Heme-binding by *Drosophila* retinoid- and fatty acid-binding glycoprotein (RFABG), a member of the proapolipophorin gene family

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Abstract **We previously have cloned and characterized a retinoid- and fatty acid-binding glycoprotein (RFABG) isolated from the heads of *Drosophila melanogaster*. The protein is composed of two glycosylated subunits (M_r = >200,000 and 70,000) and is a member of the proapolipophorin gene family. Spectral analysis of purified RFABG revealed an absolute absorbance peak at 405 nm, which is typical for a heme-containing protein. The aim of the present study was to characterize the heme-binding properties of RFABG. Upon saturation of the protein solution with carbon monoxide followed by dithionite reduction, a red shift of the Soret peak to 424 nm and the characteristic α- and β- bands at 567 and 539 nm were observed. Native RFABG contains approximately 0.175 moles of heme (mol/mol) indicating that purified RFABG is primarily the apoprotein. Hemin-agarose affinity chromatography of the native RFABG followed by Western blot analysis showed a single immunoreactive band at 70 kDa, indicating that the heme-binding domain resides in the 70 kDa subunit. Although retinoid and fatty acid also bind to the 70 kDa subunit, no competition was observed when an excess of heme was added to a solution of retinoid or fatty acid bound to RFABG. Heme added to a solution of purified RFABG bound in a saturable manner with an affinity of 3.8×10^{-7} M. Thus, the current study clearly demonstrates that retinoid- and fatty acid-binding glycoprotein is a novel heme-binding protein, which may be involved in the transport and/or metabolism of heme in *Drosophila*.—** Duncan, T., Y. Osawa, R. K. Kutty, G. Kutty, and B. Wiggert. **Heme-binding by *Drosophila* retinoid- and fatty acid-binding glycoprotein (RFABG), a member of the proapolipophorin gene family. *J. Lipid Res.* 1999.** 40: **1222–1228.**

Supplementary key words heme • lipophorin • *Drosophila* • RFABG

A novel retinoid- and fatty acid-binding glycoprotein (RFABG) from *Drosophila melanogaster* has been isolated and characterized in our laboratory (1, 2). RFABG consists of two glycosylated protein subunits with apparent molecular masses of >200 kDa and ~70 kDa. RFABG binds all-*trans* retinol and 16-[9-anthroyloxy] palmitic acid with high affinity (K'_d of 2.9×10^{-7} m and 3.5×10^{-7} m, respectively). Furthermore, the binding of retinoid and fatty acid was associated with the 70 kDa subunit of the protein (1). A gene encoding a 3351 amino acid protein that could serve as the precursor of the 70 and 200 kDa polypeptides associated with RFABG has been cloned and localized to the 102F region of *Drosophila* chromosome 4 (2). The gene encodes a >10 kb mRNA and is expressed in the amnioserosa, fat body, and apodemes of the developing *Drosophila* embryo. RFABG has also been localized by immunocytochemistry in the adult *Drosophila* compound eye, specifically the Semper (cone) cells and the intraommatidial matrix (3). The molecular evidence indicates that RFABG is likely an extracellular matrix protein belonging to the proapolipophorin gene family that could play an important role in the transport of hydrophobic molecules in *Drosophila*.

During purification of RFABG, the concentrated protein solution appeared to have a brownish cast that remained even after extensive dialysis. Upon further examination of the spectral properties of freshly purified RFABG, an absorbance peak at 405 nm was noted. A comparison of visible spectra of RFABG with those of heme-binding proteins such as myoglobin revealed that the absorbance at 405 nm could be due to the presence of heme, raising the possibility that RFABG, given its ability to bind hydrophobic ligands, could also bind heme. In the current study, evidence is presented that *Drosophila* RFABG is indeed a heme-binding protein.

Abbreviations: RFABG, retinoid- and fatty acid-binding glycoprotein; kDa, kilodalton; K'_d, dissociation constant; Con-A, concanavalin A; kb, kilobase; FPLC, fast protein liquid chromatography; RP-HPLC, reversed-phase high performance liquid chromatography; TFA, trifluoroacetic acid; CO, carbon monoxide; HIC, hydrophobic interaction chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; RPE, retinal pigment epithelium; e_o, total concentration of binding sites.

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MATERIALS AND METHODS

Preparation of RFABG and anti-RFABG antibody

Soluble protein from the heads of *Drosophila melanogaster* was subjected to concanavalin A–Sepharose 4B (Con-A) affinity chromatography followed by Superose-6 size-exclusion fast-protein liquid chromatography (FPLC) as previously described (1). Experiments were performed using the Con-A eluate and/or the FPLC-purified RFABG protein. Antibodies raised in rabbits against native RFABG (FPLC purified) and also against a peptide sequence specific to the N-terminal 70 kDa protein band (2) were used for immunoblotting.

Heme content of RFABG

The heme content of RFABG was determined using reversed-phase high performance liquid chromatography (RP-HPLC) based on the method of Osawa et al. (4). The Millennium 2010 (v2.10) Chromatography Manager (Waters) was used for instrument control, data acquisition, and data processing. Briefly, an aliquot of FPLC-purified RFABG (144 pmol total protein) was injected onto a reversed-phase C4 column (4.6 × 250 mm, Vydac) which had been pre-equilibrated with initial mobile phase. The initial mobile phase consisted of 75% solvent A (0.05% trifluoroacetic acid, TFA) and 25% solvent B (0.05% TFA in 70% aqueous acetonitrile) at a flow rate of 1 ml/min. Five min after injection, solvent B was increased linearly at a rate of 2.5% per min for 30 min. Under these conditions, any heme that is present in RFABG will dissociate from the protein and will elute as a single peak. For qualitative analysis of heme, absorbance spectra were acquired for chromatographic peaks of interest from 220–450 nm using an online photodiode-array detector. These spectra were used for peak identification and peak purity testing by comparing them against the spectral properties of a heme standard. For these experiments, myoglobin (type III, Sigma) was used as a reference standard. For quantitative analysis of heme, an external standard method was used. A calibration curve based on peak area was generated using the myoglobin (type III, Sigma) standard. Absorbance was monitored at 405 and 220 nm for detection of heme and protein, respectively. The amount of heme in RFABG was determined by integrating the peak area and by applying linear least-squares regression.

Absorbance and difference spectra of carbon monoxide-treated RFABG

Absorbance and difference spectra of FPLC purified RFABG were measured in a DW-2000 UV-VIS spectrophotometer (SLM-AMINCO) using matched cuvettes with a 1 cm optical path. All spectrophotometric measurements were made at room temperature (22–25°C) immediately after each treatment. Absorbance sprectra were obtained by placing a solution of RFABG (~0.1 mg in Tris-buffered saline, TBS; pH 7.4) in the sample cuvette and TBS in the reference cuvette. After recording the baseline, carbon monoxide (CO) was carefully bubbled through the RFABG in the sample cuvette until saturated. Reduction of the CO-saturated RFABG to produce carbonmonoxy RFABG (CO-RFABG) was achieved by the addition of solid sodium dithionite. Difference spectra were also obtained in which the sequence of treatment was reversed, that is, dithionite reduction of RFABG producing the deoxy form of RFABG preceded CO saturation.

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) of the Con-A eluate and the FPLC-purified protein was performed using a Hydropore-5 HIC column (4.6 × 100 mm; Rainin Instrument Co.) (1). Protein was eluted using a 30-min linear gradient from 0 to 100% buffer B. The gradient was initiated 5 min after injection at a flow rate of 1 ml/min (buffer A, 3 m ammonium sulfate in buffer B; buffer B, 0.1 m potassium phosphate, pH 7.0). Absorbance was monitored from 220 to 450 nm using a photodiode-array detector and spectral data for peaks absorbing at 280 nm were obtained. Fractions (0.3 ml) were collected. Aliquots were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting as described below. Fractions that were immunopositive to the 70 kDa peptide antibody and that also had an absorbance ~405 nm were subjected to hemin–agarose affinity chromatography.

Hemin–agarose affinity chromatography

Hemin–agarose affinity resin is specific for the binding of heme-binding proteins (5, 6). Hemin–agarose affinity columns were made by adding a slurry (1 ml) of hemin-agarose (Sigma) containing 7.7 μmol hemin per ml of packed agarose to a 2 ml polystyrene column. To prevent nonspecific ionic interaction between protein and the affinity resin, binding was carried out in high-ionic-strength buffer, pH 7.5. Selected HIC fractions were diluted in 10 mm sodium phosphate buffer containing 0.5 m NaCl, pH 7.5 (NP buffer) then applied to the hemin–agarose affinity columns. Then, columns were washed extensively with NP buffer. Bound protein was eluted using 1% SDS in NP buffer. Fractions of the flow through (11 × 0.5 ml), column wash (25 × 0.5 ml), and eluate (24 × 0.5 ml) were collected and aliquots from each fraction were subjected to SDS-PAGE and immunoblot analysis. Similar analysis was also performed using FPLC purified RFABG.

Western blot analysis

Fractions from HIC-HPLC and hemin–agarose affinity chromatography were subjected to SDS-PAGE using 10–20% Tricine gels (Novex) (7) then protein was transferred to a nitrocellulose membrane (Novex) (8). The membrane was blocked with 5% Carnation nonfat dry milk in TBS followed by overnight incubation at 4°C with antiserum (2). The blot was washed with TBS then incubated with an alkaline phosphatase-conjugated goat $F(ab')_2$ fragment anti-rabbit IgG (Cappel). The blot was washed again with TBS and the immunoreactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Binding studies

Heme binding to RFABG was determined by difference absorption spectroscopy according to the method described by Vincent, and Muller-Eberhard (9). Hematin stock solutions (1.1 mm) were freshly prepared by dissolving 1 mg hematin in 50 μl 1 N sodium hydroxide, then bringing the solution to volume with 50 mm sodium phosphate buffer, pH 7.5. Titration experiments were performed in matched cuvettes by adding an aliquot of hematin stock solution to the sample cuvette containing 1.4 μm RFABG. An identical aliquot of hematin was added to the reference cuvette, which contained buffer alone. The absorption spectrum was scanned from 250 to 500 nm using a DW-2000 UV-VIS spectrophotometer (SLM-AMINCO) and the increase in absorbance at 412 nm was calculated from the difference spectrum.

Competitive binding between heme, retinol, and palmitic acid for RFABG was determined. Briefly, tubes containing [11,12-^{3}H(N)] all-*trans* retinol (0.2 μm) bound to RFABG or [9,10-^{3}H] palmitic acid (0.2 μm) bound to RFABG were incubated 1 h in the dark at room temperature in the presence or absence of excess heme. After incubation, samples were subjected to Superose-6 size-exclusion FPLC. Fractions (0.3 ml) were collected then analyzed by liquid scintillation spectrometry. In similar experiments, the ability of palmitic acid to displace [11,12-^{3}H(N)] all-*trans* retinol (0.2 μm) bound to RFABG was examined. Competitive binding was also studied spectrophotometrically as described

by Vincent et al. (9). In these competitive binding studies, the change in absorbance of the RFABG·heme complex at 412 nm was measured after addition of increasing amounts of oleic acid.

RESULTS

Presence of heme in native RFABG

The heme content in native RFABG (FPLC purified) was determined by RP-HPLC. Due to the acidic nature of the mobile phase, the heme dissociates (4) from the RFABG protein and elutes as a single peak with a retention time of 28.6 min (**Fig. 1**), identical to that of heme from myoglobin. The peak apex spectral data associated with this peak matched that of the heme present in the myoglobin standard (Fig. 1, inset) confirming the presence of heme in RFABG. Quantitatively, the FPLC-purified RFABG contains approximately 0.175 moles of heme (mol/mol).

The absorption spectra obtained for the FPLC-purified RFABG are characteristic of other heme-containing proteins. For example, the absorption maxima for dithionite reduced (deoxy-RFABG) and CO-RFABG closely resembles that of myoglobin and hemoglobin (**Table 1**). Spectra from RFABG and CO saturated RFABG (**Fig. 2A–B**, respectively) show an absorbance maximum in the Soret region at 405 nm. It is apparent from Fig. 2B that CO does not bind to purified RFABG as no shift in the absorbance maximum was observed. Figure 2C represents the carbonmonoxy form of RFABG (CO-RFABG); it is the difference between the spectrum of dithionite-reduced CO-saturated RFABG and the spectrum of CO-saturated RFABG. Dithionite reduction of the CO-saturated RFABG resulted in a red shift in the Soret peak to 424 nm (Fig. 2C) and produced the characteristic α- and β-bands at 567 and 539 nm, respectively (Fig. 2C, inset). In similar experiments, dithionite reduction of RFABG prior to CO saturation producing deoxy-RFABG resulted in a stable Soret peak at 435 nm (Fig. 2D) and a broad α–band at 562 nm (Fig. 2D, inset).

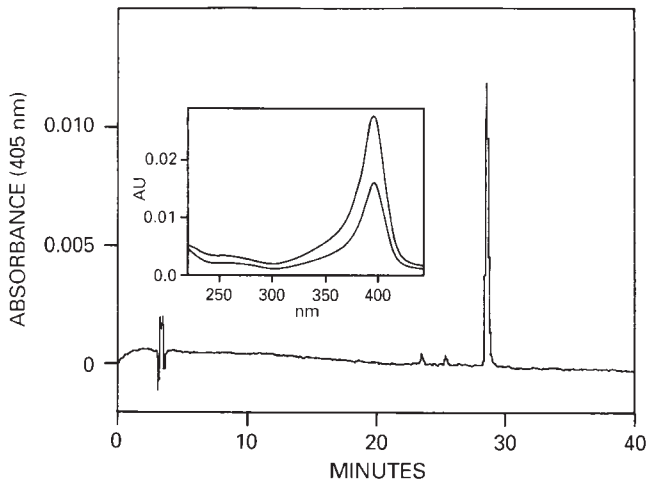

Fig. 1. Reversed-phase high performance liquid chromatography of RFABG. Purified RFABG was analyzed by RP-HPLC on a C4 column (4.6 × 250 mm) under acidic conditions. Inset: Peak-apex spectrum of the heme peak from RFABG matched against the peak-apex spectrum of the heme peak from myoglobin.

TABLE 1. Absorption maxima of myoglobin, hemoglobin, and RFABG derivatives

Derivative	α-Band	β-Band	Soret
		nm	
Deoxy-Mb[a]	556	—	434
Deoxy-Hb[b]	555	—	430
Deoxy-RFABG	562	—	435
CO-Mb	579	542	423
CO-Hb	569	539	419
CO-RFABG	567	539	424

[a] Values for myoglobin (Mb) are taken from Rothgeb, T. M., and F. R. N. Gurd. 1978. Physical methods for the study of myoglobin. *Methods Enzymol.* **52:** 473–486.

[b] Values for hemoglobin (Hb) are taken from Waterman, M. R. 1978. Spectral characteristics of human hemoglobin and its derivatives. *Methods Enzymol.* **52:** 456–463.

Heme binds to the 70 kDa subunit of RFABG

Purification of the Con-A eluate under conditions where the heme moiety remained bound to RFABG was accomplished using hydrophobic interaction chromatography. Spectral analysis of the major 280 nm absorbing peak revealed an absorbance maximum at 405 nm suggesting the presence of heme. To further test the possibility that RFABG was a heme-binding protein, this peak was subjected to a hemin–agarose affinity column. Western blot analysis of the hemin–agarose affinity column eluate revealed a single 70 kDa band immunoreactive to the antibody prepared against a peptide sequence specific to the N-terminal 70 kDa protein band. The 70 kDa subunit eluted as a peak and was collected into fractions 7–24. **Figure 3B** shows only the peak fractions of the eluate (i.e., fractions 8–11 in lanes 2–5, respectively). Analysis of the flow through (Fig. 3A) and column wash showed no immunoreactivity to the peptide antibody. Similar observations were made when blots were probed with antiserum prepared against native RFABG.

Interaction of RFABG with heme

Binding of hematin to RFABG was determined by difference absorption spectroscopy. Upon addition of hematin to purified RFABG, a marked increase in the absorbance at 412 nm was observed (**Fig. 4**). Furthermore, these data show that hematin binds to RFABG in a saturable manner. By plotting the data in linear form (Fig. 4, inset) the apparent dissociation constant (K'_d) of the RFABG·hematin complex and the concentration of binding sites (e_o) for hematin can be measured (10). Comparison of the value for e_o with the molarity of RFABG yields the number of binding sites (n) per RFABG molecule. From this line, the K'_d is 3.8×10^{-7} m and the total concentration of binding sites for hematin is equal to 8.18 μm. Therefore, the calculated number of binding sites is 5.84 per RFABG molecule.

Competitive binding studies were performed to determine whether heme could influence the binding of [11,12-^{3}H(N)] all-*trans* retinol or [9,10-^{3}H] palmitic acid to RFABG. The data in **Fig. 5A** and **B** show the binding of [11,12-

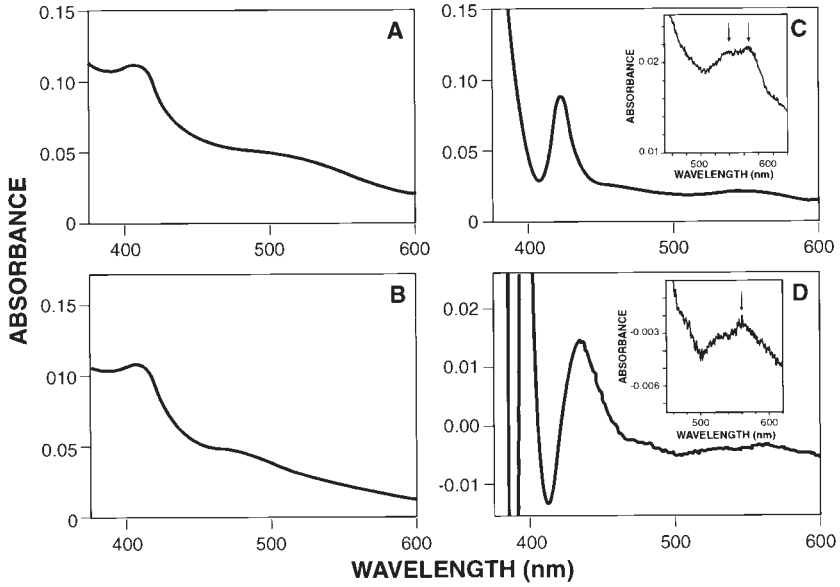

Fig. 2. Absorbance scans and carbon monoxide difference spectra of RFABG. Absorbance scan of FPLC purified RFABG (A). Absorbance scan of carbon monoxide saturated RFABG (B). Absorbance scan of carbonmonoxy RFABG (CO-RFABG) (C). Difference spectra of deoxy-RFABG (D). Insets: Spectral properties for carbonmonoxy- and deoxy-RFABG. Expanded view of the carbonmonoxy-RFABG absorbance spectrum (C, inset). The arrows indicate the α-band (567 nm) and β-band (539 nm), respectively for CO-RFABG. Expanded view of the deoxy-RFABG difference spectrum (D, inset). The arrow indicates the absorbance maximum at 562 nm of the broad α-band for deoxy-RFABG. All samples were in 10 mm Tris-buffered saline, pH 7.4 and at room temperature (~22–25°C). For absorbance spectra, the reference cuvette contained Tris-buffered saline. Difference spectra were obtained by addition of identical aliquots of RFABG to sample and reference cuvettes, measurement of the baseline, addition of dithionite to the sample cuvette, and measurement of the spectrum. The carbonmonoxy spectrum was obtained by reduction of carbon monoxide-saturated RFABG using sodium dithionite. The deoxy spectrum was obtained by addition of a few crystals of sodium dithionite to freshly purified RFABG.

^{3}H(N)] all-*trans* retinol and [9,10-^{3}H] palmitic acid to RFABG, respectively, in the absence of heme (solid circles). Addition of a 100-fold excess of heme did not compete (open circles) with either retinol or palmitic acid binding

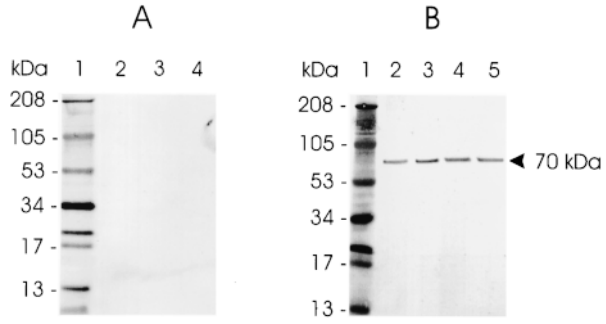

Fig. 3. Western blot analysis of the hemin–agarose affinity-purified RFABG. Hydrophobic interaction chromatography was used to purify RFABG under conditions where the heme, if present, would remain bound to the protein. Spectral analysis of the major 280 nm peak revealed an absorbance at 405 nm suggesting the presence of heme. This peak was applied to a hemin–agarose affinity column. Flow through, column wash, and eluate fractions were collected and aliquots of each fraction were subjected to SDS-PAGE and Western blot analysis. Panel A shows the flow through fractions 1–3 (lanes 2–4, respectively). Panel B shows eluate fractions 8–11 (lanes 2–5, respectively). A single 70 kDa immunoreactive band is present in the eluate fractions.

(Fig. 5A-B, respectively). In a similar experiment the ability of oleic acid to displace bound heme was examined spectrophotometrically. Upon addition of increasing amounts of oleic acid (⩾10-fold excess) to the RFABG·heme complex, no change in absorbance at 412 nm was observed (data not shown). The influence of palmitic acid on the binding of [11,12-^{3}H(N)] all-*trans* retinol to RFABG was also investigated. Figure 5C shows the binding of [11,12-^{3}H(N)] all-*trans* retinol to RFABG in the absence (solid circles) and presence (open circles) of a 50-fold excess unlabeled palmitic acid. These data indicate that unlabeled palmitic acid was unable to displace bound [11,12-^{3}H(N)] all-*trans* retinol. The reverse was also found to be true, that is, all-*trans* retinol could not displace bound [9,10-^{3}H]palmitic acid (data not shown). In summary, the competitive binding data indicate that binding of heme, fatty acid, and all-*trans* retinol are independent of each other suggesting that these ligands bind at different sites on the 70 kDa subunit of RFABG.

DISCUSSION

Heme serves as a prosthetic group for many proteins. These heme proteins play important roles in electron transfer reactions, in oxygen transport and storage, in removal of peroxides, and in oxidation of a variety of different compounds including steroids and fatty acids. Heme also

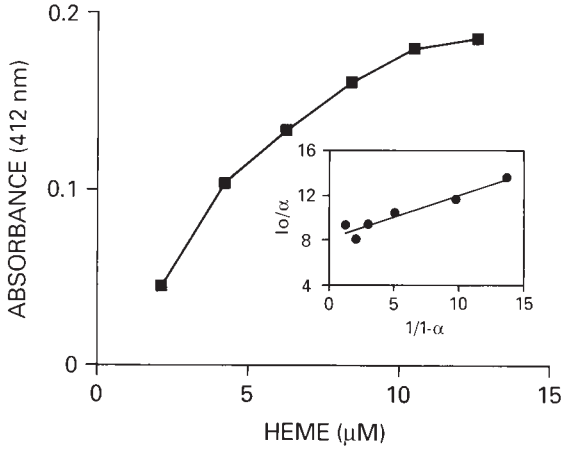

Fig. 4. Spectroscopic titration of purified RFABG with hematin. Purified RFABG was titrated with hematin and the change in absorbance at the Soret band (412 nm) was measured. Data shown are representative of three separate experiments. Inset: Spectroscopic titration data plotted in linear form according to the equation $l_o/\alpha = [K_d/(1-\alpha)] + e_o$ (10) where l_o is the concentration of added ligand, α, the fraction of sites occupied by heme, is given by $\alpha = \Delta/\Delta_{max}$ where Δ is the optical change measured. By plotting l_o/α versus $1/(1-\alpha)$ the K_d (slope) and e_o (intercept) can be measured.

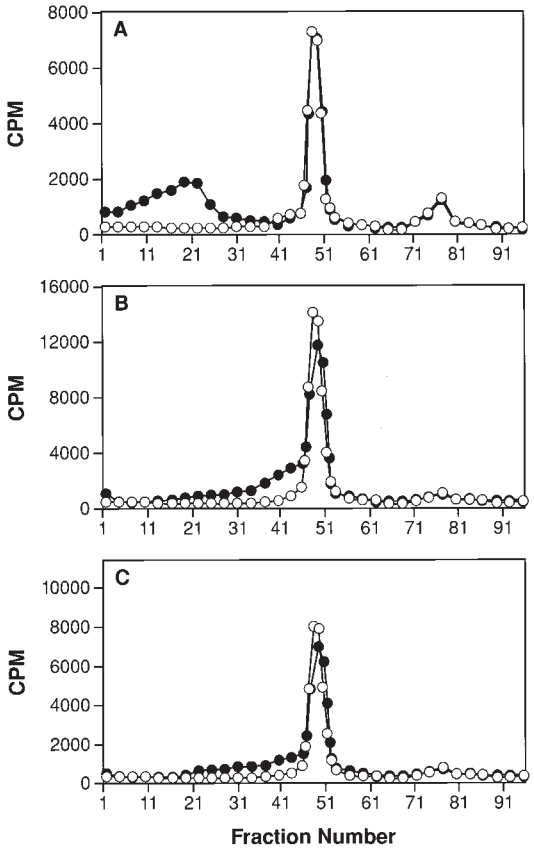

Fig. 5. Competitive binding to RFABG between heme, retinoid, and fatty acid. Tubes containing (A) [11,12-^{3}H(N)] all-*trans* retinol (0.2 μm) bound to RFABG or (B) [9,10-^{3}H] palmitic acid (0.2 μm) bound to RFABG were incubated 1 h in the dark at room temperature in the presence (○) or absence (●) of a 100-fold excess unlabeled heme. In (C), [11,12-^{3}H(N)] all-*trans* retinol (0.2 μm) bound to RFABG was incubated in the presence (○) or absence (●) of a 50-fold excess of unlabeled palmitic acid. After incubation, samples were subjected to Superose-6 size-exclusion FPLC. Fractions (0.3 ml) were collected then analyzed by liquid scintillation spectrometry.

serves as a regulatory molecule in many biological processes including protein synthesis (11–13), cellular growth, and cellular differentiation (14, 15). A specific example of heme functioning as a regulatory molecule is in the activation of soluble guanylate cyclase by nitric oxide (16, 17). That is, nitric oxide binds directly to the heme moiety of soluble guanylate cyclase, which is necessary for enzyme activation. More recently heme has been shown to regulate gene transcription and translation by enabling the binding of certain transcriptional factors to DNA. For example, HAP1, a transcriptional activator, forms a high molecular weight complex with a cellular repressive factor in the absence of heme (18, 19). The binding of heme to HAP1 causes dissociation of the cellular repressive factor enabling HAP1 to bind to DNA and activate transcription (20). Although it is not yet clear what function heme serves when bound to RFABG, the current study clearly demonstrates that RFABG is a heme-binding protein. Based on some of the known molecular and biochemical properties of RFABG possible functions will be discussed.

The presence of heme in purified RFABG is supported by several experimental parameters. The first line of evidence is that RFABG, like other heme-binding proteins, has an absolute absorbance maximum at 405 nm when analyzed at neutral pH (Fig. 2A). Heme was detected in RFABG using RP-HPLC (Fig.1). This is confirmed by the peak-apex spectrum shown in Fig. 1 inset. Here the peak-apex spectrum for the heme peak in RFABG is matched against that of heme from myoglobin. Further evidence for the presence of heme in RFABG is provided by the formation of a reduced CO difference spectrum. No spectral changes were observed upon CO treatment of RFABG indicating that the heme present on the purified protein was likely in its oxidized form (Fig. 2B). After dithionite reduction of the CO-saturated RFABG, a shift in the Soret peak to 424 nm (Fig. 2C) was noted. Although the spectra for RFABG are similar to that of myoglobin and hemoglobin (Table 1), one important difference is that the difference spectrum for deoxy-RFABG resulted in a stable (>30 min) Soret peak at 435 nm (Fig. 2D).

Previous work from our laboratory has shown that *Drosophila* RFABG consists of two major glycosylated polypeptides (M_r = >200,000 and ~70,000) (2). In addition, the ligand-binding domain, particularly for fatty acid and retinoid, appears to reside in the N-terminal 70 kDa polypeptide of RFABG (1, 2). In this study, hemin–agarose affinity chromatography of RFABG shows that the heme-binding domain also resides in the 70 kDa subunit (Fig. 3). Although the exact location of the ligand-binding domain within the 70 kDa subunit has not been determined, our competitive binding studies suggest separate binding sites for heme, retinoid, and fatty acid (Fig. 5).

Drosophila RFABG is capable of binding exogenous

heme in a saturable manner (Fig. 4) indicating that the purified protein is primarily the apoprotein. In fact, the FPLC-purified RFABG was found to be only 17.5% saturated with heme. Titration of RFABG with hematin yielded a K'_d of 3.8×10^{-7} m which is similar to that for fatty acid and retinoid binding to RFABG (1). The binding affinity of heme for RFABG is also similar to that of heme for other proteins involved in heme transport (9, 21, 22).

At the present time, we know little about the physiological role served by RFABG. It is possible that RFABG may be involved in the management of heme in *Drosophila* by binding heme and delivering it to other tissues or components present in hemolymph. It is well known that management of heme, especially free heme, is critical in biological systems. This is based on the fact that heme is a very potent oxidant capable of inducing oxidative damage to various vascular components (e.g., plasma low density lipoprotein) and other tissues. Several proteins have been identified that bind heme with high affinity and serve to protect against heme-induced oxidative damage. Recently, Oliveira et al. (23) isolated and characterized a 15 kDa protein (RHBP) from *Rhodnius prolixus* which has antioxidant activity and may also be involved in heme transport (24).

Previous work in our laboratory (1, 2) provides evidence suggesting that RFABG is a member of the lipophorin gene family. Lipophorins are high density lipoproteins present in the hemolymph of insects and, in general, are involved in the tissue-specific distribution of hydrocarbons, and other lipophilic molecules including sterols, juvenile hormones, and even carotenoids (25–31). Lipophorins are composed of two relatively large non-exchangeable apoproteins known as apolipophorin I (230–250 kDa) and apolipophorin II (70–85 kDa) and a complement of neutral and polar lipids, mostly diacylglycerols and phospholipids. In comparison to lipophorin, *Drosophila* RFABG also consists of two large glycosylated polypeptides resembling that of apolipophorin I and apolipophorin II (2). Recent evidence suggests that lipophorin could mediate the transfer of electrons to oxygen resulting in superoxide anion production (32). By inference, RFABG may also participate in the generation of reactive oxygen species.

To our knowledge RFABG is distinct from other known heme-binding proteins isolated from insects. *Drosophila* RFABG is an extracellular protein capable of binding various fatty acids, retinoids, and now we know that it binds heme. It is reasonable to postulate that RFABG may be involved in the management of heme in *Drosophila*.

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