The Ionization and Distribution Behavior of Oleic Acid in Chylomicrons and Chylomicron-like Emulsion Particles and the Influence of Serum Albumin*

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A reproducible, fairly narrow-sized population of rat lymph chylomicrons, ~100 nm, was isolated by centrifugation and combined with low levels of [1-13C]oleic acid for NMR studies. The carboxyl chemical shift was monitored as a function of aqueous pH to characterize the ionization behavior of the fatty acid in these particles. The titration curves were very similar to those for oleic acid in equivalent-sized emulsion particles composed of egg phosphatidylcholine and triolein. A simple partition-ionization model was fitted to the data to derive values for apparent ionization constant, expressed as pKapp, of 7.4-7.5 and the "true" surface to core partition coefficient of approximately 7 for oleic acid in chylomicrons. The fatty acid in chylomicrons thus appeared to be largely associated with the surface regions of these particles. Addition of bovine serum albumin to the samples showed that near physiologic pH much of the fatty acid was bound to the albumin at fatty acid to albumin-binding stoichiometries as high as 5:1 and with mass ratios of >2 in favor of the lipid or lipoprotein particles. Lowering the pH of the medium shifted the distribution of fatty acid away from albumin so that at pH 5 the emulsion, virtually all the fatty acid was associated with the lipid. The behavior observed under physiologic conditions is consistent with the rapid clearance and redistribution of fatty acid generated in these particles by lipolytic processes. However, under conditions of severe acidosis, hyperlipidemia, and hypoalbuminemia a significant portion of fatty acids might be retained in triglyceride-rich lipoproteins and their remnants and affect subsequent metabolism.

To gain some insights into the factors important in fatty acid transport processes, we attempt to characterize here the physical behavior of these species at the source of their systemic generation: in triglyceride-rich lipoprotein particles. The 13C NMR technique has been successfully applied in this laboratory to describe the ionization properties of fatty acids in aqueous systems (3) and the interactions of fatty acids with protein (4) and lipid (5, 6) moieties. This technique is utilized in the current study to detail the distribution and ionization behavior of [1-13C]oleic acid incorporated within native chylomicron particles.

Synthetic lipid emulsions have been extensively here (7-10) and elsewhere (11-13) as models of lipoprotein particles. Miller and Small (14) employed these systems for intensive compositional analyses of complex lipid mixtures, relevant to a wide range of lipoproteins. In the current work we examine how closely the ionization and distribution of fatty acid in chylomicrons might be mimicked using a simple emulsion composed of phosphatidylcholine and triolein.

A major obstacle to achieving the above objectives is the size variability within and between samples of the native and synthetic lipid dispersions, as well as their generally poor integrity for controlled studies. Preliminary efforts in this work were therefore concerned with deriving a reproducible and stable population of particles, that are sufficiently well-defined to allow some quantitative interpretation of the data.

Since most fatty acid in normal plasma is bound to serum albumin (15), much importance has been attached to the binding process (15, 16). However, the ability of serum albumin to effect the clearance of lipolytically generated fatty acid from triglyceride-rich lipoproteins has been questioned (17), and this issue is addressed in the final part of the current study.

The observations show that chylomicron fatty acid exhibits a marked preference for the surface regions of the particles and that under normal conditions serum albumin can remove fatty acid efficiently from triglyceride-rich lipoproteins.

MATERIALS AND METHODS

Triolein was obtained from Nu Chek Prep, Inc. (Elysian, MN) and egg yolk phosphatidylcholine (Grade I) from Lipid Products (South Nutfield, England). These lipids, used in emulsion preparation, were confirmed to be >99% pure by thin layer chromatography. Oleic acid, isotopically substituted to 90% 13C in position-1 (Merck) was purified by extraction from a solution in benzene/chloroform/methanol (1:0.5:1.2) with aqueous alkali (18) to eliminate non-ionizable material and back-extracted into n-hexane under acidic conditions, for isolation. The purified material was shown to be >99% pure by thin layer chromatography.
OAIE Acid Ionization in Emulsions and Chylomicrons

BSA1 specified as "essentially fatty-acid free" (Sigma) was shown to contain <0.02 mol of fatty acid/mol of albumin from previous analysis on a representative batch (4). A molecular weight of 66,000 was used for this material, and its aqueous concentration was determined from the absorbance at 279 nm using an extinction coefficient (15,000 cm^-1 M^-1)

The radioisotopes [9,10-H]tritiole and 1-palmitoyl-2-[1-14C]oleyl phosphatidylcholine were purchased from Amercham Corp.

Chylomicrons—Six male Sprague-Dawley rats (280–320 g), prepared with mesenteric lymph cannulas and gastric injection cannulas, were infused with an isotonic saline solution at 2.7 ml h^-1 for 24 h after surgery. Each then received 1 g of tritiole (practical grade, Sigma) containing ~20 µCi tritiated tritiole via the gastrostomy tube, and saline infusion was restarted. Lymph chylomicrons were collected at room temperature for 12 h in the presence of 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride to inhibit proteolysis.

Emulsions—Crude emulsions of phosphatidylcholine and tritiole were generated by a method similar to that described previously (7). Chloroform solutions containing 0.250 g of tritiole with ~0.5 µCi tritiated tritiole and 0.0625 g of egg phosphatidylcholine with ~1.25 µCi of 11C-labeled phosphatidylcholine were evaporated, and the resulting residues were stored overnight under low vacuum. The dried lipid samples were reconstituted under N2 in 9 ml of 0.16 M KCl containing 0.1 mM EDTA by sonication for 20 min at 25–30 °C using a Branson 310 Sonifier (Danbury, CT) operating at power level 4. Usually six lipid mixtures were dispersed to prepare a single sample for the studies.

Fractionation of Lipid and Chylomicron Particles—Crude chylomicron and emulsion preparations were fractionated by ultracentrifugation as follows. Aliquots of 9 ml were adjusted to a density of ~1.02 g ml^-1 with KBr and overlayed in a 2.5 × 8.3 cm ultracentrifuge tube with 0.16 M KCl containing 0.1 mM EDTA. Samples were centrifuged using an SW 28 rotor at 15,000 rpm to accumulate an a of 2.7 × 10^10 rad s^-2 (2.93 × 10^12 g s^-2), the force determined from the Stokes equation to spin-up particles >250 nm into the surface of the overlay. For these calculations, the average particle density was taken as 0.939 g cm^-3, determined for chylomicrons (20), and the overlay was assumed to have the viscosity of pure water (1.002 cp). The surface region of the overlay (~3.5 ml) was sliced from the tube and the remaining overlay (~25 ml) was transferred into a fresh tube. This suspension was adjusted to a density of 1.024 with KCl (~3.7 wt%) by addition of a 20 wt% solution and overlayed again with KC1/EDTA solution. Centrifugation was now conducted at 25,000 rpm to accumulate an a of 1.75 × 10^10 rad s^-2 or 2.11 × 10^4 g s^-2 (~45 min), a force estimated to spin-up particles of >100 nm diameter into the surface region. These fractions were sliced from the tubes and concentrated to ~300 mg of triglyceride ml^-1 by centrifugation into an overlay of the desired aqueous medium using a SW 41 rotor.

Chemical Analysis—Chylomicron samples were adjusted to their lipid compositions directly from the gel slice, as expected from the smaller size of these particles. Gel chromatography—Size estimates for fractionated lipid particles were obtained from chromatography on Sepharcl S-1000 superfine beads (Pharmacia Fine Chemicals, Upsala, Sweden) according to a procedure described for lipid vesicles (22). Gel beads were packed in

1 The abbreviation used is: BSA, bovine serum albumin.
describe a similar and fairly narrow size range for both types of particle.

The indigenous or contaminating fatty acid concentrations found in fractionated chylomicrons and emulsion, respectively, are shown in Table I. While the addition of [1-13C]oleic acid increased these amounts, total fatty acid remained less than 20 and 10 mol% with surface phospholipid in the chylomicrons and emulsion, respectively (Table I). The chylomicron sample revealed a characteristic apoprotein profile from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with major bands for apoproteins B48, A1, E, and A1. Only trace amounts of serum albumin were present in these samples.

Optical density measurements did not detect any changes in the gross physical characteristics of the fractionated samples, when exposed to conditions identical to those employed in the NMR studies. In contrast, the unfractionated samples showed poor stability under these conditions.

NMR Studies—Fig. 3 shows the entire proton-decoupled 13C NMR spectra of chylomicrons (A) and emulsion (B) containing 0.4 wt% of incorporated [1-13C]oleic acid. Differences are obvious only in the olefinic and methylene regions, where several additional peaks appear in the chylomicron spectra from polyunsaturated fatty acyl chains. The 13C-enriched carboxyl of incorporated oleic acid appears downfield from the triglyceride carbonyls (see expanded regions). Changes in the carboxyl resonance upon titration of the fatty acid are shown in Fig. 4 for both chylomicrons and emulsions. As the pH is increased, the carboxyl peak shifts downfield, broadens, and remains fairly broad at the highest pH studied. These changes are indicative of a conversion of unionized to ionized fatty acid molecules. The two forms

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**TABLE I**

Mean particle diameters and compositions of fractionated samples of chylomicrons and emulsion

<table>
<thead>
<tr>
<th>Mean diameter (nm)</th>
<th>Gel chromatography</th>
<th>Electron microscopy</th>
<th>Composition</th>
<th>Protein</th>
<th>Fatty acid</th>
<th>Phospholipid</th>
<th>Fatty acid/phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>wt%</td>
<td>wt%</td>
<td>µmol g⁻¹</td>
<td>before +[1-13C]oleic acid</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>108 ± 7</td>
<td>111 ± 9</td>
<td>102</td>
<td>1.20 ± 0.08</td>
<td>0.52 ± 0.11</td>
<td>18.6 ± 3.9</td>
<td>161 ± 7</td>
</tr>
<tr>
<td>Emulsion</td>
<td>94 ± 4</td>
<td>104 ± 5</td>
<td>96</td>
<td>1.15 ± 0.01</td>
<td>5.4 ± 0.5</td>
<td>165 ± 4</td>
<td>0.033</td>
</tr>
</tbody>
</table>

* Average and range from two samples.

* The average S.D. of particle diameter within specimens was 26 nm for both chylomicrons and the emulsion.

* Calculated as described in Ref. 11 using the above phospholipid and protein concentrations in chylomicrons and the phosphatidylycholine/triolein mole ratio of 0.171 for the emulsion at its peak elution position in Fig. 1B.

* Concentrations expressed with respect to triglyceride content.

* The fatty acid to phospholipid mole ratio before and after addition of [1-13C]oleic acid for the NMR studies.
The titrations in both chylomicrons and emulsion showed compared with... the observed chemical shift is good reversibility upon returning to pH 5.5. Further evidence that chylomicrons were unperturbed by manipulations confined to those illustrated in Fig. 4 was obtained from analyzing bulk phases from an NMR sample following titration. The aqueous phase fractionated from this sample by ultracentrifugation contained just 14% of the total protein concentration indicating that the protein compositions of the particles was not appreciably modified by oleic acid incorporation and subsequent titration.

The titration curves of oleic acid mean carboxyl chemical shift, \( \delta \), versus pH are shown in Fig. 5 to correspond closely in chylomicrons and emulsion suspended in the same aqueous system. Over much of this range, the peak is broader for oleic acid in chylomicrons, as indicated by the bars in Fig. 5. This is suggestive of slower motions or different interactions for fatty acid species in the more complex surfaces of the natural particles. However, the overall similarity in the titration plots indicates that the gross ionization behavior of oleic acid in chylomicrons may be approximated by a model in which the fatty acid partitions into the surface phospholipid region of these particles, whereupon it participates in the ionization equilibria. Mathematical derivation of such a model is outlined in the Appendix, and curves generated from the model are fitted to experimental data from chylomicron suspensions in various aqueous systems in Fig. 6.

Briefly, the modeling involves fitting to the observed behavior a single parameter that combines both \( K_{\text{app}} \), the apparent ionization constant, and \( P \), the “true” surface to core partition coefficient of the unionized fatty acid. These constants are then calculated from the fitted parameter value using an “apparent” partition coefficient of 12 \( \pm \) 2, which was determined experimentally for oleic acid at pH 7.4 in synthetic emulsions. Values are summarized in Table II with residuals for \( \delta \), expressing “goodness-of-fit.” Form I of the model equates chemical shifts for the unionized and ionized fatty acid with, respectively, the lowest and highest values measured in these experiments. This model expression achieved a good fit to titration data in unbuffered KCl solution as shown in Fig. 6. However the data from the buffered systems (“Materials and Methods”) in Fig. 6 describe a somewhat shallower curve that could not be reproduced well by the Form I expression; the best-fitting curve was very similar to that shown for the unbuffered system. In an effort to model the deviations observed in buffered systems, the chemical shift of unionized fatty acid was rigorously defined in terms of the total fatty acid concentration and contributions from surface and core components (Appendix). This Form II expression generated the curve shown in Fig. 6 which has a somewhat better fit to the data than Form I but which yielded similar values of \( K_{\text{app}} \) and \( P \) (Table II).

Whether the buffered data describe a more authentic titration behavior or reveal specific ion effects from the buffers is uncertain. However, we observed no significant change in the titration behavior of oleic acid in the emulsion when studied in either unbuffered or phosphate-buffered systems. The buffer effects would therefore appear to be a feature of the more complex chylomicron surface. While these deviations do not prohibit the application of the model, they are suggestive of more complex processes. Further evidence of a more complex process may be contained in the titration data obtained at lower pH. This region shows small but systematically lower

![Fig. 2. Electron micrographs of fractionated samples of A, chylomicrons; B, phosphatidylcholine/triolein emulsions. Bar = 250 nm.](image)
Fig. 3. Proton-decoupled $^{13}$C NMR spectra of A, chylomicrons and B, phosphatidylcholine/tri olein (PC/TO) emulsions containing [1-$^{13}$C]oleic acid (OA) (0.4 wt%) and suspended at 20 wt% in 0.12 M KCl with 0.1 mM EDTA and 0.03 M phosphate. Expanded regions show carboxyl peak for oleic acid and carbonyl peaks for triolein. Spectra were recorded at 35°C over 16,384 time domain points from 4,000 accumulations using a recycle time of 2.0 s.

Fig. 4. Carboxyl spectra recorded as a function of pH for [1-$^{13}$C]oleic acid (OA) in chylomicron (A–H) and emulsion (I–P) samples described in Fig. 3. Single spectrum for the same quantity of [1-$^{13}$C]oleic acid in the aqueous medium at pH 9.8 shown in Q. Spectra, obtained from between 4000-9000 accumulations under the conditions described for Fig. 3, are displayed over the same chemical shift range and scaled to approximately the same peak height for the triolein carbonyl (base only shown). The nontitratable peak at 173.8 ppm in spectrum J probably represents the carbonyl from egg phosphatidylcholine.
**Fig. 5.** Mean chemical shift of the oleic acid carboxyl in Fig. 4 spectra as a function of pH for the fatty acid in chylomicron (solid line) and emulsion (broken line) particles. Symbols on bars indicate the chemical shift range observed in the carboxyl peak at half-height for chylomicron (filled squares) and emulsion (open squares) samples.

**Fig. 6.** Mean carboxyl chemical shift, ~Δ, as a function of pH for oleic acid (OA) in chylomicrons suspended in various aqueous media. Curve fitted to the data from unbuffered 0.16 M KCl/0.1 mM EDTA solution (triangles) using Form I of the model expression (broken line) and using Form II (solid line) for the combined data obtained from buffering with 0.03 M phosphate (squares) and tetraborate (diamonds) in 0.12 M KCl/0.1 mM EDTA solution. Filled symbols show data used for the fitting. Parameter values from the fit used to calculate data in Table II.
chemical shifts than are provided by the best-fitting computer curves. It is possible that at low pH, core partitioning of the fatty acid is somewhat greater than predicted. Nevertheless, we fully expect our results to be applicable to the important region around physiological pH.

The pK\textsubscript{app} of 7.5–7.6 for oleic acid in the chylomicron surface is much greater than the values of 4.5 reported for aqueous solutions of monomeric carboxylic acids (3, 25, 26). The values agree well, however, with direct estimates of pK\textsubscript{app} for fatty acids in phosphatidylcholine bilayers obtained in this laboratory (7.4–7.5, Ref. 5) and by others (7.2–7.4, Ref. 27) although even higher values have been reported for phosphatidylcholine bilayers (8.4, Ref. 28). Such depressed ionization constants are reasonable when considering the extent to which dielectric properties are found to be depleted within the polar regions of phospholipid bilayers and emulsion particles (29). Because of the large proportion of the unionized species, fatty acid might realistically transfer across cell membranes in the unionized state by nonspecific mechanisms as discussed recently (1).

The preference of fatty acid for the phospholipid surface of chylomicrons, predicted here, should assist in its rapid clearance from these lipoprotein particles during triglyceride lipolysis and facilitate subsequent redistribution to endothelial cells. The possible role of albumin in this clearance and redistribution process is considered below.

Studies with BSA—\textsuperscript{13}C NMR spectra recorded for oleic acid transfer studies with BSA are shown in Figs. 7 and 8 for chylomicron and emulsion samples, respectively. Spectra of BSA with fatty acid obtained at pH 7.4 without chylomicrons or emulsion (Fig. 7A and 8A, respectively) show a prominent composite of carboxyl peaks around 180–183 ppm from protein-bound [\textsuperscript{13}C\textsubscript{oleic acid}, downfield from the broad envelope of resonances from the carbonyls of the protein backbone and similar to those previously reported for oleic acid at this stoichiometry with BSA (4). The carboxyl spectrum at pH 7.4 is not appreciably changed in the presence of chylomicrons (Fig. 7C) or emulsion (Fig. 8B). Subtracting out the protein-bound spectra reveals a small intensity depletion in the protein-bound region of the spectrum obtained with chylomicrons (Fig. 7D). Thus, a small amount of oleic acid may have transferred to the lipoprotein even though this fraction cannot be detected in the upfield region of the subtraction spectrum (Fig. 7D) at these sensitivities. An equivalent change in the protein-bound carboxyl intensity (compare Fig. 7, B and F) until at pH 5.0 in the

<table>
<thead>
<tr>
<th>Table II</th>
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<tbody>
<tr>
<td>Values of pK\textsubscript{app} for the ionization of oleic acid and P, the core to core partition coefficient of unionized oleic acid in chylomicrons, calculated from fitting Form I and Form II of the model expression (Appendix)</td>
</tr>
<tr>
<td>The residual for fit is the square of the differences between the predicted and experimental values of mean chemical shift (\delta).</td>
</tr>
<tr>
<td>pK\textsubscript{app} &amp; P &amp; Residual (in \delta)</td>
</tr>
<tr>
<td>Form I</td>
</tr>
<tr>
<td>Buffered</td>
</tr>
<tr>
<td>Form II</td>
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Upon reducing the pH, intensity from the carboxyl group of lipid-associated oleic acid begins to appear within the protein carboxyl envelope. This resonance is distinct at pH 6.0 in both samples (Figs. 7F and 8E). These changes are accompanied by a depletion in the protein-bound carboxyl intensity (compare Fig. 7, B and F) until at pH 5.0 in the

\[ \text{OA} + \text{BSA (9 wt%)} \] \[ \text{+ CHYLOMICRICONS (20 wt%)} \]

\[ [5 : 1] \]

\[ \text{pH} \]

\[ 7.4 \]

\[ 182.2 \]

\[ 180.7 \]

\[ \text{PH} \]

\[ 7.4 \]

\[ 182.5 \]

\[ 180.7 \]

\[ \text{CHYLOMICRICONS (20 wt%)} \]

\[ \text{+ EMULSION (20 wt%)} \]

\[ [5 : 1] \]

\[ \text{pH} \]

\[ 7.4 \]

\[ 182.2 \]

\[ 180.7 \]

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\[ \text{pH} \]

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\[ [5 : 1] \]

\[ \text{pH} \]

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\[ 182.2 \]

\[ 180.7 \]

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\[ \text{+ EMULSION (20 wt%)} \]

\[ [5 : 1] \]

\[ \text{pH} \]

\[ 7.4 \]

\[ 182.2 \]

\[ 180.7 \]

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\[ \text{+ EMULSION (20 wt%)} \]

\[ [5 : 1] \]

\[ \text{pH} \]

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\[ [5 : 1] \]

\[ \text{pH} \]

\[ 7.4 \]

\[ 182.2 \]

\[ 180.7 \]
emulsion sample (Fig. 8F) virtually all the intensity appears
to have been transferred to the lipid particles. Returning the
depth to 7.4 showed good reversible characteristics for oleic acid
transfer (Figs. 7G and 8G).

The results show that virtually all the fatty acid was bound
to albumin in the range of physiologic pH. This high affinity
of fatty acid for the albumin was observed at binding stoichi-
ometries much greater than normally encountered in the
circulation (16). High affinities at these elevated stoichiome-
tries, however, probably would be required for albumin to
function as a carrier during rapid lipolytic production of fatty
acid. A high binding stoichiometry would also appear to be
required to effect substantive transfer to the cell membranes
of tissues (16).

The albumin concentrations used were low in relation to the
lipid components which had a 2.2 (chylomicrons) or 4-
fold [emulsion] greater mass in these experiments. The low
fatty acid affinity noted around neutral pH for the large lipid
compartments could, however, be anticipated from the high
values of P obtained in the preceding study. These show that
only the relatively small surface regions of the particles would
generally be accessible to the fatty acid. Judging by the poor
retention of fatty acid by these surfaces in the presence of
BSA, the association between fatty acid and phospholipid is
quite nonspecific. The finding that transfer of oleic acid from
BSA to chylomicrons and emulsions occurs over a similar pH
range as the transfer to phosphatidylcholine vesicles (6) also
argues for a nonspecific association with phospholipid sur-
faces. If the fatty acid uptake by most cell membranes occurs
by this process, then transfer of fatty acid from chylomicron
surfaces to endothelial cell membranes might simply occur by
virtue of the predominant size of the membrane compartment.
It remains to be established whether this is adequate to
account for the rapid rate of cellular uptake for lipolytically
generated fatty acid.

The physiologically abundant oleic acid was a logical choice
for the detailed study of distribution behavior attempted here.
However, other important fatty acid species could exhibit a
different behavior. Spectra obtained for the saturated anal-
logue, stearic acid, with the emulsion and BSA are shown as
a function of pH in Fig. 9. While these spectra are qualitatively
similar to those for oleic acid in this system (Fig. 8), they do
suggest that partitioning of the saturated fatty acid is shifted
more toward the lipid particles at all pH values studied. Unlike
oleic acid, lipid-associated stearic acid is observable at pH 7.4
(Fig. 9, B and C, 177–178 ppm), and this is accompanied by a
pronounced depletion in the protein-bound region at this pH
(Fig. 9C). It is not possible to account for these differences at
this time, other than to mention the possibility that stearic acid
has a slightly lower affinity than oleic acid for BSA (30).

The results presented here provide some justification for
interpreting the distribution behavior of fatty acids in terms of
their fundamental physical properties in biological systems.
The work also reinforces the idea of an important participat-
ion role of serum albumin binding in this process.

Acknowledgments—We thank the analytical staff in this laboratory
for expediting chemical analyses, Howard Lilly for other technical
assistance, both David Jackson and Dr. David Atkinson for enabling
the computer modeling to be performed, and Anne Gibbons for
preparing the manuscript.

APPENDIX

General Expression for $\delta$

In the case when rapid exchange between ionized and non-
ionized states occurs during the timescale of NMR observa-
tion, the mean chemical shift, $\delta$, of the resulting single reso-
nance can be expressed as the sum of its components thus:

$$\delta = R\delta^-/(1 + R) + \delta_0/(1 + R)$$

(1)

where $R$ is the mass ratio of ionized (A$^-$) to unionized (HA)
states, having absolute chemical shifts of $\delta_0$ and $\delta^{-}$, respec-
tively.

To define $R$ in terms of constants of the physical processes
involved, we consider the following simple kinetic scheme
governing fatty acid ionization in emulsion-like particles:

\[
\begin{align*}
\text{CORE} & \quad \text{SURFACE} \\
HA_0 & \quad \overset{P}{\longleftarrow} KH_+ \quad \text{HA} \quad \text{A}^- + H^+ \\
& \quad \text{app}
\end{align*}
\]

where subscripts c and s refer to species in the core and
surface of the particle, respectively. For the distribution of
monomeric unionized species between the core and surface,
we write a surface to core "true" partition coefficient:

$$P = [HA_0]/[HA]$$

(2)

If the activities of species involved in the ionization step can
be equated with their concentrations in the surface region, we

Fig. 9. $^{14}$C NMR spectra for the transfer of [1-$^{14}$C]-stearic
acid (SA) between BSA and the emulsion particles under
conditions described for Fig. 3. The broad peak for lipid-assoc-
inated fatty acid appearing at pH 7.4 (B) is shown at 177.4 ppm in
spectrum C, obtained after subtraction of the spectrum for protein-
bound fatty acid (A). The peak at 173.9 ppm (spectrum C) corresponds
to the carbonyls from phosphatidylcholine. Spectra D–F show the
transfer of carboxyl intensity from the protein to lipid-associated
peaks with decreasing pH. Spectrum G shows good reversibility for
fatty acid transfer upon returning the pH to 7.4.
can apply the standard mass action expression for the ionization process:

\[ K_{\text{app}} = [A^-][H^+]/[HA] \]  

(3)

This ionization constant is termed "apparent" so as to be distinct from ionization within an aqueous bulk environment and also to account for measurements of bulk [H+] being used for the above relation.

Defining the mass ratio in expression 1:

\[ R = V_c[A^-]/(V_c[HA] + V_s[HA]) \]

where \( V_c \) and \( V_s \) are the core and surface volumes, respectively. Rewriting in terms of surface species:

\[ R = V_c P'[A^-]/(V_c P'[HA] + V_s P'[HA]) \]  

(4)

Rearranging:

\[ R(V_c P + V_s)/V_c P = [A^-]/[HA] \]

Introducing \( K_{\text{app}} \) from equation 3:

\[ R(V_c P + V_s)/V_c P = K_{\text{app}}/[H^+] \]

\[ R = K_{\text{app}} V_c P/[H^+](V_c P + V_s) \]

The general expression 1, incorporating the above relation, has the following form for curve fitting:

\[ f = (a10^{m_p}b_p + \delta_m)/(1 + a10^{m_p}) \]  

(5)

where

\[ a = K_{\text{app}} V_c P/(V_c P + V_s) \]  

(6)

Calculating \( P \) and \( K_{\text{app}} \)

Computer-fitted values of \( a \) can be used to calculate \( P \) using experimental values of apparent partition coefficient, defined as:

\[ P' = ([HA] + [A^-])/[HA] \]

Dividing through terms on the right-hand side of the above by \([HA]\), and rearranging gives:

\[ P' = P + P[A^-]/[HA] \]

Introducing \( K_{\text{app}} \) from expression 3:

\[ P' = P + PK_{\text{app}}/[H^+] \]

Now \( a \) can be introduced through equation 6, as follows:

\[ P' = P + a(V_c P + V_s)/(176.17 ppm) \]  

(8)

\[ \delta_{HA} = (V_c P + V_c P'/V_c P + V_s)/(V_c P + V_s) \]  

(9)

\[ \delta_{HA} = m_{\text{log}[HA]} + c \]  

with a correlation coefficient for linear regression of \( >0.99 \).

Finally, \([HA]\) is defined in terms of the total fatty acid concentration \([HA]_t\) thus:

\[ V_c[HA] = V_c[HA]_t - V_s[HA] - V_s[A^-] \]

Defining \( V_s[A^-] \) from expression 4 gives:

\[ V_s[A^-] = V_c[HA] - V_c[HA]_t - V_s[HA] - V_s[A^-] \]

or in terms of core concentrations:

\[ V_c[HA] = V_c[HA]_t - V_c P'[HA] - V_c P'[HA] - V_c P'[HA] \]

Rearranging:

\[ [HA]_t = V_s[HA]_t/(V_c + V_c P + V_s P + V_c P_{10}^{m_{HA}}) \]  

(10)

**Curve Fitting**

**Form I**—This took the general form of expression 5 for a single parameter fit for \( a \) by the nonlinear least squares Marquardt-Levenberg method available on RSI software for the MicroVaxII. \( P \) and \( K_{\text{app}} \) were evaluated using the following values for: \( V_c = 0.2202 \); \( V_s = 2.475 \times 10^{-2} \) (from chemical analyses and using reported specific volumes (26) and \( P_{176} = 12 \pm 2 \).

**Form II**—Expression 5 was used along with equation 8 for \( \delta_{HA} \), in which \( \delta = 176.17 \) ppm (Ref. 6); equation 9 for \( \delta_m \), in which \( m = 2.340 \) and \( c = 179.04 \) ppm (linear least squares fit), and equation 10 for \([HA]_t\) in which \([HA]_t = 2.955 \times 10^{-2} \) (chemical analysis). The simultaneous evaluation of these expressions for values of parameter \( a \) was achieved using a Simplex least squares fitting program developed in these laboratories by David Jackson.

**REFERENCES**


\[^3\] D. P. Cistola, J. Parks, P. Spooner, and J. A. Hamilton, unpublished data.
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