Hydrogen/Deuterium Exchange Kinetics of Apolipopophorin-III in Lipid-free and Phospholipid-bound States

AN ANALYSIS BY FOURIER TRANSFORM INFRARED SPECTROSCOPY*

(Received for publication, December 19, 1995, and in revised form, June 28, 1996)

Vincent Raussens‡§, Vasanthy Narayanaswami‡, Erik Goormaghtigh‡¶, Robert O. Ryan‡¶, and Jean-Marie Ruysschaert‡

From the ‡Laboratoire de Chimie Physique des Macromolécules aux Interfaces, Université Libre de Bruxelles CP 206/2, B-1050 Brussels, Belgium and the ¶Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Abbreviations used are, apoLp-III, apolipopophorin III; ATR, attenuated total reflection; DMPC, dimyristoylphosphatidylcholine; FTIR, Fourier transform infrared; H/D, hydrogen/deuterium.

Attenuated total reflection Fourier transform infrared spectroscopy was used to probe the kinetics of hydrogen/deuterium exchange in Manduca sexta apolipophorin-III (apoLp-III). ApoLp-III is an exchangeable apolipoprotein that is made up of five elongated amphipathic α-helices in a helical bundle conformation in the monomeric lipid-free form. Upon interaction with phospholipids, it is postulated to undergo a large conformational change whereby the hydrophobic interior is exposed, facilitating binding to the lipid surfaces. We have used the lipid-free and dimyristoylphosphatidylcholine-bound apoLp-III to study the dynamically variable domains in the two forms. Three populations of amide protons varying in their hydrogen/deuterium exchange rates were found to exist: slow, intermediate, and fast exchanging, which could correspond to completely buried, partially buried, and solvent-exposed domains on the protein in both the states. In lipid-free apoLp-III, 36, 12, and 52% of the total residues contributed to the slow, intermediate, and fast exchanging populations, respectively. In the dimyristoylphosphatidylcholine-bound form, the corresponding distribution was 20, 16, and 64%, representing a 12% increase in the number of exposed residues. The results are discussed in terms of increased solvent accessibility due to gross tertiary structural reorganization.

Apolipopophorin III (apoLp-III) from the Sphinx moth, Manduca sexta, is a 166-amino acid protein that exists in alternate lipid-free and lipid-associated states. In resting animals, this major apolipoprotein is found as a lipid-free monomeric hemolymph protein, whereas in response to flight activity, it associates with the surface of the major circulating lipoprotein, low density lipophorin (Blacklock and Ryan, 1994). Several groups have been investigating the molecular basis for the dual existence of this protein.

From circular dichroism and sequence analysis, it is known that apoLp-III is rich in α-helix (Cole et al., 1987; Kawooya et al., 1986). Furthermore, the three-dimensional structure of the homologous protein from the migratory locust, Locusta migratoria, was solved by x-ray crystallography (Holden et al., 1988; Breiter et al., 1991). It was shown that in the absence of lipid, apoLp-III exists as a bundle of five long α-helices that are connected by short loops. Each of the α-helices are amphipathic and orient with their hydrophobic faces directed toward the center of the helix bundle, whereas their hydrophilic faces are exposed to the solvent. When presented with a lipid surface, it has been proposed (Breiter et al., 1991; Wientzek et al., 1994) that the protein undergoes a major conformational change, opening about hinge regions located in the loops between helices 2 and 3 and helices 4 and 5. This structural alteration leads to exposure of the hydrophobic interior of the protein, which becomes available for lipid interaction.

In support of the above hypothesis, association with dimyrstoylphosphatidylcho line (DMPC) vesicles was shown to result in the formation of uniform disc-like structures, with an average diameter and width of 18.5 ± 2 and 4.8 ± 0.8 nm, respectively (Wientzek et al., 1994). Calculations based on cross-linking studies and molecular dimensions from electron microscopy of these complexes, in conjunction with x-ray crystallographic data from L. migratoria apoLp-III allowed the presentation of a model of lipid-protein interaction (Wientzek et al., 1994). In this model, the α-helices of the apolipoprotein adopt a unique orientation, with their helical axes lying perpendicular to the lipid acyl chains, acting as a scaffold for the whole complex. In a previous paper (Raussens et al., 1995), we presented the first experimental evidence of the validity of this model, using ATR-FTIR spectroscopy to gain information about the secondary structural organization and orientation with regard to the lipid bilayer (Goormaghtigh et al., 1994; Goormaghtigh and Ruysschaert, 1990).

It has been suggested that description of proteins in terms of their dynamically variable domains is probably more relevant functionally and in terms of evolutionary correlation than one that describes the secondary structural domains (Lumry, 1995). Hydrogen isotope exchange studies have long been used in the analysis of protein structure and dynamics (Knox and Rosenberg (1980); Gregory and Lumry (1985); Provencher and Dovi (1979); for reviews see Englander and Kallenbach (1983) and Kim (1986)). It appears to be one of the main techniques capable of identifying submolecular motional domains including fast exchanging protons of the protein surface, the somewhat slower exchanging protons of the flexible (loop) regions buried in the protein not involved in some secondary structure and slowly exchanging protons from the protein core formed by the most rigid clusters (knots) of amino acids (for a review see Lumry (1995)). Very little data are available about the hydro-
EXPERIMENTAL PROCEDURES

Materials—DMPC was purchased from Sigma. Phospholipid (cholesterol) in a lyophylized form was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Deuterium oxide was from Janssen Chimica (Geel, Belgium).

Purification of Apolipoprotein III—apoLp-III was isolated and purified from hemolymph of adult M. sexta according to the method described by Wells et al. (1985) with minor modifications (Wientzek et al., 1994). The purity of the apoLp-III was evaluated by SDS-polyacrylamide gel electrophoresis, and the pure apoLp-III was stored lyophilized at −20 °C.

Preparation of DMPC Vesicles—DMPC vesicles were prepared by modification of the method of Swaney (1980). DMPC was dissolved in chloroform/methanol mixture (3:1), evaporated to dryness as a thin film under an inert atmosphere, and dried further for 2 h, followed by the addition of 20 mM Tris-HCl, pH 7.5, to a final lipid/protein ratio of 190:1. After a brief mixing in a vortexer, the mixture was incubated at 50°C for about 20 min.

Preparation and Isolation of DMPC-apoLp-III Complexes—The preparation and isolation of DMPC-apoLp-III complexes were performed as described by Swaney (1980) and Rifici et al. (1985) with some modifications. ApoLp-III was dissolved in 10 mM Tris-HCl, pH 7.5, and added to the DMPC vesicles at a lipid/protein ratio of 2.5:1 (w/w) (molar ratio of about 70:1). After a brief mixing in a vortexer, the mixture was incubated at 4°C for 18 h. The density of the sample was adjusted to 1.21 g/ml with KBr in a final volume of 2.5 ml and placed in a 5-ml Quick-Seal tube, layered with 0.9% saline, and centrifuged at 65,000 rpm for 3 h at 4°C. At the end of the centrifugation, fractions of 0.5 ml were removed from the top of the tube and analyzed for protein and phospholipid content. The DMPC-apoLp-III complexes were localized by the coextraction of protein and phospholipid, pooled, and dialyzed extensively against 10 mM Tris-HCl, pH 7.5. The protein content of the final DMPC-apoLp-III complexes was evaluated by amino acid analysis (Beckman System 6300 Amino Acid Analyzer, System Gold Version 6010) and the content of phospholipid was determined colorimetrically as described for choline. The complex had a lipid/protein weight ratio of 7.1:1 (molar ratio of 190:1). Control DMPC alone and apoLp-III DMPC complexes were adsorbed to carbon coated grids, stained with 2% sodium phosphotungstate, and viewed under a Philips CM 400 electron microscope operated at 100 kV (Wientzek et al., 1994). In addition to apoLp-III complexes, DMPC vesicles without added apoLp-III were employed in parallel control experiments.

IR Spectroscopy—Spectra were recorded on a Perkin-Elmer infrared spectrophotometer 1726X equipped with a Perkin-Elmer microspecular reflectance accessory and a polarizer mount assembly with a gold wire grid element. The internal reflection element was a germanium ATR plate (50 × 20 × 2 mm, Harrick EJ2121) with an aperture angle of 45° yielding 25 internal reflections. 128 accumulations were performed to improve the signal/noise ratio. The spectrophotometer was continuously purged with air dried on a silicagel column (5 × 130 cm) at a flow rate of 7 liters/min. Spectra were recorded at a nominal resolution of 2 cm−1. At the end of the scan they were transferred from the memory of the spectrophotometer to a computer for subsequent treatments. All the measurements were made at 20°C.

Spectra-Oriented multilayers were formed by slow evaporation of 100 μl of the sample on one side of the ATR plate. The ATR plate was then sealed in an universal sample holder (Perkin-Elmer 186-0354).

Hydrogen/Deuterium Exchange—Films containing 75–100 μg of protein were prepared on a germanium plate as described above. Nitrogen gas was saturated with D2O (by bubbling in a water-saturated N2 flow) at a flow rate of 51 ml/min (controlled by a Brooks flow meter). Bubbling was started at least 1 h before initiating the experiment. At zero time, the tubing was connected to the cavity of the sealed chamber surrounding the film. For each kinetic time point, 12 spectra were recorded and averaged at a resolution of 4 cm−1. At the beginning of the kinetics, spectra are recorded every 15 s. After the first 2 min, the time interval was increased exponentially. After 16 min, the interval between each scan allows the linearization of the second kinetic. A second sample, placed on another ATR setup of the Perkin-Elmer sample shuttle, was then analyzed with the same time sampling with a 16-min offset by connecting the D2O-saturated N2 flow in series with the first sample. From this time on, our program changed the shuttle position to follow the two kinetics. Before starting the deuteration, 10 spectra of each sample were recorded to test the stability of the measurements and the reproducibility of the sample position. In our usual way of working, one of the samples placed on the shuttle was the lipid-free protein and the other one was the DMPC-bound protein. This procedure allowed us to test the reproducibility of the experiment under identical conditions. A background deuteration kinetic recorded with the same germanium plate at the same position in the sample shuttle but in the absence of the sample was recorded and subtracted from the kinetic recorded in the presence of the sample. This allowed us to take into account the unavoidable variations in atmospheric water content inside the spectrophotometer. Indeed, even though the spectrophotometer was purged with dry air for 20 min before starting the experiment, further removal of traces of water vapor took place for several hours, superimposing distinct sharp bands from the water vapor to the protein spectra (Goormaghtigh and Ruyschaert, 1994). Subtraction of the background kinetic was improved by adopting the following automated procedure. A subtraction coefficient is first computed as the ratio of the area of the atmospheric water band integrated between 1565 and 1551 cm−1 on the sample spectrum and on the corresponding background spectrum. Once the atmospheric water band has been removed from each spectrum, an additional correction for the amino acid lateral side chain contributions as described elsewhere.2 The area of amide I, II, and II′ was obtained by integration between 1702 and 1596, 1596 and 1502, and 1492 and 1412 cm−1, respectively. For each spectrum, the area of amide II and amide II′ was divided by the area of amide I. This permitted us to take into account small but significant variations of the overall spectral intensity due to part of the presence of D2O, which induces swelling of the sample layer, increasing the average distance between the protein sample and the germanium crystal surface. Because the ATR spectrum intensity depends on this distance (Harrick, 1967), this results in a loss of a few percent of the band intensity for all measured bands. Undeuterated spectra were recorded before the kinetic experiment as explained above. Therefore, the 100% deuteration of the protein was achieved under denaturing conditions. In order to obtain the 100% deuteration, we have completely deuterated apolipoprotein III by resuspending lyophilized protein in D2O and warming it at 70°C (above the midpoint temperature-induced denaturation of 52°C (Ryan et al., 1993)) during 1 h. After cooling, the sample was spread on the ATR plate and reflushed with D2O-saturated nitrogen during 12 h. It has been shown that after cooling the protein regains 95% of its native secondary structure (Ryan et al., 1993). On the spectrum obtained, the surface of the amide II are virtually zero after subtraction of the side chains contribution (data not shown). The area of amide II and II′ (reported to the area of amide I) was finally expressed between 0 and 100% for each kinetic time point. It must be noted that the deuteration of the core of the protein was achieved under reversibly denaturing conditions, conditions that are not part of the kinetic protocol. However, it is conceivable that 100% deuteration might be real-ized kinetically in the limit of infinite time.

RESULTS

At constant experimental conditions (pH and temperature), the rate of hydrogen/deuterium exchange is related to the solvent accessibility to the NH amide groups of the protein, which in turn is related to the tertiary structure of the protein and to the stability of secondary structure elements. Because an IR analysis of the secondary structure of the apoLp-III shows that its α-helical structure is retained as the protein alternates between lipid-free and lipid-associated states (data not shown), differences in H/D exchange rate in the two states will reflect the changes in the tertiary structure of the protein and the contact with the lipid bilayer (Weers et al., 1994; Wientzek et al., 1994). Amide hydrogen exchange of the apoLp-III was followed by monitoring the amide II absorption peak (δ(NH), maximum at 1544 cm−1) decrease as a function of time of

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exposure to D$_2$O-saturated N$_2$ flow (from 15 s to 6 h) (see “Experimental Procedures”). Figs. 1 and 2 show a series of spectra recorded as a function of deuteration time for lipid-free apoLp-III and for apoLp-III DMPC complexes, respectively. The amide I region of the spectrum (1700–1600 cm$^{-1}$) reveals an asymmetric peak with a maximum at 1653 cm$^{-1}$ characteristic of a mainly helical structure in good agreement with the CD (Ryan et al., 1993) and x-ray (Breiter et al., 1991) data. Upon exposure of the sample to D$_2$O-saturated N$_2$, the amide I region broadened and shifted from 1653 to 1646 cm$^{-1}$ after 6 h. In the presence of DMPC, the amide I shifts down to 1644 cm$^{-1}$. Compilation of literature data on the behavior of the $\alpha$-helix amide I component (Goormaghtigh et al., 1994) reveals that 1646 and 1644 cm$^{-1}$ frequencies are well within the range of frequencies characteristic of deuterated helices. Because the protein is predominantly $\alpha$-helical, even after deuteration as previously shown by CD experiments (Raussens et al., 1995), these large isotopic shifts of the amide I could indicate a relatively important and unusually fast H/D exchange of the helices. This is confirmed by the rapid and almost complete disappearance of the amide II band (1600–1500 cm$^{-1}$), especially in the case of the apoLp-III-DMPC complex (Fig. 2). In order to quantify the H/D exchange rate, the evolution of the area of amide II for each sample was computed between 0 and 100% as explained under “Experimental Procedures” and reported in Fig. 3. It appears that the hydrogen/deuterium exchange is more important for the apoLp-III-DMPC complex than for the free apoLp-III. After 6 h of deuteration, about 80% of the amide N-H has exchanged in the complex, whereas only 70% has exchanged in the lipid-free protein. Because the H/D exchange is a first order reaction, the fraction of residual amide protons $H(t)$ is expected to display a multiexponential decay corresponding to the different groups, $i$, of amides protons characterized by a common period $k_i$. Each individual site is supposed to exchange via first-order kinetics. For $N$ amino acids in a protein, the number of sites occupied by a hydrogen $H(t)$ as a function of the time $t$ is given by:

$$H(t) = \sum_{j=1}^{N} e^{-k_j t}$$

(Eq. 1)

The large number of sites, even in a small protein, makes it impossible to obtain the individual rate constant $k_j$. One ap-
approach to this problem is to fit the curve $H(t)$ function of $t$ by a small number $M$ of exponential representing each a class $A_i$ of amide groups

$$H(t) = \sum_{i=1}^{M} A_i e^{-k_i t} \quad \text{(Eq. 2)}$$

Another approach is to replace the previous sum expression by an integral

$$H(t) = \int f(k)e^{-kt}dk = L[f(k)] \quad \text{(Eq. 3)}$$

The advantage of the integral formulation lays in the fact that without further assumption on the distribution of the rate constants $f(k)$, the inverse Laplace transform $L^{-1}$ immediately yields the distribution shape

$$f(k) = L^{-1}[H(t)] \quad \text{(Eq. 4)}$$

Knox and Rosenberg (1980) suggested a dimensionless presentation of the distribution function obtained after rewriting of the integral expression $H(t)$

$$H(t) = \int f(k)e^{-kt}d(ln(k)) \quad \text{(Eq. 5)}$$

Solving the Laplace transform can be approached analytically after fitting $H(t)$ to a suitable function or numerically. For reasons detailed by Gregory and Lumry (1985), the numerical approach is subject to several artifacts if not carefully treated (Provencher 1976, 1982a, 1982b; Provencher and Dovi, 1979; Halvorson, 1992; Ameloot, 1992). We used here the approach and the program described by Provencher (1982a) to obtain the kinetics coefficient distribution. This program gives the simplest possible solution, i.e. the lowest number of exponentials that can fit the experimental curve. The results of the Laplace transform of the kinetics reported in Fig. 3 appears in Fig. 4. In both conditions, essentially three groups of exponential decays were obtained. Integration of the peaks of Fig. 4 yielded the proportion of amino acid residues in each group (Tables I and II) for lipid-free apoLp-III and in complex with DMPC.

**DISCUSSION**

Submolecular motional dynamics is an essential attribute in the determination of protein structure. ATR-FTIR offers several advantages to address issues of protein dynamics in that it requires very little quantity of sample, is non invasive, and provides high quality spectra, and, importantly, the backbone
amide protons can be selectively probed due to their absorption in distinct regions of the infrared spectrum. We have previously shown by H/D exchange analysis that the tertiary stability of native and methionine-80-modified cytochrome c is affected to a large extent without alterations in the overall secondary structure (de Jongh et al., 1995). Amide H/D exchange kinetics monitored by ATR-FTIR on thin films of protein sample were consistent with identical exchange kinetics of aqueous solutions of proteins probed by $^1$H NMR, thereby confirming the validity of the former approach using thin films of proteins (de Jongh et al., 1995). More recently, we compared the amide H/D exchange kinetics data for bovine pancreatic trypsin inhibitor, myoglobin and lysozyme, collected by both ATR-FTIR and NMR techniques, after Laplace transformation of the data. A striking similarity emerged between the solution NMR data and the thin film ATR-FTIR data, further attesting to the power and validity of the approach employed in the present paper.

Kinetic analysis of the H/D exchange of lipid-free and DMPC-bound apoLp-III reveals several interesting aspects of structural flexibility of the protein. A vast tertiary structural reorganization of the protein appears to take place when apoLp-III goes from the lipid-free to the lipid-bound state. It is seen from Fig. 4 and Table I that the slowly exchanging amide protons represent 36% (59 residues) of the total for the lipid-free protein and only 20% (33 residues) for the DMPC-bound protein; this slow exchanging population represents the core of the

![Figure 3](image-url)

**Fig. 3.** Percentage of deuteration reported as a function of the deuteration time for free apoLp-III (black triangles) and for apoLp-III-DMPC complex (open circles) in a 1 mM Tris buffer at pH 7.5. The percentage of deuteration was estimated from the amide II surface evolution as described under “Experimental Procedures.” Each curve represents the average for three independent exchange experiments. Error bars represent the standard deviation from the average.

![Figure 4](image-url)

**Fig. 4.** Hydrogen/deuterium exchange kinetic constant distribution obtained after Laplace transform of the deuteration curves (Fig. 3).

| Table I |
| --- |
| Proportion (in percent) of $k_f(k_1)$, $k_f(k_2)$, and $k_f(k_3)$ in the exchange curve for free apoLp-III and for apoLp-III-DMPC complex. Numbers in parentheses are the number of amino acid residues involved. |
| | $k_f(k_1)$ (slow) | $k_f(k_2)$ (intermediate) | $k_f(k_3)$ (fast) |
| Free apoLp-III | 36 (59) | 12 (20) | 52 (85) |
| ApoLp-III-DMPC complex | 20 (33) | 16 (26) | 64 (104) |

| Table II |
| --- |
| Maximum of the distribution function for the three components present on Fig. 4 |
| | $1/k_1$ | $1/k_2$ | $1/k_3$ |
| Free apoLp-III | slow* | 10.2 | 0.75 |
| ApoLp-III-DMPC complex | slow* | 9.3 | 0.75 |

* Values are around 1000 min ± 50%.

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protein. During lipid interaction there is a concomitant conformational change, when about 25 residues move from this core to a more solvent accessible domain of the protein, thereby contributing to an increase in the number of faster exchanging residues. The resultant slowly exchanging population of amide protons constitutes the residual core in the lipid-bound form and probably represents residues oriented toward the lipid milieu, directly interacting with the hydrophobic fatty acyl groups. The fact that the rate constant values for the slow and intermediate exchanging protons shift in opposite directions upon association with lipid (Fig. 4) cannot be explained by self-association of the protein because the complexes were isolated by ultracentrifugal flotation, which removes free protein from the system. Moreover, a self-association is unlikely when the protein is bound to the lipids (detergent-like effect). The more likely explanation relates to the significant conformational adaptation of the protein upon lipid interaction, which results in a repositioning of helices inducing changes in the relative numbers of exchanging protons. It must be mentioned here that the observed fast exchanging peak (1/k = 0.75 min) includes other faster components unresolved under our experimental conditions, which are beyond the time frame of infrared spectroscopic analysis. On the other hand, for the slow exchanging components, the analysis of the data with the Laplace transform does not allow the determination of changes in the time constants smaller than 1 order of magnitude (Table II). Regarding the nature of the differently exchanging groups, especially the intermediate one, it may be very difficult to definitely identify them as discrete groups, given our current understanding of the system. Such an assignment is beyond the scope of the present analysis. Subsequent NMR experiments may permit such characterizations.

In general, an overall increase in the rate of exchange of the amide protons may be attributed to either a structural destabilization or to an increased access of the protein backbone to the solvent. In amphipathic α-helices, it has been observed that the H-bonds between the backbone >C=O and N-H on the hydrophilic face of the helix are longer and less linear than those on the hydrophobic side (Zhou et al., 1992). In apoLp-III:DMPC disc complexes there are six molecules of apoLp-III that orient around the bilayer perimeter acting as a scaffold (Wientzek et al., 1994). To accommodate this structural organization the helices would have to adopt a curvature introducing more solvent-induced distortions in the H-bond characteristics (Blundell et al., 1983). The center of curvature is toward the hydrophobic side roughly coinciding with the direction of the hydrophobic moment. The radius of curvature of the helices appears to be about 90 Å (taking into account the molecular dimensions of the disc-like complex), which falls within the range observed for “curved” helices, 40–100 Å (Blundell et al., 1983). Thus, it is conceivable that the increased rate of H/D exchange in lipid associated apoLp-III might be explained by H-bonds on the hydrophilic face of the helices becoming increasingly susceptible to exchange. This hypothesis, however, is unlikely because helix distortion or changes in helix stability are known to result in a significant shift and variation in the width of the amide I band (for a review, see Goormaghtigh et al. (1994)).

Analysis of the spectra does not show significant differences between the free and DMPC-bound protein when compared under identical deuteration conditions. In the present situation, it is also difficult to rationalize the exchange rate increase as the result of a tertiary destabilization of the protein upon interaction with phospholipids, based on comparison between the guanidine hydrochloride denaturation profiles of lipid-free and lipid bound apoLp-III. Although lipid-free apoLp-III is sensitive to guanidine hydrochloride (midpoint of denaturation at about 0.35 M; Ryan et al. (1993)), apoLp-III bound to model phospholipid complexes exhibited midpoints of denaturation at about 2.2 and 3.7 M guanidine hydrochloride, indicative of stabilization of the protein upon lipid interaction (Wientzek et al., 1994). A similar behavior of lipid-free and -bound forms of the protein was observed with other agents such as urea (Narayanaswami et al., 1994). It is therefore more likely that the increased accessibility of the protein backbone amides to the solvent may be related to tertiary alterations of the protein that occur upon interaction with the lipids.

Study of data reported in the literature allows us to make important conclusions as to the way that apoLp-III helices interact with the membrane. Some membrane proteins exhibit very slow amide H/D exchange (e.g. bacteriorhodopsin, glycoporphin transmembrane segment and Lys2-Gly-Leu23-Lys2-Ala transmembrane model peptide. It appears that their transmembrane segments are virtually nonexchangeable (for a review see Goormaghtigh et al. (1994)) because of the shielding effect of the membrane with respect to the aqueous solvent. Soluble proteins and some other membrane proteins whose transmembrane segments are thought to form an aqueous channel pore in the membrane display faster exchange constants. Yet, a core of slowly exchanging amide population is usually found. In several proteins, this slowly exchanging cluster has been related to the initial folding core during the sequence of events leading to protein folding (Kim et al., 1993). Quite surprisingly, in the case of DMPC-bound apoLp-III, the slowly exchanging core is much reduced in the presence of lipids when compared with most membrane and soluble proteins tested so far in our laboratory. It can therefore be concluded that in the lipid-bound structure, apoLp-III looses its tertiary folding to interact with the lipids. The resulting structure is readily accessible to the solvent. These experimental data are in good agreement with the model of the complex described earlier (Wientzek et al., 1994). Similar results were obtained for cytochrome c. Muga et al. (1991) concluded from FTIR studies that its secondary structure remains essentially unchanged upon binding to lipids, although tertiary destabilization of the bound protein was indicated by an increase in the rate of amide H/D exchange. Similarly, a considerable enhancement of the rate of H/D exchange rate was demonstrated upon binding of cytochrome c to cardiolipin (Spooner and Watts, 1991) and detergent micelles (de Jongh et al., 1992). Further, the relationship between the physical state of various phosphatidylglycerol membranes and the exchange was demonstrated by Heimburg and Marsh (1993). These observations were explained on the basis of tertiary structural changes, such as opening of the heme crevice. In summary, the data are consistent with the model that coincident with the lipid interaction, apoLp-III undergoes a major conformational adaptation. Further, this adaptation likely maintains the helix boundaries present in the lipid free globular state but involves a repositioning of their orientation from the helix-helix interactions, which stabilize the bundle conformation, to helix-helix interactions.

Acknowledgment—We thank Dr. Provencher for kindly providing us with the CONTIN program.

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J. Biol. Chem. 1996, 271:23089-23095.
doi: 10.1074/jbc.271.38.23089

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