# Membrane-induced conformational change in human apolipoprotein H

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The interaction of apolipoprotein H (Apo H) with lipid membrane has been considered to be a basic mechanism for the biological function of the protein. Previous reports have demonstrated that Apo H can interact only with membranes containing anionic phospholipids. Here we study the membrane-induced conformational change of Apo H by CD spectroscopy with two different model systems: anionic-phospholipid-containing liposomes [such as 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) and cardiolipin], and the water/methanol mixtures at moderately low pH, which mimic the micro-physicochemical environment near the membrane surface. It is found that Apo H undergoes a remarkable conformational change on interaction with liposomes containing anionic phospholipid. To interact with liposomes containing DMPG, there is a 6.8 % increase in $\alpha$-helix in the secondary structures; in liposomes containing cardiolipin, however, there is a 12.6 % increase in $\alpha$-helix and a 9 % decrease in $\beta$-sheet. The similar conformation change in Apo H can be induced by treatment with an appropriate mixture of water/methanol. The results indicate that the association of Apo H with membrane is correlated with a certain conformational change in the secondary structure of the protein.

Key words: circular dichroism, $\beta_2$-glycoprotein I, lipid–protein interaction, protein adsorption.

## INTRODUCTION

Apolipoprotein H (Apo H) is a plasma glycoprotein circulating either as a free protein or associated with lipoproteins. This protein, also referred to as $\beta_2$-glycoprotein I, was first described in 1961 [1]. Human Apo H is a single-chain molecule consisting of 326 amino acid residues and 5 carbohydrate chains, with a molecular mass of approx. 54 kDa [2–5]. The amino acid sequences of Apo H in human, bovine, mouse and rat seem to be highly conserved [6–9]. The protein contains four internal repeat units and a fifth domain. The repeat units consist of approx. 60 residues, each with two internal disulphide bonds, known as the Sushi domain [10]. The 2.9 Å crystal structure of human Apo H was recently reported at the XIII International Biophysics Congress [11]. It was shown that the four internal repeat units are characterized by $\beta$-barrel structure and that the fifth domain contains three disulphide linkages stabilizing a four-stranded $\beta$-spiral with extended loop regions containing the surface-exposed lysine-rich lipid-binding region.

In contrast with current knowledge of the structural characteristics of Apo H, its biological function remains unclear. The interactions of Apo H with phospholipids have been taken into consideration as a basic mechanism related to its physiological and pathogenic roles. This glycoprotein prefers to bind to anionic phospholipids, as has been demonstrated in several laboratories [12–15]. It is believed to serve as a cofactor for the binding of anti-phospholipid autoantibodies in the patients with anti-phospholipid syndrome [16]. Previously we demonstrated that Apo H can insert spontaneously into monolayers containing anionic lipid [17]. In our recent study with fluorescence measurements [18], we reported that a certain conformational change occurs as the protein interacts with membranes containing anionic lipid, suggesting that the protein in a membrane-bound state might have a distinct structure from that in its water-soluble state. Because the membrane binding Apo H is closely related to its biological function, it is of critical significance to understand its structural characteristics in this state. Here we examine the membrane-induced conformational change in Apo H by CD spectroscopy. Two model systems were used in the experiments. The first model system was the phospholipid liposomes; the second was a solvent system with water/methanol mixtures at moderately low pH to mimic the condition near the membrane surface [19]. The results indicate that the association of Apo H with phospholipid liposomes is correlated with a certain conformational change in the secondary structure of the protein.

## MATERIALS AND METHODS

### Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and bovine heart cardiolipin (CL) were purchased from Sigma Chemical Co. (St Louis, MO., U.S.A.). The other chemicals used were of analytical grade made in China.

### Purification of Apo H

Apo H was purified from human serum by the literature of Wang et al. [17]. The purified Apo H was freeze-dried and stored at −70 °C. Before use, the protein was redissolved in 0.05 M Tris/HCl buffer, pH 7.4, containing 0.025 M NaCl; its concentration was determined by absorption spectroscopy ($\epsilon_{280}$ 1.0 litre·g$^{-1}$·cm$^{-1}$) [6].

### Preparation of liposomes

Small unilamellar vesicles (SUVs) were prepared as follows. Lipids of the desired composition were mixed in chloroform/methanol (3:1, v/v) and dried under a stream of nitrogen. Residual solvents were removed under high vacuum for 2–3 h.

Abbreviations used: Apo H, apolipoprotein H; CL, bovine heart cardiolipin; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; SUVs, small unilamellar vesicles.

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The lipid samples were incubated in a solution of 50 mM Tris/HCl/25 mM NaCl (pH 7.4) at a temperature above the phase transition point, then sonicated with a probe sonicator for approx. 5 min until the solution became optically completely clear. The concentration of phospholipid was determined by phosphate analysis [20]. The SUVs produced by this procedure were diluted to 1.0 mM and used directly in all the experiments.

### CD spectroscopy

CD measurements were performed with a JASCO J-715 spectropolarimeter. Samples containing Apo H at a concentration of 10–20 $\mu$M were scanned at least eight times at the rate of 100 nm/min and averaged. The temperature of the sample compartment was maintained at $25.0 \pm 1.0$ °C with a circulating-water bath. The cuvette used had a light path of 0.2 mm. To minimize the influences of light scattering on the spectra of Apo H, a blank run made with the liposome or buffer alone was subtracted from the experimental spectra for correction. It was established that the CD spectrum of Apo H without liposome is almost the same as that of Apo H with the DMPC SUVs, so it be considered that the effect of light scattering was minimal. All spectra were smoothed and converted into the mean residue ellipticity, $[\theta]$ (in degrees$\cdot$cm$^2\cdot$dmol$^{-1}$), by using a mean residue molecular mass of 111 Da. The fractional percentage of the secondary structure was calculated by computer fitting to a library of CD spectra of proteins of known structure [21].

## RESULTS

### Effects of lipid membranes on the conformation of Apo H

The far-UV CD spectra were used to determine the effects of lipid binding on the secondary structure of Apo H. As shown in Figure 1, the CD spectrum of lipid-free Apo H was typical of a protein containing a significant $\beta$-sheet content. The computer fitting results show that lipid-free Apo H consists of 56.7% $\beta$-sheet and no $\alpha$-helix. The mean residue ellipticity at 222 nm [22] also indicated that the lipid-free Apo H was mainly composed of $\beta$-sheet and random coil, with the $\alpha$-helix content less than 1%. Our results are in good agreement with recent reports based on X-ray crystallography [11,23]. In accordance with the results of X-ray crystallography of Apo H, which indicated that the four consensus repeats are in a $\beta$-barrel structure and the fifth domain contains four $\beta$ strands, we have calculated the content of the secondary structure of Apo H to be 2.7% $\alpha$-helix and 48.2% $\beta$-sheet.

The addition of DMPG/DMPC or CL/DMPC SUVs results in a remarkable alteration in the CD curves as also shown in Figure 1, indicating that Apo H undergoes a significant conformational change. In both cases a considerable increase in the content of $\alpha$-helix is determined by computer fitting as shown in Table 1 (DMPG/DMPC, 6.8%; CL/DMPC, 12.6%). In contrast, when the zwitterionic lipid DMPC was added, the CD curve did not alter (see the curve in Figure 1) and the secondary structure of Apo H did not change (see the results in Table 1). This might be because Apo H does not associate with the zwitterionic lipid DMPC [12,18].

The results in Table 1 indicate further that the two kinds of acidic-lipid-containing vesicle have a slightly different effect on the conformational change in Apo H. As Apo H binds to DMPG/DMPC SUVs, the content of $\beta$-sheet of the protein remains nearly constant (an approx. 2.7% increase). As Apo H binds to CL/DMPC SUVs, however, the content of $\beta$-sheet obviously decreases from 56.7% to 47.7%.

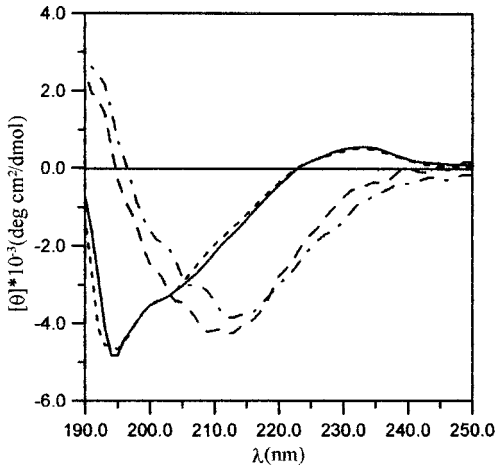


**Figure 1 CD spectra of lipid-free and lipid-bound Apo H**

Samples were prepared in 0.05 M Tris/HCl/0.025M NaCl (pH 7.4) containing 12 $\mu$M Apo H. The spectra were scanned eight times from 250 to 190 nm at 100 nm/min in a cuvette of path length 0.2 mm; the scans were averaged. Background spectra without protein were subtracted as appropriate. Solid line, Apo H in buffer without any phospholipid; dot–dashed line, Apo H in 360 $\mu$M DMPG/DMPC (80:20 molar ratio); broken line near to the dot–dashed line, Apo H in 360 $\mu$M CL/DMPC (80:20 molar ratio); dotted line near to the solid line, Apo H in 360 $\mu$M DMPC.

**Table 1 Percentages of secondary structure components of Apo H in the absence and in the presence of phospholipid liposomes**

For Yang's secondary-structure analytical method, the correlation coefficients, which describe the deviation between the experimental and the computed results, for $\alpha$-helix and $\beta$-sheet are 0.87 and 0.61 respectively. A correlation coefficient close to 1 indicates a successful prediction and a coefficient close to $-1$ indicates a total disagreement between the experimental and the computed results. The correlation coefficient is defined as:

$$r = (\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i / n) / \{[\Sigma X_{i2} - (\Sigma X_i)^2/n][\Sigma Y_i^2 - (\Sigma Y_i)^2/n]\}^{\frac{1}{2}}$$

where $X_i$ and $Y_i$ are the experimental and computed values respectively and $n$ is the number of the samples studied.

| Sample | Secondary structure predicted by Yang's method [21] (%) | |
|---|---|---|
| | $\alpha$-helix | $\beta$-sheet |
| Apo H only | 0 | 56.7 |
| | 2.7* | 48.2* |
| Apo H + PG/PC SUV | 6.8 | 59.4 |
| Apo H + CL/PC SUV | 12.6 | 47.7 |
| Apo H + DMPC SUV | 0 | 57.1 |

* Results obtained by X-ray crystallography [23].

### Influence of methanol on the conformational change of Apo H

Alcohol solvents (such as methanol and ethanol) have been used in many protein studies [19,24]. A solvent system with water/methanol mixtures can be used to mimic the physicochemical environments near the membrane surface region [19]. In the present study, the methanol/water system was also used as a model system for studying the effect of methanol-containing solvent on the conformational change of Apo H.

Figure 2 shows the CD curves of Apo H in solutions with different concentrations of methanol (from 0% to 65%, v/v) at

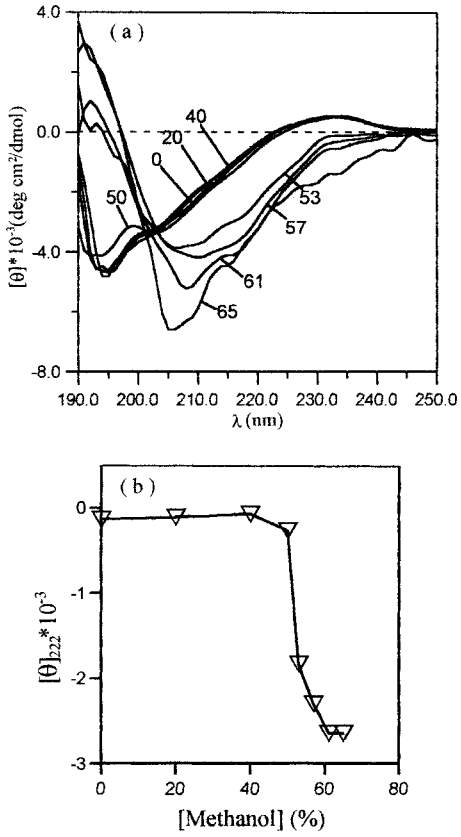


**Figure 2 CD spectra of Apo H in a methanol–water system**

(**a**) CD spectra of Apo H in solutions with various concentrations of methanol. Apo H (12 $\mu$M) was prepared in 0.05 M Tris/HCl/0.025 M NaCl (pH 7.4) containing the indicated concentrations (percentages v/v) of methanol. The spectra were scanned eight times from 250 to 190 nm at 100 nm/min in a cuvette of path length 0.2 mm; the scans were averaged. Background spectra without protein were subtracted as appropriate. (**b**) The mean residue ellipticity [$\theta$] at 222 nm is plotted against the methanol concentration. The units of [$\theta$] are degrees $\cdot$ cm$^2$ $\cdot$ dmol$^{-1}$.

pH 4.8. The secondary structure under each condition was analysed by computer fitting and the results are listed in Table 2. Figure 2(a) indicates that the CD spectra of Apo H changed little as the methanol concentration varied from 0 % to 50 %. When the methanol concentration increased further from 50 % to 65 %, the CD spectra of the protein exhibited a remarkable variation, suggesting a significant change in the secondary structure of Apo H. The rapid decrease in [$\theta$] at 222 nm, shown in Figure 2(b), demonstrated a sharp increase in the $\alpha$-helical secondary structure of Apo H from zero to approx. 8 % as the methanol concentration increased above 50 %. The midpoint of the increase in the $\alpha$-helix structure was approx. 51 % methanol. From Table 2 we can see also that, when the concentration of methanol was increased, the content of random coil showed a certain decrease and the content of $\beta$-sheet a small increase as well as an increase in $\alpha$-helix content.

It is known that the pH near the surface of an anionic membrane is approx. 1–2 units lower than that in the bulk solution. For the methanol/water system we also analysed the effect of pH on the conformation of Apo H. As the pH value varied from 7.4 to 3.0, there was no obvious change in the CD spectra of Apo H at methanol concentrations of 0 %, 30 % and 60 % (results not shown). These results suggest that the secondary structure of the protein was not sensitive to the pH of the solution.

**Table 2 Percentages of secondary structural components of Apo H in the methanol/water system**

| Concentration of methanol (%, v/v) | Dielectric constant of the system [27] | Secondary structure (%) | | |
|---|---|---|---|---|
| | | $\alpha$-helix | $\beta$-sheet | Random coil |
| 0 | 78.5 | 0 | 56.7 | 43.3 |
| 20 | 70.5 | 0 | 53.9 | 46.1 |
| 40 | 62.0 | 0 | 58.2 | 41.8 |
| 50 | 57.7 | 0 | 66.6 | 33.4 |
| 53 | 56.2 | 2.6 | 68.1 | 29.4 |
| 57 | 54.3 | 7.5 | 62.6 | 29.5 |
| 61 | 52.4 | 8.0 | 60.3 | 31.8 |
| 65 | 50.5 | 7.8 | 58.3 | 33.9 |

## DISCUSSION

The interaction of Apo H with anionic phospholipids is believed to be correlated with the biological function of the protein. With lipid vesicles, Hagihara et al. [14] reported that Apo H can interact strongly with membranes containing negatively charged phospholipids but not with those containing neutral phospholipid alone. With supported planar bilayer, Willems et al. [15] studied the binding of Apo H and the Apo H-mediated binding of anti-phospholipid antibodies to neutral and negatively charged membranes. The results in the paper cited revealed the characteristics of Apo H binding to lipid membranes, as well as the biological roles to a certain extent. We reported recently [18] that DMPG and CL vesicles can induce an obvious blue shift in the intrinsic fluorescence of Apo H. This report indicated that water-soluble and membrane-bound Apo H molecules have different structures. However, the actual behaviour of Apo H molecules on membrane surface is still little known. In the present study we therefore examined the secondary structure change in the protein on interaction with phospholipid membranes.

From the results shown in Figure 1 and Table 1 we can learn at least two points. First, the remarkable difference in the CD curves of Apo H between the presence and absence of anionic phospholipids indicates that Apo H undergoes a genuine conformational change as it binds to phospholipids; this conformational change can be characterized at the secondary structure level. Secondly, Apo H has a slight but non-negligible difference in secondary structure change when interacting with DMPG-containing liposomes and with CL-containing liposomes.

The microenvironments are distinct when the protein molecules locate in the membrane-surface region and in the bulk medium. At least two physicochemical parameters are often used to describe this difference. One parameter is pH: as already frequently mentioned previously, the low pH in the membrane-surface region is a requirement for the membrane insertion of many proteins [25]. The other parameter is dielectric constant. Smith and co-workers [26] examined the dielectric structure of lipid bilayers. They built a model to represent the lipid bilayer as three electrically distinct membrane regions, i.e. a hydrophobic region, an acetyl region and a polar-head region. The polar-head region probably has a dielectric constant of 20–50 [26]. The dielectric constant of water is 78.5 [27]. Therefore the value of the dielectric constant has a sharp change across the membrane/water interface.

As mentioned above, the secondary structure of Apo H is not sensitive to pH change; it is therefore more probably dependent on the dielectric features of the microenvironment. By varying the mixture of methanol and water we can change the dielectric constant of the solution so that the dielectric feature of the

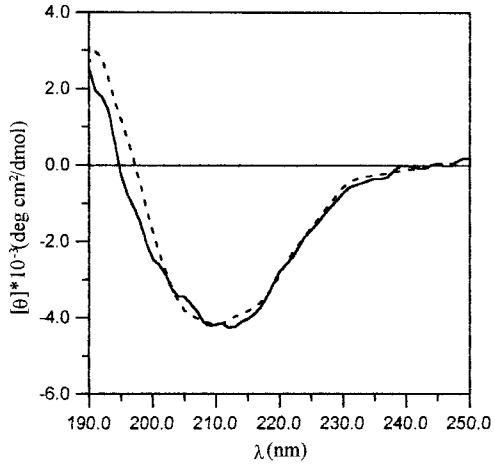


**Figure 3 Comparison of the CD spectra of Apo H in the presence of DMPG/DMPC SUV and in 57% (v/v) methanol solvent**

Solid line, CD spectrum of Apo H in the presence of DMPG/DMPC (80:20 molar ratio) SUV (data from Figure 1); dotted line, CD spectrum of Apo H in 0.05 M Tris/HCl/0.025 M NaCl (pH 4.8) containing 57% (v/v) methanol (data from Figure 2).

membrane surface region can be simulated. The measurement curves of Apo H in various mixtures are exhibited in Figure 2 and the data of computer fitting are shown in Table 2. From the results shown in Table 2, we can see that as the the dielectric constant of the solution decreases, the $\alpha$-helix content of Apo H remains zero at first and then increases sharply when the dielectric constant reaches 57. During this transition process it is accompanied by an increase in $\beta$-sheet structure and a decrease in random coil. This feature is quite similar to the behaviour observed when the protein binds to DMPG/DMPC vesicles. The plots in Figure 3 show such a comparison. From Figure 3 we can see that the CD curve of the binding of Apo H to DMPG/DMPC lipid bilayer is probably similar to that of Apo H in the 57% methanol/water mixture.

According to Akerlof [27], the 57% methanol/water (v/v) mixture has a dielectric constant of approx. 54. This value is located just at the point of the membrane/surface region. We can therefore deduce that the change in secondary structure of Apo H on interaction with DMPG/DMPC membrane is due mainly to the change of the dielectric constant near the membrane surface. This conclusion is consistent with our recent paper [18], in which we found that the negatively charged DMPG does not serve as a specific binding site but rather provides a suitable microenvironment for Apo H interaction.

The interesting phenomenon is the feature of the secondary structure change of Apo H on interaction with liposomes containing CL. In comparison with the change on binding to liposomes containing DMPG, Apo H exhibits a decrease in $\beta$-sheet content when binding to CL-containing lipid membrane, as well as a remarkable increase in $\alpha$-helix content and an identical decrease in random coil. The change in $\beta$-sheet content is opposite to the change that occurs as the protein binds to DMPG-containing liposomes. Further more, the CD curve of Apo H after binding to CL-containing liposomes (as shown in Figure 1) is not comparable with any of the curves shown in Figure 2. This suggests that the CL-induced conformational change of Apo H cannot be interpreted simply as indicating a change in dielectric constant.

Received 7 October 1999/7 February 2000; accepted 29 February 2000



Apo H has been reported to function as a cofactor for the binding of some anti-phospholipid antibodies to membranes [16]. Such anti-phospholipid antibodies, which appeared at high titres in some autoimmune diseases and anti-phospholipid syndrome, were considered to be pathogenic *in vivo*. What these antibodies recognized *in vivo* would not be the phospholipid alone, as discussed in [16]. It has also been established [28] that the anti-phospholipid antibodies do not recognize Apo H alone. It is therefore supposed that the antibodies might actually bind to the new determinants of Apo H that were specifically exposed during the interactions between Apo H and membrane. This idea gets good support from our findings that (1) Apo H undergoes a conformational change on interaction with anionic membranes, and (2) this membrane-induced conformational change might depend specifically on the specific type of anionic phospholipid, such as CL. Nevertheless, the exact physiological and pathogenic roles of Apo H, for example the exact role of Apo H in the formation of the anti-phospholipid–antibody/phospholipid complex, are still poorly known. The biological function of Apo H *in vivo* warrants further investigation.

This work was supported by the National Natural Science Foundation of China.

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