Hydrophobic Interactions of the apo-Gln-I Polypeptide Component of Human High Density Serum Lipoprotein*

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SUMMARY

Apo-Gln-I, the major polypeptide component of human serum high density lipoprotein, has four noninteracting hydrophobic sites which associate with alkanes, anionic detergents, and cationic detergents. Hexane and octane bind to these sites with association constants of $6.8 \times 10^3$ and $1.8 \times 10^3$ liters/mol, respectively, and compete with the anionic detergent, sodium dodecyl sulfate ($C_{12}SO_3^-$), at low detergent ligand binding ratios (i.e. $\leq 1.0$ mol of $C_{12}SO_3^-$ per mol of protein). At higher detergent binding ratios (>$2$ mol of $C_{12}SO_3^-$ per mol of protein) the polypeptide cooperatively binds alkanes and a conformational change is induced.

The similarity between the anionic and cationic detergent binding suggests that the binding sites on aqueous apo-Gln-I are primarily hydrophobic. In the studies reported here these binding sites were further investigated by using the purely hydrophobic ligands, hexane and octane. In addition, the possible competitive or cooperative interaction between these alkanes and $C_{12}SO_3^-$ was measured by studying alkane binding in the presence of varying concentrations of $C_{12}SO_3^-$.

EXPERIMENTAL PROCEDURE

Materials—Human HDL was isolated from fresh serum by the ultracentrifugation flotation method of Scanu et al. (7). The density fraction between 1.063 and 1.21 g/ml was washed by reflootation at 1.21 g/ml until 0.1% $C_{12}SO_3^-$ polyacrylamide gels showed no contamination by serum albumin. After delipidation according to Scanu et al. (7) the apoHDL was dissolved in sufficient $C_{12}SO_3^-$ to saturate the polypeptides at 1.4 g of $C_{12}SO_3^-$ per g of protein (8). Separation of the polypeptide components was achieved by gel filtration on an ascending flow Sephrose 6B column (0.8 x 90 cm) with a flow rate of 10.5 ml/hour. The elution buffer was 0.1% $C_{12}SO_3^-$, 0.02 M Tris-HCl, 10 mM NaN$_3$, and 1 mM EDTA, ionic strength 0.088. The elution pattern for apoHDL in 0.1% $C_{12}SO_3^-$ was similar to that previously reported for 5% guanidine hydrochloride (5). The apo-Gln-I fractions were pooled and the $C_{12}SO_3^-$ was removed by filtration through Dowex AG 1-X8, 200 to 400 mesh anion exchange resin (International Chemical and Nuclear Corp.). Before use, the Dowex resin was sequentially washed with 0.1 N NaOH, distilled water, 0.1 N HCl, and distilled water until the wash had a neutral pH. Approximately 1 ml of settled bed resin was used for each 20 mg of $C_{12}SO_3^-$ to be removed. By use of $^1$H labeled $C_{12}SO_3^-$ it was determined that less than 0.05 mol of $C_{12}SO_3^-$ per mol of apo-Gln-I remained bound after filtration through the anion exchange resin. The protein solution was extensively dialyzed against a pH 8.3, 0.02 M Tris-HCl, 10 mM NaN$_3$, 1 mM EDTA buffer. When necessary, a stirred Amicon Diaflo cell with a PM 10 membrane filter was used to concentrate the protein solution.

As judged by 0.1% $C_{12}SO_3^-$ polyacrylamide gel electrophoresis the apo-Gln-I polypeptide was homogeneous. Less than 0.1 mol of phospholipid per mol of protein was present as assayed by the Bartlett method (9).

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The abbreviations used are: apoHDL, delipidated high density lipoprotein; lipoHDL, high density lipoprotein; apo-Gln-I, the major polypeptide component of apoHDL which has also been referred to as apoA-I, AI, and Fraction III; $C_{12}SO_3^-$, sodium dodecyl sulfate; $C_{14}NMe_3^+$, tetradecyl trimethylammonium ion; detergent $C_{14}NMe_3^+$ binds to apo-Gln-I with the same number of sites and association constant as the anionic detergent $C_{12}SO_3^-$. The similarity between the anionic and cationic detergent binding suggests that the binding sites on aqueous apo-Gln-I are primarily hydrophobic. In the studies reported here these binding sites were further investigated by using the purely hydrophobic ligands, hexane and octane. In addition, the possible competitive or cooperative interaction between these alkanes and $C_{12}SO_3^-$ was measured by studying alkane binding in the presence of varying concentrations of $C_{12}SO_3^-$. A. The major polypeptide component of apoHDL which has also been referred to as apoA-I, AI, and Fraction III; $C_{12}SO_3^-$, sodium dodecyl sulfate; $C_{14}NMe_3^+$, tetradecyl trimethylammonium ion; detergent $C_{14}NMe_3^+$ binds to apo-Gln-I with the same number of sites and association constant as the anionic detergent $C_{12}SO_3^-$.
n-[1-14C]hexane to β-lactoglobulin (Nutritional Biochemicals Corp.) at pH 5.0 was measured and found to be identical with that previously reported (12).

Methods—The binding of volatile hydrophobic ligands to proteins can be conveniently measured by use of a gas solubility cell as described by Wishnia and Pinder (13). All glass apparatus was used having four interconnected chambers that permit exchange of the gas but not the liquid phase. The radioactive solute, either n-[1-14C]hexane or n-[1-14H]octane was introduced into the cell in liquid form using a 10-μl Hamilton syringe by puncturing a serum stopper. At least 1 hour of equilibration time was allowed for each microliter of alkane injected into the cell. Calibrated 100-μl Hamilton syringes were also used to sample the equilibrated solutions which were immediately dispensed into a counting vial containing 20 ml of scintillation fluid (8 g of 2,5-diphenyloxazole (PPO), 0.4 g of 1,4-bis[5(phenyloxazoyl)benzene (POPOP), 1.0 liter of Toluene X-100, 2.0 liters of toluene). The amount of free alkane was calculated from duplicate aliquots of the liquid phase from the dialysate-containing chamber. One chamber was reserved for each protein solution. The solubility of the alkane in C12-OSO4- micelles (in 0.15 M NaCl) was measured in a similar fashion. A 10 mm stock solution of C12-OSO4- was used and corrected for the concentration of monomeric C12-OSO4- at ionic strength of 0.15 (14). Scintillation counting was done in a Beckman model LS-100 counter. Protein concentrations of apo-Gln-I were determined with a Cary 15 spectrophotometer using an extinction coefficient, E1%1cm = 10.94 dl/g-cm and a monomer molecular weight of 28,500.

Sedimentation equilibria were determined in a Spinco model E ultracentrifuge equipped with a photoelectric scanner. A partial specific volume, υ, of 0.734 was calculated from the amino acid composition of apo-Gln-I. Circular dichroism was determined using a Cary Model 60 Spectropolarimeter.

The aqueous solubilities of n-[1-14H]octane and n-[1-14C]hexane were measured by injecting 3 to 4 μl of the respective alkane into a 10-ml serum stopped vial filled with double-distilled water. Aliquots of the aqueous phase were withdrawn with a 100-μl calibrated Hamilton syringe and injected into a scintillation vial and counted. The specific activities of n-[1-14H]octane and n-[1-14C]hexane were 7.47 X 10^6 and 1.77 X 10^7 pmol/cpm, respectively.

Results

Renaturation of apo-Gln-I from 0.1% Sodium Dodecyl Sulfate—The pooled fractions of apo-Gln-I from the 0.1% C12-OSO4- column were filtered through a Dowex anion exchange resin in order to rapidly remove the denaturing detergent. When the detergent-free apo-Gln-I was reapplied to the same column, without C12-OSO4- in the eluting buffer, a Stokes radius of 26 Å from both 6 M guanidine hydrochloride (5). The near ultraviolet circular dichroism of apo-Gln-I in aqueous solution, obtained from the Dowex anion exchange resin, is shown in Fig. 5 (solid curve 1) and is the same as that previously observed by stepwise renaturation from 6 M guanidine hydrochloride (6).

Sedimentation equilibrium studies of apo-Gln-I renatured from both 6 M guanidine hydrochloride (by stepwise dialysis) and from 0.1% C12-OSO4- (by Dowex anion exchange resin) at pH 8.3 and μ = 0.083, show an identical, but weak monomer-dimer association. On a molar basis there is approximately 85% monomer (28,500 MW) and 15% dimer (57,000 MW) at a total protein concentration of 0.7 mg/ml. These sedimentation equilibrium results will be discussed in detail in a forthcoming publication. The above results reaffirm the previous observation that apo-Gln-I regains an ordered structure independent of the original denaturating solvent (5, 6). In the case of C12-OSO4- the renatured state appears to be the same whether the detergent is removed slowly by stepwise dialysis or rapidly by use of an anion exchange resin.

Binding of n-Octane and n-Hexane to apo-Gln-I—The binding data for n-[1-14H]octane and n-[1-14C]hexane at 0°C are presented in Figs. 1 and 2, respectively. Reversible binding was demonstrated as shown in Fig. 2 where points obtained by either increasing or decreasing the concentration of alkane fall on the same binding isotherm. In addition, the binding of both alkanes is independent of the apo-Gln-I concentration. The binding isotherm for n-hexane was obtained over a 9-fold range of protein concentrations, i.e. from 0.79 mg/ml to 7.02 mg/ml. This indicates that the alkane binding sites are the same for both monomer and dimer apo-Gln-I and are unaffected by monomer-dimer association.

Binding isotherms for C12-OSO4- and C12NMe4+ have previously been fit to four independent and identical binding sites with an association constant of 2 X 10^4 liters/mole (5, 6). An analysis of the binding data in Figs. 1 and 2 by iterative curve fitting (10) has been made by assuming both one and four independent sites. The curves assuming four sites (solid) rather than one site (dashed) give a better fit to the experimental data. The calculated association constants, assuming four sites, are 6.8 X 10^4 liters/mole and 1.8 X 10^5 liters/mole for n-hexane and n-octane, respectively.

Although the assumption of four independent and identical sites adequately fits most of the data in Figs. 1 and 2 it is likely that a more complex combination of nonequivalent and slightly cooperative sites might fit the data equally well. In this regard it should be kept in mind that the free concentration of alkane is limited by its saturation vapor pressure at 1 atm in the solubility cell. The experimentally observable portion of the protein-alkane binding isotherm is thus limited by the competing process of alkane phase separation. (A complete discussion of hydrocarbon binding to water-soluble proteins is given by Tanford (16).)

Alkane Binding to apo-Gln-I in Presence of Detergent—The binding of n-hexane and n-octane to apo-Gln-I was measured at varying initial concentrations of C12-OSO4- as shown in Figs. 3 and 4. When 0.85 mol of C12-OSO4- per mol of apo-Gln-I was initially bound, a noticeable reduction in hexane binding was observed compared to binding in the absence of detergent (see Fig. 3). This result is compatible with the suggestion that the alkane and detergent binding sites of apo-Gln-I are identical. At higher initial C12-OSO4- concentrations, however, a coopera-

![Fig. 1](https://example.com/fig1.png)  
Fig. 1 (left). The interaction between n-octane and apo-Gln-I at 0°C, pH 8.3 and μ = 0.083. The solid curve is a fit to four independent sites, dashed curve is for one site. Data points are: O, forward; □, reverse.

![Fig. 2](https://example.com/fig2.png)  
Fig. 2 (right). The interaction between n-hexane and apo-Gln-I at 0°C, pH 8.3 and μ = 0.083. The solid curve is a fit to four independent sites, dashed curve is for one site. Data points are at the following protein concentrations: O, at 0.79 mg/ml; □, at 1.18 mg/ml; ●, at 3.14 mg/ml; ●, at 7.02 mg/ml.

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The binding of \( \text{n-hexane} \) to apo-Gln-I in the presence of \( \text{C}_{12}\text{OSO}_3^- \). Initial binding and equilibrium concentrations of detergent were: \( C_0, \bar{v} = 0.85, C_{eq} = 1.7 \times 10^{-4} \text{ M}; \) \( C_0, \bar{v} = 4.4, C_{eq} = 1.0 \times 10^{-4} \text{ M} \). Solid curve is \( \text{n-hexane} \) binding in the absence of detergent.

Fig. 4 (right). The binding of \( \text{n-octane} \) to apo-Gln-I in the presence of \( \text{C}_{12}\text{OSO}_3^- \). Initial binding and equilibrium concentrations of detergent were: \( C_0, \bar{v} = 2.0, C_{eq} = 5.0 \times 10^{-3} \text{ M}; \) \( C_0, \bar{v} = 3.0, C_{eq} = 7.9 \times 10^{-3} \text{ M} \); \( \Delta, \bar{v} = 6.0, C_{eq} = 1.2 \times 10^{-4} \text{ M} \). Solid curve is \( \text{n-octane} \) binding in the absence of detergent.

The near ultraviolet circular dichroism spectra of apo-Gln-I. Curve 1 (---) is aqueous protein; Curve 2 (-----) is protein + \( \text{n-octane} \), \( C_{eq} = 8.8 \times 10^{-4} \text{ M} \); Curve 3 (---) protein + \( \text{C}_{12}\text{OSO}_3^- \) at \( \bar{v} = 6.0 \); Curve 4 (----) protein + \( \text{C}_{12}\text{OSO}_3^- \) at \( \bar{v} = 6.0 \) + \( \text{n-octane} \), \( C_{eq} = 5.2 \times 10^{-4} \text{ M} \).

**DISCUSSION**

Apo-Gln-I has two different modes of interaction with hydrophobic ligands, and they will be considered separately.

**TABLE I**

| Ligand          | \( K_A \) | \( \mu''_P - \mu''_w \) | \( \mu''_{HC} - \mu''_w \) | Temperature |
|-----------------|-----------|-------------------------|---------------------------|-------------|
| \( \text{n-Hexane} \) | \( 6.8 \times 10^6 \) | \(-5.7\) | \(-6.9\) | \( 0^\circ\) |
| \( \text{n-Octane} \) | \( 1.8 \times 10^4 \) | \(-7.5\) | \(-8.6\) | \( 25^\circ\) |
| \( \text{C}_{12}\text{OSO}_3^- \) | \( 2.0 \times 10^4 \) | \(-8.3\) | \( -8.2\) | \( 25^\circ\) |
| \( \text{C}_{12}\text{MN} \text{Me}_3^+ \) | \( 2.0 \times 10^4 \) | \(-8.2\) | \(-8.2\) | \( 25^\circ\) |

* Assuming four independent binding sites.

* The aqueous solubilities were \( 186 \times 10^{-4} \) and \( 5.2 \times 10^{-4} \text{ M} \) for \( \text{n-hexane} \) and \( \text{n-octane} \), respectively.
entropy of transfer (19) which reflects some molecular organization near the surface of the micelle (16).

The hydrophobic binding sites on apo-Gln-I are clearly not like the interior "pockets" of B-lactoglobulin, myoglobin, and hemoglobin, i.e., \( \mu^F - \mu^w > \mu^HC - \mu^w \) for apo-Gln-I, whereas \( \mu^F - \mu^w < \mu^HC - \mu^w \) for B-lactoglobulin, myoglobin, and hemoglobin. Most probably the apo-Gln-I sites are surface-localized and when occupied may still have some contact with water. Alternatively, the sites could have a slightly smaller entropy of transfer due to some degree of molecular organization, as has been found for C12OSO3- micelles.

By studying the binding of ligands with varying alkyl chain lengths it was determined that the hydrophobic binding regions of serum albumin and B-lactoglobulin have a limited size or binding capacity (13, 15). We have already demonstrated that the anionic or cationic polar group on two detergent ligands of similar hydrophobicity (C12OSO3- and C14NMe3+) has no measurable effect on the free energy of association to apo-Gln-I. The data in Table I for alkane binding (and in Fig. 6) may, therefore, be used to calculate the free energy of binding of C12OSO3- to apo-Gln-I. Each A° of hydrophobic surface area of the dodecyl chain is \(-540 A^2\) (18), so the pre-existing pop-pow for C12OS03 binding to apo-Gln-I is \(-8.6 \text{ kcal/mol} at 0.0^\circ \text{C and } -9.7 \text{ kcal/mol} at 25.0^\circ \text{C}\). The experimentally determined value of \( \mu^F - \mu^w \) for C12OSO3- binding to apo-Gln-I is \(-8.6 \text{ kcal/mol at } 0.0^\circ \text{C and } -9.7 \text{ kcal/mol at } 25.0^\circ \text{C}\). The experimentally determined value of \( \mu^F - \mu^w \) for C12OSO3- binding at 25.0°C is \(-8.2 \text{ kcal/mol (see Table I)}, \) significantly less favorable than predicted. We must, therefore, conclude that the binding sites on this polypeptide, while primarily hydrophobic, exhibit an apparent restriction in size such that they can optimally accommodate alkyl chains shorter than C6 or 18.

2. As we have pointed out previously, these four sites cannot directly account for the large number of lipid molecules bound in vivo. Furthermore, the conformation of apo-Gln-I in this state is different from that in vitro as evidenced by circular dichroism data (Fig. 5, Refs. 6 and 17). This polypeptide, however, undergoes a conformational change in the presence of hydrophobic ligands at unbound ligand concentrations higher than those responsible for occupying the four sites discussed above. The final state of apo-Gln-I in the presence of C12OSO3-, C14NMe3+, and deoxycholate at saturation levels is similar to that found in vitro (6) as evidenced by circular dichroism. (It should be noted that in Fig. 1 the onset of a highly cooperative binding phenomenon is observed at the highest levels of octane binding, but is limited by the solubility of octane itself.) We now observe that two hydrophobic ligands at low binding ratios can operate synergistically to induce the cooperative transition at lower concentrations of unbound ligand. Thus, as is shown in Figs. 3 and 4, the presence of low concentrations of C12OSO3- leads to the cooperative binding of either octane or hexane to apo Gln-I.

The implications of this phenomenon to the binding of lipid in vivo are significant. While the free energy of binding of long diacyl phospholipids to apo-Gln-I would not be sufficiently favorable to lead to significant interaction (5, 6), a synergistic effect could operate in vivo in which the essential conformational change results from the binding of a few moles of lysolecithin in the presence of other biological lipids. HoloHDL contains some 4 mol of lysolecithin molecules per HDL particles (1). Preliminary studies of lysolecithin binding to apo-Gln-I in this laboratory by Dr. M. E. Haberland indicates a cooperative interaction accompanied by a conformational change.

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