

Binding of Straight-Chain Saturated Dicarboxylic Acids to Albumin

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Abstract

Dicarboxylic acids are prominent features of several diseases, including Reye's syndrome. Long-chain dicarboxylic acids have profound effects on the function and structure of isolated mitochondria, suggesting that they could contribute to the mitochondrial dysfunction in Reye's syndrome. Binding of fatty acids to albumin and the intracellular fatty acid-binding proteins is important in regulating the transport and metabolism of fatty acids and protects against the toxic effects of unbound fatty acids. We studied the binding of dicarboxylic acids to defatted albumin using equilibrium dialysis to assess to what extent dicarboxylic acids are likely to be bound in the plasma of patients. Dicarboxylic acids bind weakly to albumin in a molar ratio of 3.8, 4.2, 1.6, 0.8, and 0.7 to 1 for octadecanedioic, hexadecanedioic, tetradecanedioic, dodecanedioic, and decanedioic acid, respectively. The dissociation constants for long-chain dicarboxylic acids are 100–1,000-fold larger than those of comparable monocarboxylic acids. Oleate competes with dicarboxylic acid and reduces the moles of dicarboxylic acid bound per mol of albumin to < 1. Octanoate inhibits dicarboxylic acid binding. Our observations indicate that in Reye's syndrome, substantial concentrations of dicarboxylic acids of patients may be free and potentially toxic to mitochondria and other cellular processes.

Introduction

Dicarboxylic acids are the product of omega-oxidation in the microsomes. Only 5–10% of FFA are metabolized by this pathway in ketotic rats (1). However, dicarboxylic acids have recently been shown to be a prominent feature of several diseases, including Reye's syndrome (2–4), neonatal adrenoleukodystrophy (5), Zellweger's syndrome (5), and defects of fatty acid metabolism (6, 7). In Reye's syndrome, dicarboxylic acids make up as much as 55% of the total serum FFA (4).

The hallmark of Reye's syndrome is a transient generalized impairment of mitochondrial enzymes and swelling and distortion of mitochondrial ultrastructure (8). Several toxins, including aspirin, have been suggested to predispose children to this illness (9). Serum from patients with Reye's syndrome impairs ATP formation and induces swelling and distortion of isolated mitochondria (10). These effects correlate directly

with the concentration of dicarboxylic acids in the serum samples (10). Studies with isolated mitochondria have demonstrated that dicarboxylic acids, particularly long-chain dicarboxylic acids, at concentrations comparable to those in plasma of patients with Reye's syndrome, profoundly inhibit the enzymes of the terminal respiratory pathway (11), inhibit ATP formation (10), and induce an irreversible expansion of mitochondria characteristic of an uncoupler of oxidative phosphorylation (12). Dicarboxylic acids thus may contribute to the mitochondrial dysfunction that is central to Reye's syndrome (10). The regulation of dicarboxylic acid metabolism may therefore be important in Reye's syndrome and other diseases in which dicarboxylic acid formation is prominent.

The binding of monocarboxylic fatty acids to albumin and the intracellular fatty acid-binding proteins modulates the transport and metabolism of these fatty acids (13, 14). Binding of potentially toxic ligands to albumin protects against the toxic effects of the ligands, as toxicity is proportionate to the concentration of unbound ligand (13, 15, 16). We undertook this study to determine the affinity and capacity of albumin for dicarboxylic acids.

Methods

Monocarboxylic and dicarboxylic acids. Unlabeled monocarboxylic and dicarboxylic acids were purchased from Sigma Chemical Co. (St. Louis, MO), Applied Science (Warrenville, IL), and Foxboro Analabs (North Haven, CT). The purity of the unlabeled acids was assessed by gas-liquid chromatography and was found to be at least 99.5%. [³H]-Dicarboxylic acids, 1-[¹⁴C]C_{8,0},¹ and 1-[¹⁴C]C_{18,1} were purchased from Amersham Corp. (Arlington Heights, IL) and 1-[¹⁴C]decanedioic acid was purchased from Pathfinder Laboratories, Inc. (St. Louis, MO). The [³H]dicarboxylic acids prepared by Amersham Corp. were obtained by reaction with tritiated water (method TR.8; Amersham International, Amersham, England) at a very high specific activity. The chemical purity of the labeled compounds was assessed by gas-liquid chromatography (3) and found to be at least 97%. The radiopurity of the labeled compounds was assessed by thin-layer chromatography (17). Silica G plates were spotted with a mixture of labeled and unlabeled methyl esters of the dicarboxylic acids, separated using pentane/ether/acetic acid (92:7:1), and visualized with iodine vapors. The plates were scraped in 1-cm bands and the gel fractions were solubilized with water and then the radioactivity was measured. The radiopurity of the dicarboxylic acids was at least 93.4%.

Potassium salts of a mixture of labeled and unlabeled monocarboxylic or dicarboxylic acid were made by dissolving the acid in a small amount of ethanol and adding 10 meq of 1 M methanolic KOH to 1 meq acid. Phenolphthalein was added as a pH indicator. The flask containing the solution was connected to a refluxing apparatus and heated at 80°C for 30 min in a water bath. The solution was cooled, then dried with N₂, and then redissolved in phosphate buffered solution containing 0.116 M NaCl, 0.0049 M KCl, and 0.016 M sodium

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1. *Abbreviations used in this paper:* C_{18,1}, oleic acid; C_{18,0}, stearic acid; C_{16,0}, palmitic acid; C_{14,0}, myristic acid; C_{12,0}, lauric acid; C_{10,0}, decanoic acid; C_{8,0}, octanoic acid.

phosphate, pH 7.4. This salt solution was rinsed with chloroform to remove the monocarboxylic or dicarboxylic acids that were not in salt form. The concentration of the carboxylate salt was determined by gas-liquid chromatography by comparing the detector response to a calibration curve of a known amount of the unlabeled compound. The extraction efficiency was determined by comparing the radioactivity of aliquots before and after extraction. The specific activity of the carboxylate salt was determined from the counts per minute of the extracted, derivatized sample divided by the concentration of the carboxylate salt.

Albumin. Essentially fatty acid-free (< 0.005%) crystalline BSA was from Sigma Chemical Co. and further purified as described by Spector, John, and Fletcher (18). The monocarboxylic fatty acid content of the purified albumin was determined by gas-liquid chromatography (4) and found to have < 0.02 mol fatty acid/mol of albumin.

Equilibrium dialysis. Binding of dicarboxylic acids to defatted BSA was determined using equilibrium dialysis as described by Ashbrook, Spector, and Fletcher (19). Dialysis chambers and membranes were from Bel-Art Products (Pequannok, NJ). The chamber contains two 1-ml compartments separated by a dialysis membrane that is impermeable to compounds with a molecular weight > 6,000; albumin does not cross the dialysis membrane (19). The dicarboxylic acids reached equilibrium within 18 h at 37°C. We chose a 26-h incubation period to ensure equilibrium. Binding was assessed by varying concentrations of dicarboxylic acid (0.05–1.5 mM) in a near physiologic salt solution containing 0.116 M NaCl, 0.0049 M KCl, and 0.016 M sodium phosphate, pH 7.4. Dicarboxylic acid was added to one side of the chamber and defatted albumin in the same salt solution was added to the other side to a final concentration of 0.05 mM. Because octadecanedioic acid is less soluble than the other dicarboxylic acids (20), binding of this fatty acid was assessed using 0.010 mM albumin and 0.010–0.200 mM octadecanedioic acid. Dialysis chambers were incubated in a shaking water bath at 37°C and at the end of the incubation period, 100 µl was removed from each side, and the radioactivity of the aliquot was measured using an Isocap/300 liquid scintillation counter (G. D. Searle & Co., Des Plaines, IL). The concentration of free dicarboxylic acid was determined from the counts per minute in the aliquot from the side of the chamber that did not contain albumin, corrected for the volume of the two sides of the chamber into which the free dicarboxylic acid was distributed, and multiplied by the specific radioactivity of the dicarboxylic acid solution. The concentration of bound dicarboxylic acid was determined by subtracting the counts per minute in the aliquot from the side of the equilibrium chamber that did not contain albumin, from the counts per minute in the aliquot from the albumin-containing side. This was then corrected for the volume of the albumin-containing chamber and multiplied by the specific radioactivity of the dicarboxylic acid solution. Experiments were performed in triplicate. The recovery of radioactivity was between 88 and 101%. The data shown are the results of between four and eight separate experiments.

Monocarboxylic acid competition. Experiments involving the competition of the 1-[¹⁴C]C_{18,1} with ³H-labeled dicarboxylic acids were performed as described by Ashbrook, Spector, and Fletcher (19). It has previously been observed that C_{18,1} does not readily cross the dialysis membrane (15, 19). This observation was confirmed in preliminary experiments. The competition experiments were performed as described above, except that either 0.1, 0.3, or 0.5 mM 1-[¹⁴C]C_{18,1} was added to the albumin-containing side of the equilibrium chamber. The extent to which C_{18,1} was bound was determined by gel filtration (21, 22). At the end of the incubation, 100 µl was removed from the albumin-containing side of the chamber and added to a 1-ml Sephadex G-25 column (Pharmacia Fine Chemicals, Piscataway, NJ). The column was prepared by placing a slurry of Sephadex G-25 in buffered salt solution in a 1-ml plastic syringe. The buffer was removed by centrifugation at 150 g for 10 min at 25°C. 100 µl from the albumin side of the chamber was added to the column and the column was centrifuged again at 150 g for 10 min. Protein content of the eluate was monitored spectrophotometrically at 280 nm and fatty acid content was assessed by scintillation counting. At least 90% of the albumin is eluted after a single centrifugation. Albumin was completely eluted

from the column with two additional rinses of 100 µl of salt solution. Fatty acids not associated with albumin remained bound to the column when the column was eluted with additional rinses of aqueous buffer. Recovery of the FFA was achieved by rinsing the column three times with 0.5 ml of 2:1 chloroform/methanol. The buffer and chloroform rinses were collected separately and transferred to scintillation vials. The samples were evaporated to dryness and the amount of [¹⁴C]C_{18,1} was determined by liquid scintillation. The mean recovery of albumin was determined to be 95.8%. The mean recovery of [¹⁴C]C_{18,1} was 98.3%. We demonstrated that reequilibration of bound fatty acid is sufficiently slow that it does not occur to any significant extent, as albumin is propelled by centrifugation through the column.

In contrast to C_{18,1}, C_{8,0} freely diffuses across the dialysis membrane (19). Equilibration of C_{8,0} occurs within 24 h. Therefore, binding of [¹⁴C]C_{8,0} was assessed from the partitioning of ¹⁴C counts per minute as described previously for the dicarboxylic acids.

Analysis of binding. Binding isotherms were analyzed using a non-linear least-squares fit computer program; in general, the damping Gauss-Newton method was used (23). The points in the figures are experimental data and the lines are the best fit of the data to the theoretical equation as determined by the least-squares fit computer program. Dissociation constants are expressed as the mean ± SD.

Results

The binding of dicarboxylic fatty acids to albumin was analyzed using the equation of reversible, saturable binding to one, two, or three classes of noninteracting equivalent sites, i.e., the equation of a Langmuir isotherm (24). For the case of one class of sites, $K_d = (F_f)(S_f)/C_x = (F_f)(S_t - F_b)/F_b$, where K_d = dissociation constant (micromolar), F_f = free dicarboxylic acid (micromolar), F_b = bound dicarboxylic acid (micromolar), C_x = dicarboxylic acid-site complex (micromolar), and S_f and S_t = free and total binding sites, respectively. The number of sites/albumin molecule can be calculated from S_t and the albumin concentration. We used the Akaike information coefficient (23) to ascertain whether the best fit of the data could be obtained by assuming one, two, or three classes of binding sites.

Affinity of dicarboxylic acids for albumin. We first examined the binding of hexadecanedioic acid (Fig. 1). Analysis of the binding shows two different types of binding sites with dissociation constants ± SD of 1.2 ± 1.4 and 67.8 ± 15.6 µM, respectively (Table I). The first site binds 1 mol of dicarboxylic acid and the second class of binding sites binds the remaining acid. We observed a molar ratio of bound hexadecanedioic acid to albumin of 4.2:1. The calculated saturation of albumin occurs at a molar ratio of 5:1 (0.9 ± 0.3 plus 4.2 ± 0.2 mol, Table I).

Figure 1. Binding of hexadecanedioic acid to defatted albumin. Binding was assessed at pH 7.4, using varying concentrations of [³H]-dicarboxylic acid in an equilibrium dialysis chamber in which one side contained 0.050 mM defatted albumin. The points are experimental data and the lines are the best fit of the data to the theoretical equation.

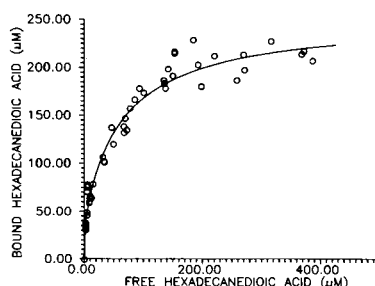


Table I. Equilibrium Constants for Saturated Straight-Chain Dicarboxylic Acids

Fatty acid	Bound dicarboxylic acid mol/mol BSA*	Dissociation constants		Predicted saturation		Number of data points
		K_{d1}	K_{d2}	Site 1	Site 2	
		$\mu\text{M} \pm \text{SD}$		mol/mol BSA $\pm \text{SD}$		
Octadecanedioic pH 7.4	3.8	1.1 \pm 1.7	19.4 \pm 18.2	1.4 \pm 1.4	3.2 \pm 1.1	28
Hexadecanedioic pH 6.8	4.2	2.5 \pm 1.4	27.8 \pm 6.1	1.1 \pm 0.4	3.3 \pm 0.3	35
pH 7.4	4.2	1.2 \pm 1.4	67.8 \pm 15.6	0.9 \pm 0.3	4.2 \pm 0.2	51
pH 8.0	4.2	3.9 \pm 0.9	216.0 \pm 46.0	2.1 \pm 0.2	4.3 \pm 0.9	41
Tetradecanedioic pH 7.4	1.6	23.2 \pm 9.8	292.6 \pm 26.0	1.0 \pm 0.1	1.1 \pm 0.1	49
Dodecanedioic pH 6.8	0.80		30.1 \pm 6.2		0.9 \pm 0.1	22
pH 7.4	0.88		75.2 \pm 4.3		1.0 \pm 0.02	28
pH 8.0	0.80		83.3 \pm 13.2		1.0 \pm 0.1	21
Decanedioic pH 7.4	.70		31.5 \pm 9.3		0.8 \pm 0.1	17

Equilibrium constants for saturated straight-chain dicarboxylic acids. Binding was assessed in the presence of 0.050 mM albumin for all of the dicarboxylic acids except octadecanedioic acid, which was incubated with 0.010 mM albumin. * Indicates the mol ratio of fatty acid to albumin at saturation as observed experimentally.

The binding of octadecanedioic acid is similar to that of hexadecanedioic acid (Fig. 2). Octadecanedioic acid is bound in a 3.8:1 molar ratio (Table I). As with hexadecanedioic acid, there appear to be two distinct types of binding sites with a theoretical saturation of 4.6 \pm 1.4 mol of the dicarboxylic acid.

In contrast to the longer chain dicarboxylic acids, albumin only binds 2 mol of tetradecanedioic acid per mol of albumin (Table I, Fig. 2). There are again two distinct binding sites, each binding 1 mol of tetradecanedioic acid per mol of albumin with dissociation constants of 23.2 \pm 9.8 and 292.6 \pm 26.0 μM (Table I). There is only a single binding site for dodecanedioic and decanedioic acids. The maximal binding achieved

experimentally for dodecanedioic and decanedioic acids is 0.8 and 0.7 mol/mol of albumin (Fig. 2, Table I). The dissociation constants for dodecanedioic and decanedioic acid are 75.2 \pm 4.3 and 31.5 \pm 9.3 μM , respectively.

Effect of pH on dicarboxylic acid binding. The effect of pH of the buffer solution on dicarboxylic acid binding was also examined. Incubations were performed at pH 6.8 and 8.0 with hexadecanedioic acid and dodecanedioic acid and compared with incubations at pH 7.4 (Table I). The pH of the buffer solution does not affect the saturation level of the albumin. The binding curves for both hexadecanedioic and dodecanedioic acid shift slightly to the left with a more acidic pH (Fig. 3). The pH of the buffer has no significant effect on the K_{d1} for hexadecanedioic acid but the K_{d2} decreases at the more acidic pH. The dissociation constant for dodecanedioic acid also decreases at pH 6.8 (Table I).

Competition with monocarboxylic fatty acids for binding. We examined the effect of the monocarboxylic acid, oleic acid ($C_{18,1}$) on the binding of dicarboxylic acids using 0.1, 0.3, and 0.5 mM $C_{18,1}$ (Fig. 4, Table II). $C_{18,1}$ competes with hexadecanedioic acid for binding; as a result, the apparent dissociation constant of the dicarboxylic acid increases 15–35-fold (from 1.2 \pm 1.4 to 41.0 \pm 7.3 μM , Table II). When the monocarboxylic acid concentration is increased to 0.5 mM (a 10:1 $C_{18,1}$ /albumin ratio), all but 0.70 mol of hexadecanedioic acid is displaced (Fig. 4, Table II). A similar result was obtained when the binding of octadecanedioic acid was examined in the presence of $C_{18,1}$. All but 0.7 mol of octadecanedioic acid is displaced in the presence of near saturating concentrations of $C_{18,1}$. As shown in Fig. 5, when the concentration of long chain dicarboxylic acid is high enough, long-chain dicarboxylic acid can bind to albumin and displace some $C_{18,1}$.

The effect of monocarboxylic fatty acids on the binding of dodecanedioic acid was also examined (Fig. 6, Tables II and

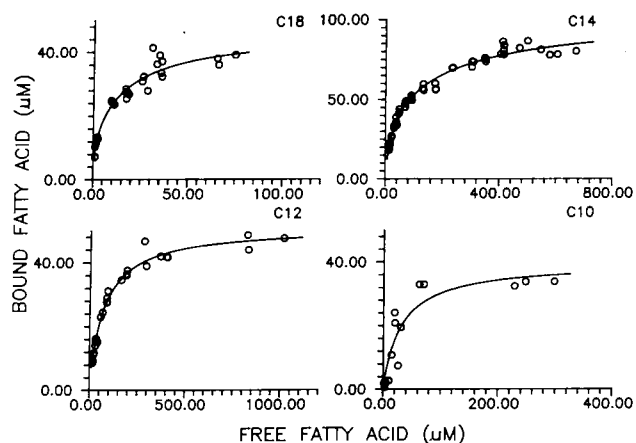


Figure 2. Binding of dicarboxylic acids to defatted albumin. Binding was assessed as described in Fig. 1 with the exception that the binding of octadecanedioic acid was assessed with 0.010 mM albumin. Octadecanedioic acid (C18), tetradecanedioic acid (C14), dodecanedioic acid (C12), and decanedioic acid (C10).

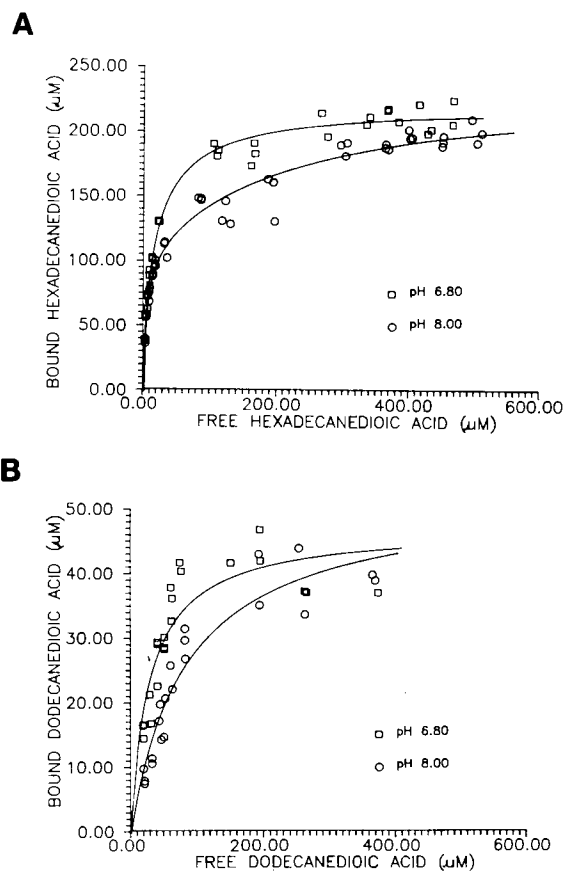


Figure 3. The effect of buffer pH on the binding of dicarboxylic acids. Binding of hexadecanedioic acid (A) and dodecanedioic acid (B) was assessed at pH 6.8 (□) and 8.0 (○) as described in Fig. 1.

III). In the absence of any competition, between 0.8 and 0.9 mol are bound per mol of albumin. When 2–3 mol of $C_{18,1}$ are bound to albumin (0.10–0.15 mM $C_{18,1}$), the affinity for dodecanedioic acid is reduced almost fivefold (Table II), but there is no significant effect on the maximal binding capacity (Tables II and III). When 4–5 mol of $C_{18,1}$ are bound to albumin (0.3–0.5 mM $C_{18,1}$) the affinity is reduced ninefold (Table II)

Table II. Competition of Oleic Acid with Dicarboxylic Acids for Binding

Fatty acid	Bound dicarboxylic acid <i>mol/mol BSA*</i>	Dissociation constants		Predicted saturation		Number of data points
		K_{d1}	K_{d2}	Site 1	Site 2	
		$\mu M \pm SD$		<i>mol/mol BSA ± SD</i>		
Hexadecanedioic with	4.4	1.2 ± 1.4	67.8 ± 15.6	0.9 ± 0.3	4.2 ± 0.2	51
0.1 mM oleic	3.3		41.0 ± 7.3		3.7 ± 0.1	29
0.3 mM oleic	1.4		15.0 ± 1.2		1.4 ± 0.2	14
0.5 mM oleic	0.7		34.4 ± 9.6		0.8 ± 0.1	14
Dodecanedioic with	0.9		75.2 ± 4.3		1.0 ± 0.02	28
0.1 mM oleic	0.9		339.3 ± 116.0		1.4 ± 0.2	15
0.5 mM oleic	0.4		707.4 ± 171.7		0.7 ± 0.1	22

Competition of oleic acid with dicarboxylic acids for binding. * Indicates the mol ratio of fatty acid to albumin, as observed experimentally.

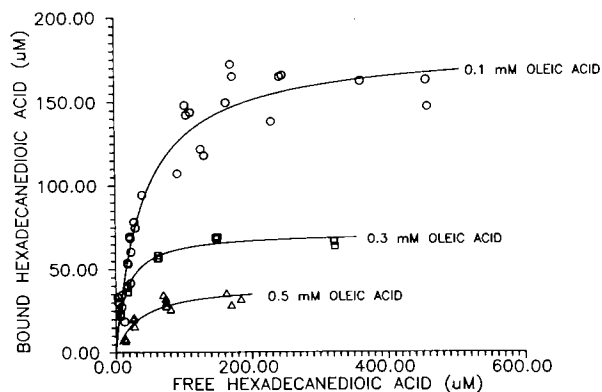


Figure 4. Competition of oleic acid and hexadecanedioic acid for binding to albumin. Binding of [3H]dicarboxylic acid was assessed in the presence of 0.05 mM albumin incubated with 0.1 (○), 0.3 (□), and 0.5 (Δ) mM [^{14}C]oleic acid.

and the experimentally observed binding capacity of dodecanedioic acid is reduced by more than half (Table III).

The medium-chain length monocarboxylic acid, octanoic acid ($C_{8,0}$) has a more profound effect on the binding of dodecanedioic acid (Table III). When 0.5 mol of $C_{8,0}$ per mol of albumin are bound, only 0.1–0.2 mol of dodecanedioic acid is bound and when 1 mol of $C_{8,0}$ per mol of albumin is bound, the binding of dodecanedioic acid is further inhibited.

Discussion

Our results indicate that dicarboxylic acids bind to albumin with lower affinity than monocarboxylic acids of the same chain length. There is a single low-affinity site for dodecanedioic and decanedioic acid. There is a single higher affinity site for the longer chain dicarboxylic acids (C_{18} – C_{14}) with between one and four additional low-affinity sites, depending on the chain length of the dicarboxylic acid. The affinity of long-chain dicarboxylic acids for albumin is 100–1,000-fold less than that of long-chain monocarboxylic fatty acids and is more comparable to the affinity of many drugs for albumin (25). The molar ratio of bound dicarboxylic acid to albumin observed is also significantly less than that reported for monocar-

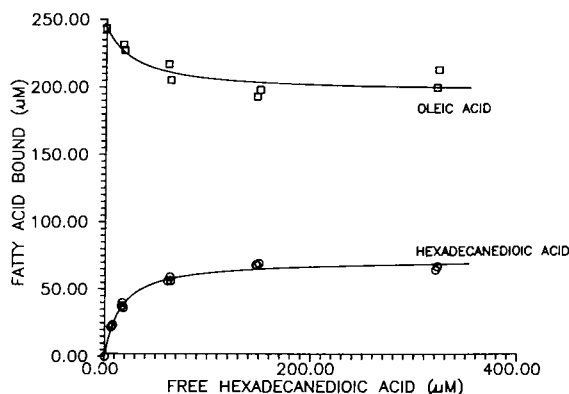


Figure 5. Competition of 0.3 mM oleic acid with hexadecanedioic acid for binding to albumin. Binding was assessed as described in Fig. 4. The amount of bound oleate was determined after separation of free and bound fatty acid by centrifugation through a Sephadex G-25 column. □, micromoles of bound oleic acid; ○, micromoles of bound hexadecanedioic acid. The binding of hexadecanedioic acid is analyzed using the equation of a Langmuir isotherm. The points are experimental data and the lines are the best fit of the data to the theoretical equations obtained using the nonlinear least-square computer program as described in the text.²

boxylic fatty acids of the same chain length. Spector and co-workers (18, 19) reported binding ratios of 6.5, 7.2, 7.0, 8.4, and 13.9 for C_{18,0}, C_{16,0}, C_{14,0}, C_{12,0}, and C_{10,0}, respectively compared with our observations of 3.8, 4.2, 1.6, 0.8, and 0.7 for dicarboxylic acids of the same chain length.

The pH of the buffer exerts only a modest effect on the binding of dicarboxylic acids. The pK of each of the carboxyl groups for octanedioic acid is 4.52 (26). The dissociation constants for longer chain dicarboxylic acids are probably close to this value. Thus, the dicarboxylic acids are > 99% deprotonated in the physiological pH range we examined. The modest shift in binding at pH 6.8 may be due to a change in amino acid charge at one or more binding sites which facilitates binding of dicarboxylic acids.

As might be expected from a comparison of the dissociation constants of monocarboxylic and dicarboxylic acids, monocarboxylic acids competitively inhibit dicarboxylic acid binding. However, the competition studies demonstrate that at high enough concentrations, dicarboxylic acid can bind and displace some C_{18,1}.

2. The binding of hexadecanedioic acid is analyzed using the equation of a Langmuir isotherm, $K_d^{app} = (S_t^{app} - S_b) (D_f) / D_b$, where K_d^{app} = apparent dissociation constant, S_t^{app} = total sites on albumin available for binding hexadecanedioic acid under these experimental conditions, S_b = concentration of sites occupied by hexadecanedioic acid, D_b = concentration of bound hexadecanedioic acid, and D_f = concentration of free hexadecanedioic acid.

The desorption of oleic acid at various concentrations of hexadecanedioic acid is analyzed using the equation $K_{d_2} = (S_{fd})(D_f) / O_{bd} = (O_b - D_b^{max})(D_f) / O_{b,d=0} - O_b$. In this equation, the total sites from which oleic acid can be displaced = $O_{b,d=0} - D_b^{max}$, where $O_{b,d=0}$ = oleic acid bound in the absence of hexadecanedioic acid, and D_b^{max} = maximal hexadecanedioic acid able to bind to displaceable oleic acid sites on albumin. D_f = concentration of free hexadecanedioic acid, O_b = concentration of bound oleic acid, and K_{d_2} = dissociation constant (M) for hexadecanedioic acid under these experimental conditions.

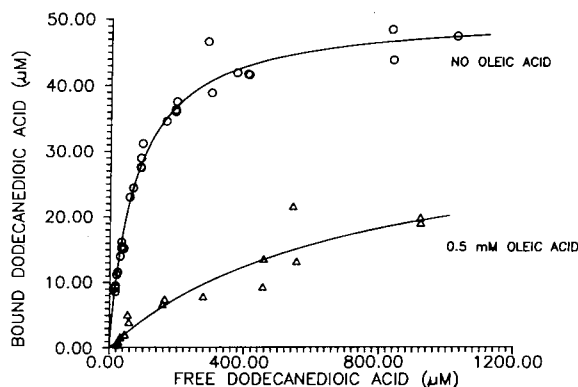


Figure 6. Competition of 0.5 mM oleic acid with dodecanedioic acid for binding to albumin. Binding was assessed as described in Fig. 1. Binding in the presence of 0.5 mM oleic acid (Δ) is compared with binding in the absence of competition (○).

The albumin molecule is composed of three nonidentical cylindrical domains (13), each of which has a narrow hydrophobic channel able to accommodate only one or two hydrocarbon chains. The ends of each domain form nonidentical subdomains containing positively charged amino acid side chains. Recent studies indicate that medium-chain monocarboxylic fatty acids bind almost exclusively via hydrophobic interactions, whereas the binding of long-chain monocarboxylic acids is dependent on both electrostatic and hydrophobic interac-

Table III. Competition of Dodecanedioic and Monocarboxylic Fatty Acids

Concentration of fatty acids	Bound dodecanedioic	Bound oleic	Bound octanoic
$\mu\text{M dodecanedioic} / \mu\text{M oleic or octanoic}$	<i>mol/mol BSA</i>		
10/0	0.03	—	—
200/0	0.50	—	—
300/0	0.60	—	—
0/150	—	2.60	—
10/150	0.02	2.50	—
200/150	0.48	2.70	—
300/150	0.65	2.70	—
0/320	—	4.88	—
10/320	0.01	4.66	—
200/320	0.19	4.67	—
300/320	0.27	4.53	—
0/100	—	—	0.46
200/100	0.16	—	0.49
300/100	0.25	—	0.47
0/500	—	—	1.25
200/500	0.16	—	1.10
300/500	0.19	—	1.10

Competition of dodecanedioic with the monocarboxylic acids octanoic, and oleic. Binding was assessed as described in Fig. 5. The concentration of albumin was 0.050 mM.

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