 333 nm to 357 nm for both apoproteins and the intensity of the maxima is decreased. Moreover, the spectra of normal and Tangier apoA-I, as well as of the respective lipid-apoprotein mixtures, become identical in the presence of 5 M GdmCl. The kinetics of denaturation of normal and Tangier apoA-I by GdmCl are a fast process completed in less than 5 min. Compared to the lipid-free apoA-I, the denaturation kinetics of the normal apoA-I-dimyristoylphosphatidylcholine complex presents a slower component due to the disruption of the apoA-I-lipid association by GdmCl (15). This component is not observed when the Tangier apoA-I-lipid mixture is exposed to 5 M GdmCl; the denaturation kinetics are identical to those monitored with lipid-free normal and Tangier apoA-I.

The denaturation patterns of normal and Tangier apoA-I are monitored by plotting the maximal wavelength for the tryptophanyl emission as a function of the GdmCl concentration (**Fig. 6**). After 30 min incubation the behavior of normal and Tangier apoA-I towards the denaturation by GdmCl are close (Fig. 6A), though the midpoint for Tangier apoA-I lies at a higher GdmCl concentration (1.2 M versus 1.0 M). This difference is significant and has been consistently observed for different preparations of Tangier apoA-I, suggesting a more labile conformation for normal than for Tangier apoA-I. The addition of either dimyristoylphosphatidylcholine or dimyristoylphosphatidylcholine-cholesterol vesicles to Tangier apoA-I has no effect on the denaturation profile of the Tangier apoprotein as the curves of native and re-

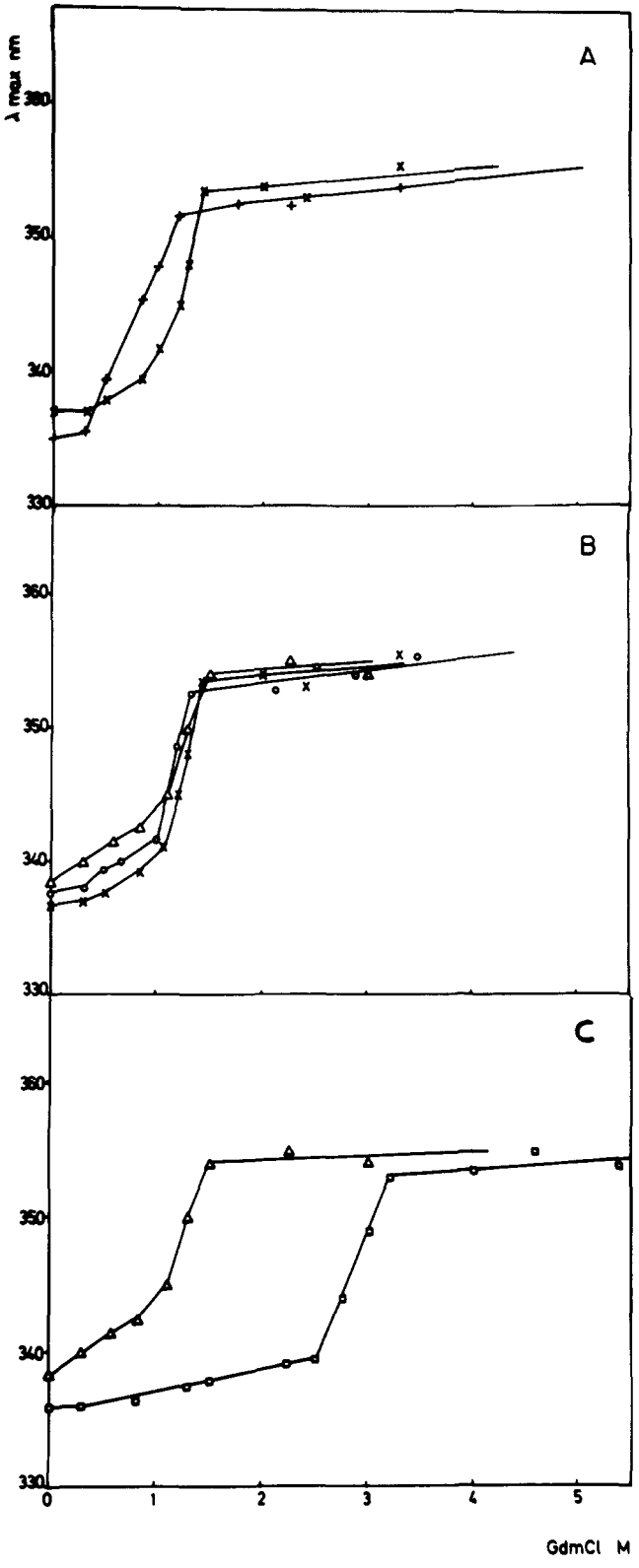

Fig. 6. Denaturation patterns of normal and Tangier apoA-I by GdmCl. Maximal wavelength of tryptophanyl fluorescence. A, normal (+) and Tangier (×) apoA-I; B, Tangier apoA-I (×); Tangier apoA-I + dimyristoylphosphatidylcholine vesicles (△); Tangier apoA-I + dimyristoylphosphatidylcholine-10 mol % cholesterol vesicles (○). C, Complexes between DMPC, normal (□) and Tangier (△) apoA-I.

lipidated apoA-I coincide within experimental error at all GdmCl concentrations (Fig. 6B). The protective effect of phospholipid against the denaturation of normal apoA-I by GdmCl is depicted on Fig. 6C and compared to that of Tangier apoA-I. The mid-point of the transition curve for the normal apoA-I is shifted from 1.0 to 4–5 M GdmCl in agreement with previous reports (21, 22). By contrast, the addition of phospholipid and phospholipid-cholesterol vesicles to Tangier apoA-I did not affect the denaturation of the Tangier apoprotein by GdmCl.

DISCUSSION

The observation of a decreased HDL concentration and the presence of apoA-I not associated with lipids in the plasma of patients with Tangier disease (2, 4), has led to the hypothesis of a defective lipid-protein association in this disease. The differences observed by Zannis et al. (7) in the distribution of the isoproteins of normal and Tangier apoA-I further emphasize the relevance of the study of the lipid-apoprotein association in Tangier disease.

The isoprotein-2 of normal apoA-I represents a proprotein form of the isoprotein-4; a defective conversion of the isoprotein-2 to isoprotein-4 in Tangier disease might account for the lack of association of Tangier apoA-I with HDL lipids and especially with phospholipids. In order to check this hypothesis we compared the physicochemical behavior of these isoproteins and characterized the products generated after incubation of the isoproteins 2 and 4 with phospholipids. The concentrations of apoA-I (0.5–0.7 mg/dl) (23) and of the isoproteins 2 and 4 in Tangier plasma are so low that only fluorescence techniques could be applied to the study of the protein conformation. Previous studies by Gwynne et al. (18, 19) have shown that the monitoring of the tryptophanyl fluorescence emission provides information about the protein conformation which is comparable to the circular dichroism measurements. The lipid-apoprotein complexes were isolated by density gradient ultracentrifugation which enabled the resolution of a mixture of 20 μg of protein and 50 μg of lipid in the various components.

In normal individuals apoA-I and its major isoprotein-4 easily associate with dimyristoylphosphatidylcholine to generate small discoidal complexes, as previously described (22). The association of the isoprotein-2 of normal apoA-I, and of the isoforms 2 and 4 of Tangier apoA-I with lecithin yields both a lipid-rich and a protein-rich complex. The isoprotein-2 of Tangier apoA-I associates only to a limited extent with lipids as only 30% of the protein present in the original mixture is incorporated into a lipid-rich complex. This was confirmed by the results of the denaturation experiments at high temperature

and at high pH, showing that the denaturation pattern of the DMPC-isoprotein T2 complex is identical to that of the native isoprotein-2 of Tangier apoA-I.

The apoA-I protein is synthesized with a signal sequence which is cleaved upon translation to yield a proapoprotein (24). Zannis et al. (7) have shown that the isoprotein-2 represents the proapoprotein A-I, containing a hexapeptide prosegment, followed by the N-terminus of the apoprotein A-I in normal individuals. Schmitz et al. (25) have recently sequenced the amino terminal portion of the proapoprotein A-I in three homozygous patients with Tangier disease. They found a sequence identical to that of the normal proapoprotein A-I. The differences observed in the lipid association properties of the normal and Tangier proprotein A-I-2 are therefore likely to arise from differences in the amino acid sequence and the conformation of Tangier apoA-I-4. The behavior of the proprotein 2 and of the mature isoform 4 of normal apoA-I is different from that reported for phospholipase A_2 and its zymogen (26). The enzyme associates with phospholipid micelles and bilayers, whereas its zymogen binds only to lipid monomers. This was attributed to a conformational difference of the proprotein, containing a heptapeptide at its N-terminal end, and thereby lacking the "interface-recognition site" present on phospholipase A_2.

Our data suggest that differences exist in the structure of the isoprotein-2 compared to that of the whole Tangier apoA-I protein. We have observed that the isoprotein-2 of Tangier apoA-I is more sensitive to pH denaturation than the isoprotein-4, indicating that the titration of the lysines and tyrosines induces an unfolding of the protein structure at lower pH. The isoprotein-4 of Tangier apoA-I associates with lipids although these lipids seem less tightly bound, as suggested from the denaturation experiments carried out at high pH. The structure of the apoA-I-T4-DMPC complex is unfolded at pH 11.6 compared to pH > 12 for normal apoA-I-DMPC complex. The presence of the isoproteins 5 and 6 in whole apoA-I probably induces protein-protein interactions within the complex which might reduce the accessibility of the lysine residues to titration. In plasma, Tangier apoA-I consists of 47% of the isoform-4 which associates weakly with lipids and of 50% of the isoform-2 which shows minimal lipid association; this might account for the fact that Tangier apoA-I does not associate with normal HDL (25) and exists as lipid-free protein in the plasma of the patients with Tangier disease.

The results of the pH denaturation experiments, monitoring the exposure of the tryptophanyl residues to the solvent, can be interpreted in terms of the ionization behavior of apoA-I, either lipid-free or associated with lipids. We have previously shown that the lysines and tyrosines of apoA-I titrate in two groups in the pH range 9–13. The first group consists of twelve lysines which titrate at a pK of 9.6 both in the apoprotein and in the complex. The remaining residues, which titrate at a pK of 10.8 in apoA-I, are masked by lipid in the complex. Out of the seven tyrosines in the apoA-I, three titrate at a pK of 9.5 and four at a pK of 11. These pK's are shifted, respectively, to 10.5 and 12.1 in the lecithin-apoA-I complex (27).

The denaturation pattern of the whole apoprotein A-I from both normal subjects and Tangier patients indicates that a structural unfolding occurs around pH 11, coinciding with the pK of the nine lysines and four tyrosine residues less exposed to the solvent. In the lipid-apoprotein complex these residues are less accessible to titration and therefore the native structure is preserved up to pH > 12 in the normal apoA-I-lecithin complex and up to pH 11.5 in the isoprotein-4-lipid complexes. According to the model of Segrest et al. (28), these residues are probably located in the helical regions of apoA-I. Their ionization results in a decreased helical content of apoA-I at higher pH. In the isoform-2 of Tangier apoA-I the denaturation occurs around pH 9.8, coinciding with the titration of the twelve lysines and three tyrosines not involved in lipid binding.

These data demonstrate that normal and Tangier apoA-I and the isoform 4 in each have a similar behavior towards pH and temperature denaturation and in their association with lecithin. The isoform-4 of normal and Tangier apoA-I is, however, less tightly associated with lipids than the corresponding normal apoA-I. The proprotein of Tangier apoA-I denatures at lower pH and has a limited binding capacity for lipids.

As apoA-I consists of several isoforms, the lipid-apoA-I complexes might involve both lipid-protein association to each of the isoforms and protein-protein association between these isoforms. This would result in a more tightly structured complex with a higher stability against denaturing agents. This would also imply that differences in the ratios of the apoA-I isoforms among individuals might result in differences in the lipid-protein ratio in HDL and the lecithin and cholesterol uptake by high density lipoproteins. This hypothesis will be tested further on lipid-apoprotein mixtures consisting of various proportions of normal and Tangier apoA-I isoforms.

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