pH-dependent interactions of Apolipophorin-III with a lipid disk

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Received 11 June 2020
Accepted 9 July 2020
Published 18 September 2020

Apolipophorin-III (ApoLp-III) is required for stabilization of molecular shuttles of lipid fuels in insects and is found to contribute to the insect immune reaction. Rearrangement of its five α-helices enables ApoLp-III to reversibly associate with lipids. We investigate computationally

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the conformational changes of ApoLp-III and the pH-dependence of the binding free energy of ApoLp-III association with a lipid disk. A dominant binding mode along with several minor, low population, modes of the ApoLp-III binding to a lipid disk was identified. The pH-dependence of the binding energy for ApoLp-III with the lipid disk is predicted to be significant, with the pH-optimum at pH = 8.0. The calculations suggest that there are no direct interactions between the lipid head groups and titratable residues of ApoLp-III. In the physiological pH range from 6.0 to 9.0, the binding free energy of ApoLp-III with the lipid disk decreases significantly with respect to its optimal value at pH 8.0 (at pH = 6.0, it is 1.02 kcal/mol and at pH = 9.0 it is 0.23 kcal/mol less favorable than at the optimal pH = 8.0), indicating that the pH is an important regulator of ApoLp-III lipid disk association.

**Keywords:** pH-dependence; apolipophorin-III; lipids; molecular dynamics; pKa’s calculations; free energy.

1. Introduction

A living organism produces hydrophobic lipids, such as triacylglycerols and cholesterol, which are not solvable in aqueous biofluids. To move lipids from one site of the organism to the other, animals develop special shuttles-lipoproteins that can be loaded with lipids and are able to circulate through the animal blood vessels. Lipoprotein is a special assembly of lipids and proteins representing a single noncovalently bound particle. In mammals and insects, lipoproteins have similar structures and multiple functions. Some lipoprotein particles form very complex structures consisting of other lipoproteins, which are bound together and cannot be broken; these are nonexchangeable lipoproteins. There are exchangeable lipoproteins which, upon a physiological command of the organism, are able to temporally and reversibly bind the nonexchangeable proteins and then leave them to move to another nonexchangeable lipoprotein. While in mammals, the lipid transport involves different types of lipoproteins, the number of lipoproteins in insects is much smaller.

Insect lipoproteins are called lipophorins. The nonexchangeable lipoprotein in insects is called High Density Lipophorin particle (HDLp). It is assembled from two proteins that bind lipids, Apolipophorin-I (ApoLp-I) and Apolipophorin-II (ApoLp-II) and phospholipids, sterols, and hydrocarbons. In insects, the lipids are stored in the fat body in the form of triglycerides (TAG). The insects use a set of complex biochemical reactions to convert TAG into diglycerides (DAG). The latter is loaded to HDLp to be transported to the flight muscles where the DAG is used to generate metabolic energy required for sustained flight. As more and more DAGs are collected by HDLp, the particle increases in size and needs some molecular belts to keep it intact. Fortunately, at the surface of the DAG-loaded HDLps, the phospholipid packing is disrupted by the hydrophobic fatty acid end groups. These groups attract a surfactant-like exchangeable protein called Apolipophorin-III (ApoLp-III), which is freely available in insect blood. In the blood, free ApoLp-III resides as a compact globular protein composed of a bundle of five amphiphilic α-helices. Interacting with the surface of HDLp, ApoLp-III undergoes large conformational changes opening up its structure to form molecular belts interacting with the hydrophobic fatty acid end groups of DAG. Up to 16 ApoLp–III, belts
can be attached to a single HDLp preventing this particle against breakup.\textsuperscript{24,25} This new structure — the HDLp covered with DAGs and ApoLp-III has a lower density and is consequently named Low Density Lipophorin particle (LDLp).

The mechanism of interactions of ApoLp-III with HDLp generated a considerable interest not only in the community of entomologists, but in much broader community, because of its structural and functional similarity with mammalian proteins ApoE\textsuperscript{26} and TIP47/perilipin 3.\textsuperscript{27–29} To model interactions of ApoLp-III with HDLp, different phospholipid model vesicles have been proposed. For example, dimyristoylphosphatidylcholine (DMPC) vesicles mimic the HDLp surface fairy well and hence become quite popular.\textsuperscript{23} These experiments led to the development of the so-called disc assay where association of a lipoprotein in question with DMPC vesicles results in the formation of stable nanodiscs.\textsuperscript{26,30–35}

The experiments showed that ApoLp-III unfolds by preserving the $\alpha$-helical secondary structure and exposing its hydrophobic residues to the hydrophobic acyl groups of lipids.\textsuperscript{23,36} Thus, the conformational change involves helices rearrangement via connecting loops.\textsuperscript{36–41}

Here, we will investigate the conformational change of ApoLp-III via molecular dynamics (MD) simulations to provide mechanistic interpretation of experimental data. In our modeling protocol, the size of the lipid disk is 20 nm and its thickness is 5 nm.\textsuperscript{23} Such a large macromolecular assemblage along with several copies of ApoLp-III is a challenge for MD simulations. However, we apply nano-scale molecular dynamics simulations with NAMD\textsuperscript{42,43} of free and the disk-bound-ApoLp-III in open conformation to investigate the following questions: (i) is the lipid disk coated with the ApoLp-III belt a stable structure; (ii) which conformation is more stable, when an open ApoLp-III belt fastens the lipid disk over the disk border or when an open ApoLp-III belt resides free in water; (iii) which helical topology of ApoLp-III results in the most stable association with the disk?

We study these questions by investigating the free energy differences between a compact soluble ApoLp-III and the-disk-bound ApoLp-III. Both the lipids and ApoLp-III have titratable groups in which ionization state depends on pH of the environment. However, the lipid headgroups pKa’s are much lower than neutral pH, while the ApoLp-III titratable groups may titrate at such a pH. In addition, the binding may induce pKa’s changes, i.e. it may be pH-dependent. In this work, we investigate how the solution pH affects ApoLp-III binding to a lipid disk. Addressing these questions, we expect to get a deeper insight into the pH dependence of the transition from HDLP to LDLP.

2. Methods

2.1. Compact structure of Apolipophorin-III

The NMR structure of Manduca Sexta ApoLp-III (PDB:1EQ1) was used for the calculation of compact (a lipid free) state of ApoLp-III. The PDB file contains
25 models, which are quite similar except for the conformations for the N-terminal short loop. The first model was used in our MD simulations. However, for the pKa calculations and pH-dependence of the binding, all 25 models were investigated, and the results were averaged.

2.2. Construction of Apolipophorin-III open conformation models

In order to bind to the lipid disk, the ApoLp-III must open its compact structure. According to numerous experimental works, the opening involves rearrangement of $\alpha$-helices without structural changes of $\alpha$-helices themselves. Thus, we used the NMR structure mentioned above as a starting structure to build models of open conformations of ApoLp-III. In the compact state, the ApoLp-III adopts a bundle of five $\alpha$-helices connected by short loops (following the convention discussed in Ref. 45, the helices are labeled as 1, 2, ..., 5 beginning from the N-terminal, Fig. 1). The previous studies have pointed out that the opening should involve two $\alpha$-helices resulting in formation of two sub-bundles, which allow the hydrophobic interior of the protein to interact directly with lipids. Based on the topology of ApoLp-III, there are four plausible open conformations (Fig. 2). Here, starting from the original NMR structure (PDB ID:1EQ1), we manipulated torsion angles of the loops connecting the $\alpha$-helices with Chimera and rearranged five $\alpha$-helices bundles in two sub-bundles. For each model, we rotated the two $\alpha$-helices in a way to make their

![Fig. 1. Structure of ApoLp-III of Manduca Sexta in compact conformation ((PDB ID:1EQ1). The $\alpha$-helices are counted starting from the N-terminal.](image-url)
hydrophobic interior facing outward. In total, we generated four open conformation models (Fig. 2).

2.3. Model for Apolipophorin-III bound to a lipid disk

The lipid disk was constructed with VMD membrane plugin. Association of ApoLP-III with DMPC vesicles results in the formation of uniform discs with an average diameter of 18.5 ± 2.0 nm. Therefore, the disk radius was selected to be 100 Å and the disk is made of POPC lipids. As a result, the disk had a total of 683 POPC lipids. Then using Chimera, five copies of the open ApoLP-III were manually wrapped over the lipid disk border, fitting the entire circumference of the lipid disk (Fig. 3, and the movie apolipophorin-disk.mp4). Thus, four lipid-disk — ApoLP-III models were created, using each of the four open conformation modes of ApoLP-III described above. The resulting 3D structures can be downloaded via http://compbio.clemson.edu/downloads.

Fig. 2. Four plausible structural model of unfolded conformation of ApoLP-III.
2.4. Electrostatics potential calculations

The electrostatic potential calculations were performed with the Delphi program. The dielectric constants for protein and solvent were set to 2 and 80, respectively, and the salt concentration was set to be 0.15 M. The percentage filling of the box was 70 with the scale of 1 grid/Å. The surface potential of the protein was calculated with DelPhi, the phimap output file and the electric field lines were drawn with VMD.

2.5. Calculations of the pH-dependence of the Apolipophorin-III net charge

The net charge of ApoLp-III was calculated with DelPhiPKa. The ion concentration was set at 0.15 M and the dielectric constants for protein and water phase were 8 and 80, respectively. The pH range was set from 0 to 14 with an interval of 1. Based on the modeling of pKa’s of ionizable groups, the DelPhiPKa program calculates the net charge of the protein at each pH. Two sets of calculations were done. A calculation of the pH-dependence of the net charge of compact ApoLp-III and of open ApoLp-III bound to a lipid disk. In the last case, the calculations were done by keeping the lipid charges constant, while allowing ApoLp-III ionizable groups to titrate. As mentioned above, in the pKa’s calculations of compact ApoLp-III, all NMR models (25 models) were used and then the results were averaged. To have multiple conformations for the bound state, for each Model, five conformations were extracted from the MD simulations at $t = 20, 40, 60, 80$, and 100 ns, resulting in five snapshots per model. Due to the large size of the ApoLp-III-lipid-disk complex, adding more snapshots was computationally prohibited. These snapshots were used for the pKa’s calculations, and the results were averaged.

fig. 3. The lipid disk — ApoLp-III complex. The left panel is the top view, the right panel is the side view.
2.6. *pH*-dependence of binding free energy of Apolipophasrin-III to a lipid disk

The change of the net charge of a system from one state to another as a function of pH can be used to compute the pH-dependence of the free energy between the states. In the previous paragraph, we outlined the modeling steps for calculations of the net charge of compact and bound-to-the-lipid-disk ApoLp-III. The net charge difference $\Delta q(pH)$ has been identified from these calculations and analyzed as a function of pH. Then the pH-dependence of the free energy difference between compact ApoLp-III in water and open ApoLp-III bound to the lipid disk is calculated as follows:

$$
\Delta \Delta G(pH) = \int_{pH_0}^{pH} \Delta q dpH + \Delta G(pH_0),
$$

where $\Delta \Delta G(pH)$ is the pH-dependence of the free energy, $\Delta G(pH_0)$ is the free energy at pH$_0$, and the integral goes from pH$_0$ to the desired pH.

2.7. Molecular dynamics simulations

We performed the all-atom MD simulations for each of the four open models of ApoLp-III. To compare the structural dynamics of a free and the disk bound ApoLps, the MD simulations were done on ApoLp-III alone and five ApoLp-III-lipid disk complexes. All MD simulations were performed by NAMD 2.11 with CHARMM36m force field and TIP3P water model. The input files were prepared with VMD. The systems were solvated with 0.15 M NaCl in a cubic water box with no less than 10 Å spacing from the protein to the edge of the box. The Langevin dynamics with periodic boundary conditions were applied in the simulations. The van der Waals and electrostatic interactions were truncated at 12 Å distance with a switching function from 10 Å. The particle mesh ewald was applied for calculations of long-range electrostatic interactions.

In simulations of the open ApoLp-III conformations alone, the system first underwent a 5000-step minimization with the backbone fixed and then followed by a subsequent 5000-step minimization without any constraints. Furthermore, all atoms of the protein were fixed for 100 ps equilibration of the water molecules and ions and then the system was gradually heated from 0 K to 310 K with 1000-step/K in NVT. Next, the system was subjected to the 1 ns equilibration with the alpha carbon atoms (CA) constrained and another 2 ns equilibration without any constraints at 310 K. Lastly, the system was switched to NPT simulations and all constraints were removed for the 100 ns production run.

In the simulation of ApoLp-III–disk complexes, the system first underwent a 5000-step minimization by fixing all the atoms except of the lipid tails, and then a subsequent 5000-step minimization without constraints. Next, the systems were subjected to 500 ps equilibration at 310 K in NVT simulations and a harmonic constraint of 1 kcal/mol was applied to the CAs. The system was then equilibrated
without any constrains for another 500 ps in NPT simulation and after that, we performed the 100 ns production run.

2.8. Analysis of trajectories

The analysis of trajectories were performed using TCL scripts in VMD. We first calculated the Root Mean Square Deviation (RMSD) and Radius of Gyration ($R_g$) for the simulations of ApoLp-III alone, where the frames are superimposed onto the initial structures and then the RMSD of $C_\alpha$ atoms were calculated. The $R_g$ was calculated using the following equation:

$$R_g^2 = \frac{\sum m_i(r_i - R_c)^2}{\sum m_i},$$

where $m_i$ is the mass of the $i$th nonhydrogen atoms and $r_i$ is its coordinates. $R_c$ is the center of mass of the protein.

In the simulations of ApoLp-III -disk complexes, we calculated the RMSD and $R_g$ for each of ApoLp-III separately and then took the mean values.

2.9. Surface area calculations

The solvent accessible surface area (SASA) was calculated using VMD with a probe distance of 1.4 Å using the last frames from each simulation runs. The binding interfacial area (measured in squared Å) of ApoLp-III to lipid disk was calculated as the subtraction between the SASA of free ApoLp-III and SASA of bound ApoLp-III.

3. Results

We question (a) what is the most favorable conformational changes induced by the lipid disk binging and (b) what is the pH dependence of the binding free energy?

3.1. Investigating the most favorable open conformation of ApoLp-III

To bind a lipid disk, ApoLp-III has to undergo large conformational change to partially unfold its bundle. To test the ability of MD simulations to sample such a change, we simulated ApoLp-III in water and in a medium mimicking a lipid bilayer. It is known that in water the ApoLp-III bundle is not very stable since the folding free energy is only 1.24 kcal/mol. We carried MD simulations of the compact structure of ApoLp-III in water, modeled as a high dielectric constant medium ($\epsilon_{\text{out}} = 80$), and in lipid environment modeled as a low dielectric constant medium ($\epsilon_{\text{out}} = 4$). This way, one can evaluate whether the ApoLp-III stability depends on the presence of a lipid phase. We did not observe any significant difference between the RMSD of ApoLp-III in high and in low dielectric media. In both cases, the protein demonstrated instability manifested by significant conformational deviations of its initial structure after 100 ns simulation time. This indicates that the protein is not very stable, indeed. Despite these observations, we were unable to model a
conformational change expected for the lipid-bound protein. Perhaps much longer simulations are needed along with enhanced sampling techniques. To avoid this, the following strategy was adopted: instead of modeling the process of the protein opening, the open state was generated manually (see below).

As mentioned in Sec. 2, four plausible open conformations of ApoLp-III were generated and docked to a lipid disk. The open conformations and those bound to the disk were subjected to MD simulations and the outcome was investigated in terms of RMSD, \(R_g\) and the interfacial area of ApoLp-III bound to the lipid disk (Figs. 4 and 5 and Table 1).

It is expected that ApoLp-III should unfold its bundle in the presence of a lipid bilayer exposing its hydrophobic core to the disk border be able to fasten it up. However, during this unfolding process prior to binding to the lipid disk, the open ApoLp-III will still be in water, suggesting that the open ApoLp-III should be stable in water as well.

Thus, it can be anticipated that the optimal configuration of the unfolded ApoLp-III bundle should be the one with the smallest RMSD and the smallest RMSD fluctuations. This conformation would provide the most probable configuration of the ApoLp-III belt which is about to land on the disk border. We also assume that the best model should be such that the RMSD of the disk-bound ApoLp-III is the smallest, and the \(R_g\) remains almost a constant while radius of gyration of unfolded ApoLp-III in water should have a tendency to decrease (indicating that ApoLp-III would tend to fold back into a soluble compact configuration). Lastly, the best

Fig. 4. The RMSDs for *Manduca Sexta* ApoLp-III in unfolded conformations. The RMSDs were calculated for ApoLp-III alone (red color) and for the disk-bound states for all four models (blue color).
configuration should result in a relatively tight binding to the lipid disk, which should result in the largest interfacial area covered (Table 1).

An analysis of the results suggests that the Model 3 is the best candidate following experimental observations. However, the other modes cannot be ruled out completely. Indeed, the Model 2 has the second largest interfacial area and also small RMSD of the bound state. This indicates that while Model 3 is putatively correct and describes the anticipated mode of ApoLp-III association with the lipid disk, there may be a mixture of binding modes with low populations coming from the Models 1, 2 and 4.

### 3.2. Investigating the pH-dependence of binding free energy

The first step in this investigation is to check the validity of the computational protocol by benchmarking it against the experimental data. We used the PDB

![Fig. 5](image_url)
structure of compact soluble ApoLp-III and performed pKa’s calculations with DelPhiPKa. The results are shown in Table S1. We are not aware of any experiments to compare our calculated pKa’s of ionizable residues with. However, there are experimental data on the net charge of ApoLp-III at a particular pH and about the isoelectric point, pI (the pH as which the net charge is zero). Figure 6 shows the calculated net charge of a compact ApoLp-III as a function of pH. One can see that the pI is calculated to be 6.2 which is very close to experimentally measured pI of 5.7. At pH = 8.6, the experiment gives the net charge of $-4.7e$ which is again closely matched by our calculations resulting in the net charge of $-3.0e$. These results indicate that the computational protocol is adequate.

The pH dependence of the transition from the compact soluble ApoLp-III to the ApoLp-III lipid disk complex was modeled using Eq. (1) and computing the net charge differences as a function of pH. The pH dependence of the net charges of the compact ApoLp-III bundle and four models of to the ApoLp-III lipid disk complexes are provided in Fig. S1. The results are shown in Fig. 7 for each of the models separately (for completeness, Fig. 7(a) shows the pH-dependence in the range of pH from 0 to 14, while Fig. 7(b) shows the results in the range of physiological pH). In most of the cases, the pH-dependence of the free energy is an inverted bell-shaped curve and has a minimum at a particular pH. This pH is termed the pH-optimum since the binding affinity is the most favorable at this pH. There is no much difference between pH-dependence of biding free energy among the four models, indicating that there are no specific interactions between ApoLp-III titratable residues and the lipid disk that differ among the models (Note that pKa calculations were done at atomistic level of detail, thus including interactions between all atoms of ApoLp-III and lipids).

It is important to notice that we are talking about the relative energy values since Eq. (1) has a constant, the binding free energy at a particular pH, which in this case.
Fig. 7. The calculated pH-dependence of the free energy difference between the compact soluble ApoLp-III bundle and ApoLp-III bound to a lipid disk. The profiles are adjusted to result in zero energy at pH = 0. (a) pH ranges from 0.0 to 14.0. (b) pH ranges from 6.0 to 10.0.
it taken to be zero at pH = 0.0. In the range of physiological pH, from 6.0 to 9.0, the magnitude of the binding free energy for Model 3 changes from $-5.67 \text{kcal/mol}$ at pH = 6.0 to $-6.69 \text{kcal/mol}$ at pH = 8.0 to $-6.46 \text{kcal/mol}$ at pH = 9.0. In the other models, these changes are very similar. Having in mind that the folding free energy of ApoLp-III is only $1.24 \text{kcal/mol}$, such a change represents a significant barrier for the transition of ApoLp-III from the free to the lipid-bound state. Thus, the pH is an important regulator of the ApoLp-III association with a lipid disk and perhaps the same is valid for ApoLp-III binding to LDLp.

The pKa's of ionizable residues of the soluble compact ApoLp-III and in the ApoLp-III-disk complexes are provided in Tables S1, S2, S3, S4 and S5. A titratable group that has different pKa in compact versus bound ApoLp-III will have contribution to the pH-dependence, while a titratable group that has the same pKa in both states will not contribute. Thus, one can compare the individual pKa's and seek change in pKa's from soluble to bound state to identify which titratable groups cause the pH-dependence of the binding free energy. While there are many such changes, we are mostly interested in the cases affecting the pH dependence around the pH-optimum = 8.0. There is no pKa = 8.0 in any of the Tables S1, S2, S3, S4 and S5, however there are several Histidine and Lysine residues that undergo pKa shifts that make them not fully ionized at pH = 8.0. Comparing pKa's of such residues across all the models, one cannot spot any specific trend. Thus, Histidine 15, 151 and 160 have mostly unperturbed pKa (with respect to pKa in the unbound state) in all the Models, while Histidine 94 is predicted to have some shifted pKa in Models 2 and 4, but not in Models 1 and 3. Similarly, the behavior of Lysine residues varies across the models. This indicates that the ApoLp-III binding to lipid disk is not specific, indeed, and it is driven by hydrophobicity which is nonspecific effect. Thus, while almost the same pH-dependence profile is predicted for all the models, the ionizable groups contributing to it are different in different models.

### 3.3. Role of electrostatics in Apolipophorin III binding to a lipid disk

While the hydrophobic effect is the main reason for ApoLp-III physical binding to a lipid disk, the electrostatics provides long-range guiding force to initiate the process. Indeed, the electrostatic potential mapped onto the compact soluble ApoLp-III bundle is shown in Fig. S2 and it can be seen that ApoLp-III surface is mostly negatively charged and in its compact form the protein will be repelled from the negatively charged lipids. The electrostatic field lines indicate the same phenomena — there are electrostatic funnels that will provide guidance for binding to the positively charged partners, but not for the negatively charged ones.

In the case of open ApoLp-III conformation (Fig. S3), the negatively and positively charged surface patches are equally distributed over the protein surface. Especially in the case of Models 3 and 4, the positively charged patches dominate. These patches will provide guidance toward binding to the negatively charged partners like lipids.
When ApoLp-III is bound to the lipid disk (Fig. S4), the negatively charged patches face away from the binding interface, while the positively charged patches are mostly facing the lipid disk. This stabilizes the ApoLp-III-lipid-disk complex and makes it soluble in water.

4. Discussion

The work suggests that the ApoLp-III binding to a lipid disk may be multi-modal binding. While we confirm the experimental observation that the dominant binding mode involves an opening of hydrophobic code of ApoLp-III such that helices 3, 4 move away their original positions adopted in the 3D structure of soluble ApoLp-III, we suggest that other open conformations may be present as well. This prompted us to consider that ApoLP-III association with a lipid disk is nonspecific and is mostly governed by the hydrophobic effect. Indeed, the binding involves an opening of ApoLp-III such that the hydrophobic core turns into a curved patch of hydrophobic residues and the curvature of the patch matches the curvature of the lipid disk (see the video). Thus, the ApoLp-III hydrophobic patch binds to the hydrophobic lipid tails and completes the physical binding. It is known that hydrophobic effect is nonspecific, thus, any structural rearrangement of ApoLp-III that results in such hydrophobic patch will promote the binding to the lipid disk.

From electrostatics point of view, we predict strong pH-dependence of the binding free energy of ApoLp-III to a lipid disk. The predicted free energy change as pH varies in physiological pH ranges is comparable with the folding free energy of ApoLp-III. This indicates that pH is an important regulator for ApoLp-III binding to lipid disk and perhaps to LDLP. It should be mentioned that all four open ApoLp-III models resulted in very similar pH-dependence of the binding free energy to a lipid disk, which further support the hypothesis of nonspecific binding.

Acknowledgments

This work was supported in part by National Science Foundation SC EPSCoR/IDeA Program under NSF Award No. OIA-1655740. The views, perspectives and content do not necessarily represent the official views of the SC EPSCoR/IDeA Program nor those of the NSF. E. A and Y. P. were supported by a grant from NIH, grant number R01GM093937. We thank Alex Alexov for generating the movie illustrating ApoLp-III binding to a lipid disk.

References

1. Ryan RO, van der Horst DJ, Lipid transport biochemistry and its role in energy production, Annu Rev Entomol 45:233–260, 2000.
2. Van der Horst DJ, Rodenburg KW, Lipoprotein assembly and function in an evolutionary perspective, Biomol Concepts, 1(2):165–183, 2010.
3. Van der Horst DJ, Roosendaal SD, Rodenburg KW, Circulatory lipid transport: Lipoprotein assembly and function from an evolutionary perspective, *Mol Cell Biochem* **326**(1–2):105–119, 2009.

4. Van der Horst DJ, Ryan RO, Lipid transport, in Gilbert LI (ed.), *Insect Molecular Biology and Biochemistry*, Elsevier Academic Press Inc., San Diego, pp. 317–345, 2012.

5. van der Horst DJ et al., Alternative lipid mobilization: The insect shuttle system, *Mol Cell Biochem* **239**(1–2):113–119, 2002.

6. Plump AS, Breslow JL, Apolipoprotein E and the apolipoprotein E-deficient mouse, *Annu Rev Nutr* **15**:495–518, 1995.

7. Breslow JL, Human apolipoprotein molecular biology and genetic variation, *Annu Rev Biochem* **54**:699–727, 1985.

8. Soulagés JL, Wells MA, Lipophorin — the structure of an insect lipoprotein and its role in lipid transport in insects, *Adv Protein Chem* **45**:371–415, 1994.

9. Zdybicka-Barabas A, Cytrynska M, Apolipophorins and insects immune response, *Isy-Invertebrate Survive J* **10**(1):58–68, 2013.

10. Weers, PMM, Ryan RO, Apolipophorin III: Role model apolipoprotein, *Insect Biochem Mol Biol* **36**(4):231–240, 2006.

11. Narayanaswami V, Kiss RS, Weers PMMM, The helix bundle: A reversible lipid binding motif, *Comparative Biochem Physiol A Molecular Integrative Physiol* **155**(2):123–133, 2010.

12. Law JH, Ribeiro JMC, Wells MA, Biochemical insights derived from insect diversity, *Annual Rev Biochem* **61**:87–111, 1992.

13. Law JH, Wells MA, Insects as biochemical models, *J Biol Chem* **264**(28):16335–16338, 1989.

14. Canavoso LE et al., Fat metabolism in insects, *Annual Rev Nutrition*, **21**:23–46, 2001.

15. Arrese EL, Soulagés JL, Insect fat body: Energy, metabolism, and regulation, *Annual Rev Entomol* **55**:207–225, 2010.

16. Mirheydari M, Mann EK, Kooijman EE, Interaction of a model apolipoprotein, apoLp-III, with an oil-phospholipid interface, *Biochim Et Biophys Acta-Biomembranes*, **1860**(2):396–406, 2018.

17. Rathnayake SS et al., Insertion of apoLp-III into a lipid monolayer is more favorable for saturated, more ordered, acyl-chains, *Biochim Et Biophys Acta-Biomembranes*, **1838**(1):482–492, 2014.

18. Breiter DR et al., Molecular structure of an apolipoprotein determined at 2.5 Å resolution, *Biochemistry*, **30**(3):603–608, 1991.

19. Wang J, Sykes BD, Ryan RO, Structural basis for the conformational adaptability of apolipophorin III, a helix-bundle exchangeable apolipoprotein, *Proc Natl Acad Sci USA*, **99**(3):1188–1193, 2002.

20. Fan DP et al., NMR solution structure and dynamics of an exchangeable apolipoprotein, Locusta migratoria apolipophorin III, *J Biol Chem* **278**(23):21212–21220, 2003.

21. Ryan RO, Oikawa K, Kay C, Conformational, thermodynamic, and stability properties of Manduca sexta apolipophorin III, *J Biol Chem* **268**(3):1525–1530, 1993.

22. Weers PMM, Ryan RA, Apolipophorin III: A lipid-triggered molecular switch, *Insect Biochem Mol Biol* **33**(12):1249–1260, 2003.

23. Wientzek M et al., Binding of insect apolipophorin III to dimyristoylphosphatidylcholine vesicles. Evidence for a conformational change, *J Biol Chem* **269**(6):4605–4612, 1994.

24. Wells MA et al., The role of apolipophorin III in *in vivo* lepopterin interconversions in adult Manduca sexta, *J Biol Chem* **262**(9):4172–4176, 1987.

25. Kawooya JK et al., Lipophorin structure analyzed by in vitro treatment with lipases, *J Lipid Res* **32**(11):1781–1788, 1991.
26. Saito H et al., Lipid binding-induced conformational change in human apolipoprotein E. Evidence for two lipid-bound states on spherical particles, J Biol Chem 276(44):40949–40954, 2001.
27. Hickenbottom SJ et al., Structure of a lipid droplet protein: The PAT family member TIP47, Structure, 12(7):1199–1207, 2004.
28. Gimenez-Andres M, Copic A, Antonny B, The many faces of amphipathic helices, Bio- molecules, 8(3):14, 2018.
29. Itabe H et al., Perilipins: A diversity of intracellular lipid droplet proteins, Lipids Health Dis 16:11, 2017.
30. Wan CPL et al., Apolipoprotein-induced conversion of phosphatidylcholine bilayer vesicles into nanodisks, Biochim Et Biophys Acta Biomembranes, 1808(3):606–613, 2011.
31. Weers PMM et al., A laboratory exercise to illustrate protein-membrane interactions, Biochem Mol Biol Edu 44(1):86–94, 2016.
32. Nath A, Atkins WM, Sligar SG, Applications of phospholipid bilayer nanodiscs in the study of membranes and membrane proteins, Biochemistry, 46(8):2059–2069, 2007.
33. Mizuguchi C et al., Fluorescence analysis of the lipid binding-induced conformational change of apolipoprotein E4, Biochemistry, 51(28):5580–5588, 2012.
34. Fukuda M et al., Conformational change of apolipoprotein A-I and HDL formation from model membranes under intracellular acidic conditions, J Lipid Res, 49(11):2419–2426, 2008.
35. Wang SX, Sun YT, Sui SF, Membrane-induced conformational change in human apolipoprotein H, Biochem J 348 Pt 1:103–106, 2000.
36. Sahoo D et al., Lipid-triggered conformational switch of apolipophorin III helix bundle to an extended helix organization, J Mol Biol 321(2):201–214, 2002.
37. Chetty PS et al., Role of helices and loops in the ability of apolipophorin-III to interact with native lipoproteins and form discoidal lipoprotein complexes, Biochemistry, 42(51):15061–15067, 2003.
38. Soulages JL, Arrese EL, Interaction of the alpha-helices of apolipoporphin III with the phospholipid acyl chains in discoidal lipoprotein particles: A fluorescence quenching study, Biochemistry, 40(47):14279–14290, 2001.
39. Soulages JL et al., Essential role of the conformational flexibility of helices 1 and 5 on the lipid binding activity of apolipoporphin-III, J Biol Chem 276(36):34162–34166, 2001.
40. Soulages JL, Arrese EL, Dynamics and hydration of the alpha-helices of apolipoporphin III, J Biol Chem 275(23):17501–17509, 2000.
41. Raussens V et al., Alignment of the apolipophorin-III alpha-helices in complex with dimyristoylphosphatidylcholine. A unique spatial orientation, J Biol Chem 270(21):12542–12547, 1995.
42. Wang Y et al., Implementation of accelerated molecular dynamics in NAMD, Comput Sci Discov 4(1):015002, 2011.
43. Phillips JC et al., Scalable molecular dynamics with NAMD, J Comput Chem 26(16):1781–1802, 2005.
44. Wijeratne TU, Weers PMM, Lipid-bound apoLp-III is less effective in binding to lipopolysaccharides and phosphatidylglycerol vesicles compared to the lipid-free protein, Mol Cell Biochem 458(1–2):61–70, 2019.
45. Leon LJ et al., Apolipoporphin III: Lipopolysaccharide binding requires helix bundle opening, Biochem Biophys Res Commun 348(4):1328–1333, 2006.
46. Dwivedi P et al., Deletion of the N- or C-terminal helix of apolipoporphin III to create a four-helix bundle protein, Biochemistry, 55(26):3607–3615, 2016.
47. Narayanaswami V et al., A molecular trigger of lipid binding-induced opening of a helix bundle exchangeable apolipoprotein, Proc Natl Acad Sci USA, 96(8):4366–4371, 1999.
pH-dependent interactions of Apolipophorin-III with a lipid disk

48. Pettersen EF et al., UCSF Chimera—a visualization system for exploratory research and analysis, J Comput Chem 25(13):1605–1612, 2004.
49. Humphrey W, Dalke A, Schulten K, VMD: Visual molecular dynamics, J Mol Graph, 14(1):33–38, 27–28, 1996.
50. Li C et al., DelPhi Suite: New developments and review of functionalities, J Comput Chem 40(28):2502–2508, 2019.
51. Li L et al., DelPhi: A comprehensive suite for DelPhi software and associated resources, BMC Biophys 5:9, 2012.
52. Wang L, Li L, Alexov E, pKa predictions for proteins, RNAs, and DNAs with the Gaussian dielectric function using DelPhi pKa, Proteins, 83(12):2186–2197, 2015.
53. Pahari S et al., DelPhiPKa: Including salt in the calculations and enabling polar residues to titrate, Proteins, 86(12):1277–1283, 2018.
54. Huang J et al., CHARMM36m: An improved force field for folded and intrinsically disordered proteins, Nat Meth 14(1):71–73, 2017.
55. Ryan RO, Oikawa K, Kay CM, Conformational, thermodynamic, and stability properties of Manduca sexta apolipophorin III, J Biol Chem 268(3):1525–1530, 1993.
56. Soulages JL, Wells MA, Lipophorin: The structure of an insect lipoprotein and its role in lipid transport in insects, Adv Protein Chem 45:371–415, 1994.
57. Alexov E, Numerical calculations of the pH of maximal protein stability. The effect of the sequence composition and three-dimensional structure, Eur J Biochem 271(1):173–185, 2004.
58. Mitra RC, Zhang Z, Alexov E, In silico modeling of pH-optimum of protein-protein binding, Proteins, 79(3):925–936, 2011.
59. Talley K, Alexov E, On the pH-optimum of activity and stability of proteins, Proteins, 78(12):2699–2706, 2010.