A THEORETICAL ANALYSIS OF THE BINDING OF PALMITATE BY HUMAN SERUM ALBUMIN

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SUMMARY: The distribution of palmitate between the form bound by human serum albumin and the free form in plasma was calculated by use of 12 stepwise equilibrium constants and a computer program. Computations were carried out for molar ratios of palmitate to serum albumin of 0.5,1,2,3, and 4. At most 0.0003% of the palmitate would be in the unbound form, and the remainder distributed among different complexes with albumin. At low molar ratios, the complexes with 1 to 2 moles of palmitate/albumin would predominate while at the highest ratio the complexes of 3 and 4 moles of palmitate/albumin would be most abundant. In the delivery of palmitate to tissues the relative contribution of the different complexes would change, as the molar ratio of fatty acid to albumin changed.

INTRODUCTION

It is well established that serum albumin is the major carrier of the free fatty acids (FFA) in the plasma. The binding of FFA by serum albumin has been characterized by Scatchard constants, (1) and more recently by stepwise equilibrium constants (2). The Scatchard analysis provides association constants and binding capacities which are characteristic of the different sets of sites. In contrast, the stepwise equilibrium constants are related to the moles of ligand bound per mole of macromolecule without reference to individual sites. Spector (3) pointed out that stepwise equilibrium constants are superior to Scatchard constants for the FFA and for those reasons were used in this study. The purpose of the present report is to describe the distribution of palmitate between the free form and palmitate bound by human serum albumin (HSA) as multiple complexes of palmitate and HSA; the various distribution values were computed using previously published stepwise equilibrium constants (2) and a recently developed computer program (4). The implications of this theoretical analysis with respect to the delivery of FFA

by serum albumin to tissues are also considered along with competition for binding sites by other ligands.

METHODS

The distribution of palmitate between the free or unbound form, the total amount bound, the amount bound by the different complexes and the concentration of free protein was computed with 12 stepwise equilibrium constants, on an IBM 370/168 computer using a program which was developed for such computations (4), and is more suitable for this purpose than the one used previously for bovine serum albumin (5). The stepwise equilibrium constants used in the computations were 0.6150, 0.2340, and 0.1190×10^8; 0.3100, and 0.1480×10^7; 0.9560, 0.4350, 0.3800, 0.3380, 0.3040, 0.2770 and 0.2540×10^6 (2). The computations were carried out in single precision, however, the output data were rounded off for simplicity to show the trends.

RESULTS

Distribution of Palmitate Between Bound and Unbound Forms and Concentration of Free Albumin. Table 1 shows the changes in the computed distribution of palmitate between the bound and unbound form at five concentrations of palmitate and at a fixed concentration of serum albumin of 580 μmole/L (4 g%), which is in the normal range in plasma. The computations showed that

Table 1 The Distribution of Palmitate and Concentration of Free Albumin at Different Molar Ratios of Palmitate to Human Serum Albumin[a]

	Molar Ratio of Palmitate/HSA				
	0.5	1	2	3	4
Bound Palmitate (μmole/L)	289.9	579.98	1159.9	1739.8	2319.6
Free Palmitate (μmole/L)	0.009	0.021	0.062	0.16	0.4
Free Albumin (μmole/L)	341	186	38	3.3	0.15

[a]The concentration of palmitate was varied from 290 to 580, to 1160, to 1740, to 2320 μmole/L and the concentration of albumin was kept constant at 580 μmole/L for the computations.

more than 99.99% of the palmitate would be bound. Also note that for each doubling of the molar ratio of palmitate to albumin, the amount of free palmitate would increase by a factor greater than two. For example, in going from a ratio of 0.5 to 1, 1 to 2, and 2 to 4 the calculated relative increases in unbound palmitate were 2.3, 3.0, and 6.0 respectively.

Another point of interest is that as the concentration of palmitate increases there would be a progressive decrease in the amount of protein without ligand (Table 1), which is expected, but the values may be of some significance with regard to competive displacement interactions with other ligands. Thus far, little consideration has been given to the concentration of free protein and its possible significance. Computations showed that at a molar ratio of 0.5 about 59% of the albumin would be without any palmitate, while at the molar ratio of 4 only 0.03% would be free. Thus, as the total concentration of palmitate increases, the concentration of free protein progressively decreases.

Distribution of Palmitate Among Different Complexes. Since 12 stepwise equilibrium constants were reported, it was of interest to determine how many complexes of palmitate and HSA would account for the binding of the fatty acid. As shown in Table II at a molar ratio of palmitate to albumin of 0.5, 192.8 μmoles/L would be present as a complex of one palmitate/albumin, and about 83 μmoles/L as the complex of two palmitate /albumin. As the concentration of palmitate increased to 580 μmoles/L there would be marked increase in the concentration of complexes containing 2,3, and 4 moles of palmitate/mole of albumin. A comparison of these data with the concentration of free protein (Table I) shows that as the molar ratio of palmitate to albumin increases, there is an increase in the concentration of the complexes with greater numbers of palmitate and an associated increase in the concentration of free protein. The concentrations of the complexes and free protein are not linear functions of total palmitate.

Table II. The Distribution Of Palmitate Among Complexes at Different Molar Ratios Of Palmitate To Albumin[a]

Complexes[b]	Concentration Of Palmitate (μmole/L) At Molar Ratio Of Palmitate To Albumin				
	0.5	1	2	3	4
1	192.8(66.5)[c]	242.0(41.7)	145.3(12.4)	33.5(19)	3.6(0.16)
2	83.0(28.6)	239.4(41.3)	419.8(36.2)	257.2(14.8)	68.4(2.9)
3	13.6(4.7)	90.3(15.6)	462.4(39.9)	752.7(43.3)	489.5(21.1)
4	0.5(0.2)	7.9(1.4)	117.9(10.2)	510.1(29.3)	811.4(35.0)
5		0.3(0.05)	13.5(1.2)	154.7(8.9)	601.9(25.9)
6			1.0(0.09)	29.1(1.7)	276.9(11.9)
7			0.03(0.003)	2.4(0.14)	56.3(2.4)
8				0.2(0.01)	9.8(0.4)
9					1.5(0.07)
10					0.2(0.009)
11					0.02(0.001)

a. Computations were carried out for concentrations used in Table I Values less than 0.01 μmole/L are not included.
b. The complexes indicate the number of moles of palmitate/mole of albumin.
c. Bracketed items indicated the percent of the total bound palmitate in the complexes.

The Contribution of the Different Complexes in the Delivery of Fatty Acids. The tight binding of palmitate and other FFA by serum albumin can be viewed as a transport function, because a large fraction of the total amount of palmitate is removed in transit through as organ; for example, about one-fourth of the palmitate was reported to be removed by the liver *in vivo* (6) and in perfusion studies *in vitro* (7,8). In order to study the changes in the distribution of palmitate among the complexes, computations were carried out at the molar ratios used above and also at 0.75 of each of

these values which would correspond to a removal of 0.25 of the total amount of palmitate. The net differences in the concentration of the complexes were calculated for molar ratios of 0.5 and 0.375, 1 and 0.75, 2 and 1.5, 3 and 2.25, and finally 4 and 3 (Table III). At a molar ratio of 0.5 the complexes with 1 and 2 moles of palmitate/albumin would contribute the greatest amount of palmitate. As the concentration of palmitate increased the

Table III. Net Contribution of Each Complex of Palmitate-HSA When One-Fourth Is Removed at Different Molar Ratios of Palmitate/Albumin

	Palmitate Removed (μmole/L) at Molar Ratio of Palmitate to Albumin[a]				
Complexes[b]	0.5	1	2	3	4
1	31.9(0.17)[c]	11.0(0.05)	-65.5(-.45)	-77.2(-2.30)	-29.9(-8.31)
2	32.7(0.39)	80.0(0.33)	49.7(0.12)	-150.1(-0.58)	-188.8(-2.76)
3	7.6(0.54)	48.4(0.54)	214.6(0.46)	181.0(0.29)	-263.1(-0.54)
4	0.4(0.80)	5.3(0.67)	79.5(0.67)	324.3(0.64)	301.3(0.37)
5	0.01(1.00)	0.2(0.67)	10.8(0.80)	127.7(0.83)	447.2(0.74)
6		0.01(1.00)	0.8(0.80)	26.7(0.92)	247.8(0.90)
7			0.03(1.00)	2.3(0.96)	53.9(0.96)
8				0.2(1.00)	9.6(0.98)
9				0.01(1.00)	1.5(1.00)
10					0.2(1.00)
11					0.02(1.00)
12					0.00

[a]The calculated amount of palmitate removed at molar ratios of 0.5,1,2,3, and 4 was 72.4, 145, 290, 435, and 580 μmoles/L respectively at an albumin concentration of 580 μmoles/L. See text for details.

[b]The complex indicates the number of moles of palmitate/mole of albumin.

[c]The bracketed terms indicate the fraction of palmitate removed from each complex; the minus signs designate opposite changes resulting from redistribution.

greatest changes would come from the complexes with progressively greater values but some redistribution would take place which is indicated by a minus sign. In general, at lower molar ratios of palmitate to albumin the contributions of the complexes with lower number would provide the greatest amount of palmitate. As the molar ratio of palmitate to albumin increased, the contribution of the FFA would come from the complexes with higher numbers of palmitate.

DISCUSSION

The present study, based upon the use of stepwise equilibrium constants shows a change in the concentration of various complexes of palmitate/albumin as the total concentration of palmitate changes. Although such changes might be expected, the relationships between the association constants, the physiological concentrations of FFA and the concentrations of the different complexes are not ready apparent. The relationships can be shown by calculations which are facilitated by use of a computer.*

It has been suggested that the unbound fraction of a fatty acid is an obligatory intermediate in the translocation of a fatty acid from the albumin to a cell surface (9, 10). Since the amount of free or unbound palmitate would be very small (0.009 to 0.4 μmole/L) in comparison with the amount released in transit through an organ, it would appear that the rate of dissociation of palmitate from albumin may be of great physiological importance. For example, calculations showed that at a molar ratio of 0.5, 72.5 μmole/L of palmitate would be removed which is about 8,000 times the amount which would be free (0.009 μmole/L). At a molar ratio of 4 the ratio of the amount removed to the amount free is about 1400. (Table I and III).

The present calculations showed marked differences in the net changes in the concentrations of the different complexes at different molar ratios of

*Similar changes have been found for other long chain of fatty acids, (12:0, 14:0, 16:0, 18:0, 18:1, 18:2) with, however, quantitative differences. Therefore the comments suggested by the present work appear to apply not only for palmitate but other long chain fatty acids as well.

palmitate/albumin when the same fraction of FFA was removed (Table III). Perhaps these differences could explain, in part, some previous observations which showed that the uptake of FFA by perfused liver was markedly affected by the flow rate at a molar ratio of 2.1, but only slightly at a molar ratio of 0.7 (11). The net removal of FFA bound to the complexes with different number of moles of FFA per mole of albumin could be limiting or not depending upon both the rate of blood flow and the FFA/albumin molar ratio. Thus, hemodynamic variations could modulate the effect of concentration of FFA on the uptake of FFA and in turn the relative utilization of FFA by different tissues in the body. From this perspective the binding of FFA by serum albumin can be regarded as playing a regulatory role in the uptake of FFA by tissues and not solely a passive transport system for FFA.

It should be pointed out that in vivo the binding would be affected by the presence of: a) other long chain FFA, and other materials competing simultaneously for the same binding sites in serum, b) red cells (12-14) and lipoproteins (15-18) which also have some affinity for FFA and c) an albumin bound pool of FFA which has a very slow turnover rate and which is not in rapid equilibrium with the total pool of FFA (9,19).

The data reported in the present paper also has pharmacological implications because many drugs are bound by serum albumin in a multiple fashion by serum albumin, and some of which appear to compete with FFA. Reports (20-25) have shown that at high molar ratios of FFA/albumin, competition can take place effectively between FFA and drug but not at lower ratios. According to Gugler et al. (25) a FFA/albumin molar ratio of 2:1 to 3:1 was needed in order to show a significant displacement of diphenylhydantoin and Warfarin from their binding sites on plasma albumin. At these molar ratios the concentration of free albumin would be only 6.5 and 0.6% of the total protein, respectively (see actual values in Table I), and this reduced concentration of free protein could explain, in part, why it is necessary to reach certain molar ratios of FFA to albumin in order to obtain a significant displacement.

Thus, the present studies could aid in our understanding of certain physiological and pharmacological phenomena.

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