Biochem. J. (1990) 270, 325–330 (Printed in Great Britain)

Binding of porphyrin to human serum albumin

Structure–activity relationships

Smadar COHEN* and Rimona MARGALIT†
Department of Biochemistry, George S. Wise Life Science Center, Tel-Aviv University, Tel-Aviv 69978, Israel

The equilibrium binding of hydroxyethyl vinyl deuteroporphyrin (HVD) and of irreversible porphyrin aggregates to human serum albumin was studied at the molecular level. This protein may function as an endogenous drug carrier for porphyrins in photodynamic therapy of tumours. HVD–protein binding studies revealed two types of binding sites, which are attributed to the two HVD isomers. The binding constant for the high-affinity isomer, $2.1 (\pm 0.3) \times 10^8$ M^{-1}, is similar to that previously determined for protoporphyrin. At the same time the binding constant for the lower-affinity HVD isomer, $1.8(\pm 0.3) \times 10^6$ M^{-1}, is similar to that previously determined for haematoporphyrin. Irreversible porphyrin aggregates were purified from the haematoporphyrin derivative and from Photofrin and are defined by spectral and chromatographic data. Gel-exclusion studies indicate that the dominant size of these aggregates is ten porphyrin monomeric units. The protein-binding constant of these aggregates is $1.7(\pm 0.2) \times 10^5$ M^{-1}, with four binding sites per protein molecule. The distinction between the HVD isomers along the porphyrin–protein affinity sequence gives insight into the relationship of porphyrin structure to porphyrin–albumin binding. On the basis of this study an evaluation of human serum albumin as an endogenous carrier for porphyrins (at various aggregation states) in photodynamic therapy of tumours is presented.

INTRODUCTION

The affinity of serum albumin and serum lipoproteins for porphyrins (Muller-Eberhard & Morgan, 1975; Morgan *et al.*, 1980; Lamola *et al.*, 1981; Reddi *et al.*, 1981; Smith & Neuschatz, 1983; Grossweiner & Goyal, 1984; Jori *et al.*, 1984; Reyftmann *et al.*, 1984; Moan *et al.*, 1985; Rotenberg & Margalit, 1985; Kessel, 1986*a*; Beltramini *et al.*, 1987; Rotenberg *et al.*, 1987; Kongshaug *et al.*, 1989) indicates a role for these proteins as endogenous carriers of porphyrins administered for PDT (Kessel, 1985; Pottier & Truscott, 1986; Potter *et al.*, 1987; Manyak *et al.*, 1988). As a drug carrier, a protein may aid in the selective delivery of porphyrins to a tumour region and, as suggested for the lipoproteins, may facilitate drug access into the cell via receptor mechanisms (Jori *et al.*, 1984; Reyftmann *et al.*, 1984; Kessel, 1986*a*, Beltramini *et al.*, 1987; Kongshaug *et al.*, 1989). On the other hand, the same carrier may cause a decrease in the amount of porphyrin available for PDT, by its rapid removal from the circulation. The balance between these two activities might differ from one protein to another and from one porphyrin species to another.

The porphyrin preparations currently employed in PDT, such as HPD, Pf, DHE and HPE, are complex porphyrin mixtures (Moan & Sommer, 1981; Kessel & Chou, 1983; Dougherty *et al.*, 1984; Margalit *et al.*, 1985; Swincer *et al.*, 1985; Kessel *et al.*, 1985; Kessel, 1986*b*; Dougherty, 1987; Dougherty & Mang, 1987; Kessel *et al.*, 1987*a*,*b*; Bryne *et al.*, 1987; Potter *et al.*, 1987). In general, these preparations contain two types of species: (*a*) well-defined porphyrins such as HP, HVD (two isomers) and PP, each of which can be present as an equilibrium mixture of monomers and reversible aggregates (Brown & Shillcock, 1976; Karns *et al.*, 1979; Brown *et al.*, 1980; Margalit *et al.*, 1983; Margalit & Cohen, 1983; Margalit & Rotenberg, 1984; Margalit *et al.*, 1985; Cohen & Margalit, 1986); (*b*) irreversible aggregates, which are not well-defined and are porphryin clusters distinguished by the following properties (Moan & Sommer, 1981; Grossweiner & Goyal, 1984; Dougherty *et al.*, 1984; Margalit *et al.*, 1985; Swincer *et al.*, 1985; Dougherty, 1987; Dougherty & Mang, 1987; Kessel *et al.*, 1987*a*,*b*; Bryne *et al.*, 1987; Potter *et al.*, 1987): (i) they contain covalent dimers; (ii) they are of a size range considerably larger than a single porphyrin dimer; (iii) their dilution into aqueous or organic solutions does not lead to dissociation, as is observed with reversible aggregates of HP, HVD and PP.

In view of their complex composition, the intravenous administration of porphyrin preparations such as HPD, Pf, DHE and HPE for PDT should be taken as the simultaneous administration of two systems. One system contains an equilibrium mixture of monomers and reversible aggregates, all of which (i.e. the monomers and the reversible aggregates) could, potentially, bind to a given serum protein. After administration, owing to dilution in plasma, the equilibrium mixture would be shifted in favour of monomers. Another source for changes in the monomer–dimer distribution is the binding to serum proteins, depending on whether monomers alone or both monomers and reversible dimers participate in such binding.

The other system is the irreversible aggregates, which might (with respect to protein binding) be taken as a single, albeit large and not yet well-defined, porphyrin species. As long as protein binding does not induce dissociation of irreversible aggregates, these should exist as a distinct system, not in equilibrium with the other one defined above.

The final analysis and assessment of the advantages and disadvantages of a serum protein as an endogenous porphyrin

Abbreviations used: PDT, photodynamic therapy of tumours; HSA, human serum albumin; DP, deuteroporphyrin IX; HP, haematoporphyrin IX; HPD, haematoporphyrin IX derivative; HPE, haematoporphyrin ester; HVD, hydroxyethyl vinyl deuteroporphyrin; Pf, Photofrin; PP, protoporphyrin IX. It should be noted that we make use of the term 'irreversible aggregates', which is descriptive of a distinction of ill-defined porphyrin clusters from other porphyrin aggregation states, since at present there is no uniformly accepted terminology for them. The term 'DHE' has been used frequently, but we suggest that it should be avoided, as it has seen triple use: it has been used to name the ether-bonded dimer, the ester-bonded dimer and aggregates larger than a dimer.

* Present address: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.
† To whom correspondence should be addressed.

carrier requires investigations in living systems. Nevertheless we find molecular-level studies to be an essential step in providing data for the ultimate evaluation of serum proteins for this task. In previous papers (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987) we have reported on the thermodynamics of binding of monomers and reversible dimers of DP, HP and PP to serum albumin. In the present paper we report our studies on the binding of HVD and of irreversible aggregates isolated from HPD and Pf to HSA. In addition, chromatographic and spectral data are provided, in order to define the specific preparation of the irreversible aggregates used in this study.

EXPERIMENTAL

Materials

HVD and HPD were from Porphyrin Products (Logan, UT, U.S.A.). Pf was from Photofrin Medica (Cheektowaga, NY, U.S.A.). HSA was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bio-Gel P-10 (100–200 mesh) was from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Sephadex LH-20 was from Pharmacia Fine Chemicals (Uppsala, Sweden). All other materials were of analytical grade. Absorbance spectra were recorded on a Kontron 810 spectrophotometer. Fluorescence spectra were recorded on a Perkin–Elmer model MPF-44B fluorimeter.

Methods

Isolation of irreversible aggregates from HPD and Pf. Irreversible porphyrin aggregates were isolated from HPD and from Pf by non-aqueous gel chromatography on Sephadex LH-20 columns, essentially by a method devised by D. Kessel (personal communication). The eluent consisted of tetrahydrofuran/methanol/5 mM-phosphate buffer, pH 7.2 (4:3:3, by vol.). Methanol and tetrahydrofuran were added to porphyrin samples (originally at a concentration of 10 mg/ml in 0.9% NaCl) to give final proportions 1:1:2 (by vol.). The column was operated in the dark. Twelve fractions were collected and analysed by chromatography on mini Bio-Gel P-10 columns and on reverse-phase t.l.c. The fractions containing the irreversible aggregates were pooled, evaporated to dryness under N_2 at 37 °C and redissolved in phosphate-buffered saline, according to our regular procedures (Margalit *et al.*, 1983).

Gel-exclusion chromatography. Gel-exclusion chromatography on Bio-Gel P-10 mini-columns (0.5 cm × 5 cm) was performed essentially by the procedure of Dougherty *et al.* (1984). The eluent was water, adjusted to pH 8. The column was operated in the dark, and the absorbance of each fraction was determined at 370 nm and 390 nm.

Reverse-phase t.l.c. T.l.c. (reverse-phase) of the porphyrin fractions collected from the Sephadex LH-20 or Bio-Gel P-10 columns was essentially by the procedure of Kessel (1982). The plate was Whatman KC18 and the mobile phase was methanol/3 mM-tetrabutylammonium phosphate, pH 3.5 (13:7, v/v).

Binding of HVD monomers to HSA. Binding of HVD monomers to HSA was studied according to our previously reported method, titrating the porphyrin with protein and recording the changes in porphyrin fluorescence (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987). HVD concentration was in the region of 5 nM and HSA concentrations were varied over the 0.01–10 μM range. Excitation was at 405 nm and emission was recorded for the 600–640 nm range. Peak emissions of free and protein-bound HVD were found to be at 620 nm and 634 nm respectively. The emission intensity at 634 nm, observed for each point in the titration, is the contribution of both free and protein-bound porphyrin. The contribution of the protein-bound porphyrin alone could be extracted from the observed value by use of the following data and parameters: the total porphyrin concentration in the system, a calibration curve (run anew for each titration) of free porphyrin at 634 nm and the ratio of the emission intensity of protein-bound HVD to that of free HVD (at 634 nm) for equimolar concentrations. This ratio, which was determined from titrations of porphyrin by albumin, pursued to saturation, was found to be 4:1 (for further details see Rotenberg & Margalit, 1985).

Binding of irreversible aggregates to HSA. Binding of the irreversible aggregates to HSA was studied by titrating the protein with increasing concentrations of the irreversible aggregates and monitoring the changes in the differential absorption spectra of protein-bound versus free porphryin. Dual-chamber spectrophotometric cells, with an optical path of 0.5 cm for each chamber, were used. At the start of a titration both reference and sample cells held HSA (at a concentration in the region of 3 μM) in phosphate-buffered saline in one chamber and phosphate-buffered saline alone in the other chamber. Each titration point consisted of the simultaneous addition of portions of irreversible aggregates, in the concentration range 1–300 μM (expressed in units of 600 Da), to the protein-containing chamber in the sample cell and to the buffer-containing chamber in the reference cell. In addition, portions of buffer of identical volume were added to the protein-containing chamber in the reference cell.

The absorption spectrum over the 350–450 nm range was recorded for each point in the titration. We found the difference spectrum to peak at 406 nm and have determined the difference between the absorption coefficients of protein-bound and free irreversible aggregates at 406 nm to be $2.6(\pm 0.1) \times 10^3$ $M^{-1} \cdot cm^{-1}$.

RESULTS

Binding of HVD monomers to HSA

Typical results of the fluorimetric titration of HVD with increasing concentrations of protein, monitored by the changes in porphyrin fluorescence, are illustrated in Fig. 1. The data show quite clearly two titration steps, indicative of two binding processes. This pattern contrasts with those that we have found in similar titrations with DP, HP and PP monomers, which were

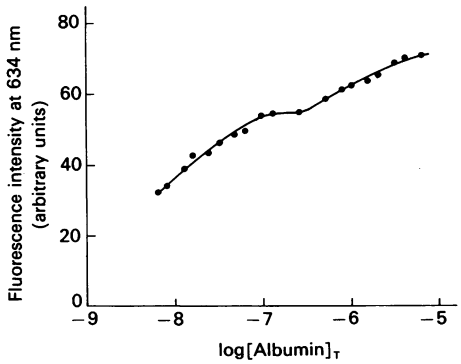

Fig. 1. Increase in fluorescence intensity of HSA-bound HVD with increase in protein concentration

Excitation was at 405 nm and peak emission at 634 nm. The points are the experimental data, and the continuous curve is non-theoretical, drawn in order to emphasize the pattern of the data.

invariably single-step titrations (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987).

The phenomena of two binding steps observed for the association of HVD with albumin can be accounted for on the following basis. There are two HVD isomers, with equal distribution in the HVD preparation studied here. We propose that both compete for the single high-affinity site that HSA has for porphyrin monomers (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987). If the HVD isomers differ in their affinity to the protein, this would be expressed as two binding processes.

The two binding processes can be simply described as follows:

$$HVD_h + A \xrightarrow{K_h} HVD_h\text{–}A \quad (1)$$

$$HVD_l + A \xrightarrow{K_l} HVD_l\text{–}A \quad (2)$$

HVD_h and HVD_l denote the isomers with the higher (relatively) and lower affinities respectively, A, HVD_h–A and HVD_l–A denote the free protein, the high-affinity and the low-affinity protein–HVD complexes respectively, and K_h and K_l are the corresponding equilibrium binding constants. The distinct division into two titration steps (as illustrated in Fig. 1), together with the allowed assumption of equal quantities of the two isomers in a racemic solution, made it possible to determine the magnitude of the two binding constants. Those are listed in Table 1 along with similar data that we have previously determined for other porphyrin monomers (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987).

Characterization of the irreversible aggregates

Before the presentation of protein-binding data, we found it prudent to provide chromatographic and spectral data establishing the nature of the fractions isolated from the Sephadex LH-20 column, especially those that should contain irreversible aggregates.

Each of the 12 fractions isolated from the Sephadex LH-20 column was subjected to reverse-phase t.l.c. (as described in the Experimental section). Typical results of chromatogram scanning (by fluorescence) for complete HPD and for fractions 2, 6 and 10 are given in Fig. 2. Clearly, and in agreement with previously established data (Kessel, 1986*b*), the material contained in the early Sephadex LH-20 fraction (i.e. fraction 2) remains at the origin. The material from the late Sephadex LH-20 fraction (i.e. fraction 10) contains monomers, and the material of the intermediate fraction is not well-resolved.

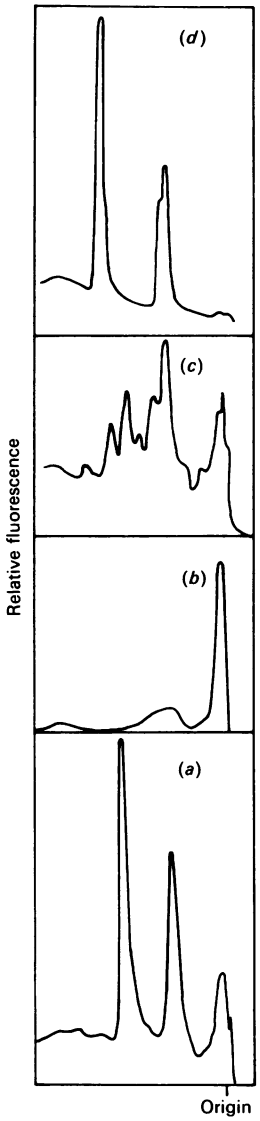

Fig. 2. Fluorescence scanning of a reverse-phase t.l.c. plate of complete HPD and HPD fractions isolated by chromatography on Sephadex LH-20 columns

(*a*) Complete HPD; (*b*) fraction 2 (early); (*c*) fraction 6 (intermediate); (*d*) fraction 10 (late).

Table 1. Equilibrium constants of porphyrin binding to HSA

Data were determined in phosphate-buffered saline, pH 7.2, at 37 °C except for 25 °C for the irreversible aggregates. All data are for monomers except those for the irreversible aggregates. $K_{eq.}$ and n are the equilibrium binding constants and the number of sites respectively. The numbers in parentheses are standard deviations.

Porphyrin	n	$10^{-6} \times K_{eq.}$ (M^{-1})	Substituents of variable side chains
HP	1	1.4 (±0.1)*	2 × -CH(OH)CH_3
HVD_l	1	1.8 (±0.3)†	1 × -CH(OH)CH_3 1 × -CH=CH_2
DP	1	51 (±16)*	2 × -H
HVD_h	1	210 (±30)†	1 × -CH(OH)CH_3 1 × -CH=CH_2
PP	1	280 (±70)*	2 × -CH=CH_2
Irreversible aggregates	4	0.17 (±0.02)†	

* Data are from Margalit & Rotenberg (1985) and Rotenberg *et al.* (1987).

† The present work.

Typical absorption spectra of the early and the late Sephadex LH-20 fractions are shown in Fig. 3. As expected for porphyrins, the Soret peak of the aggregated form is shifted to the blue. In addition, early and late Sephadex LH-20 fractions of matching absorbance were found to be far apart, in the expected direction, in the corresponding fluorescence intensities. The emission intensities of the late Sephadex LH-20 fractions were 5–30-fold higher than those of the corresponding early Sephadex LH-20 ones (results not shown).

A third comparison of the early and the late Sephadex LH-20 fractions was done by gel-exclusion chromatography on mini-columns of Bio-Gel P-10. This chromatography requires complete replacement (via drying and dissolution) or organic solvents (legacy of the Sephadex LH-20 chromatography) by water. The transfer from organic to aqueous phase should have no effect on the irreversible aggregates, but should allow monomers to form reversible aggregates (Brown & Shillcock, 1976; Brown *et al.*, 1980; Margalit *et al.*, 1983; Margalit & Cohen, 1983; Margalit & Rotenberg, 1984; Margalit *et al.*, 1985; Cohen & Margalit,

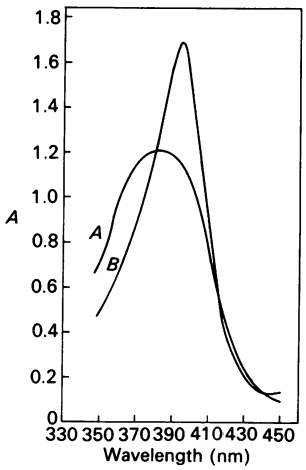

Fig. 3. Absorption spectra of fractions isolated by chromatography of HPD on Sephadex LH-20 columns, dissolved in methanol

Curve *A*, early fraction; curve *B*, late fraction. See the text and the legend to Fig. 2 for definitions of early and late fractions.

1986). As shown by the typical chromatograms presented in Fig. 4 (left-hand side), these expectations were met. The early Sephadex LH-20 fraction was eluted as a single peak with a shoulder, which probably indicates the size-heterogeneity within the irreversible aggregates. This peak, denoted peak A, corresponds to the first peak of complete HPD. The latter was previously shown to be enriched with irreversible aggregates (Dougherty *et al.*, 1984; Margalit *et al.*, 1985). The late Sephadex LH-20 fraction was also eluted as a single peak, denoted peak B, corresponding to the second peak of complete HPD. The latter was previously shown to contain reversible aggregates (Dougherty *et al.*, 1984; Margalit *et al.*, 1985).

Further support for the nature of fractions A and B is given by dilution tests, also presented in Fig. 4 (right-hand side). If dilution drives aggregates to dissociate, the Soret absorption should be red-shifted (Brown & Shillcock, 1976; Brown *et al.*, 1980; Margalit *et al.*, 1985) (see also Fig. 3). The higher the dilution, the larger the shift. A measure of this shift is given by the ratio of the absorbance at 390 nm to that at 370 nm. As shown in Fig. 4, this ratio increases with the increase in the extent of dilution for samples taken from peak B. This trend is

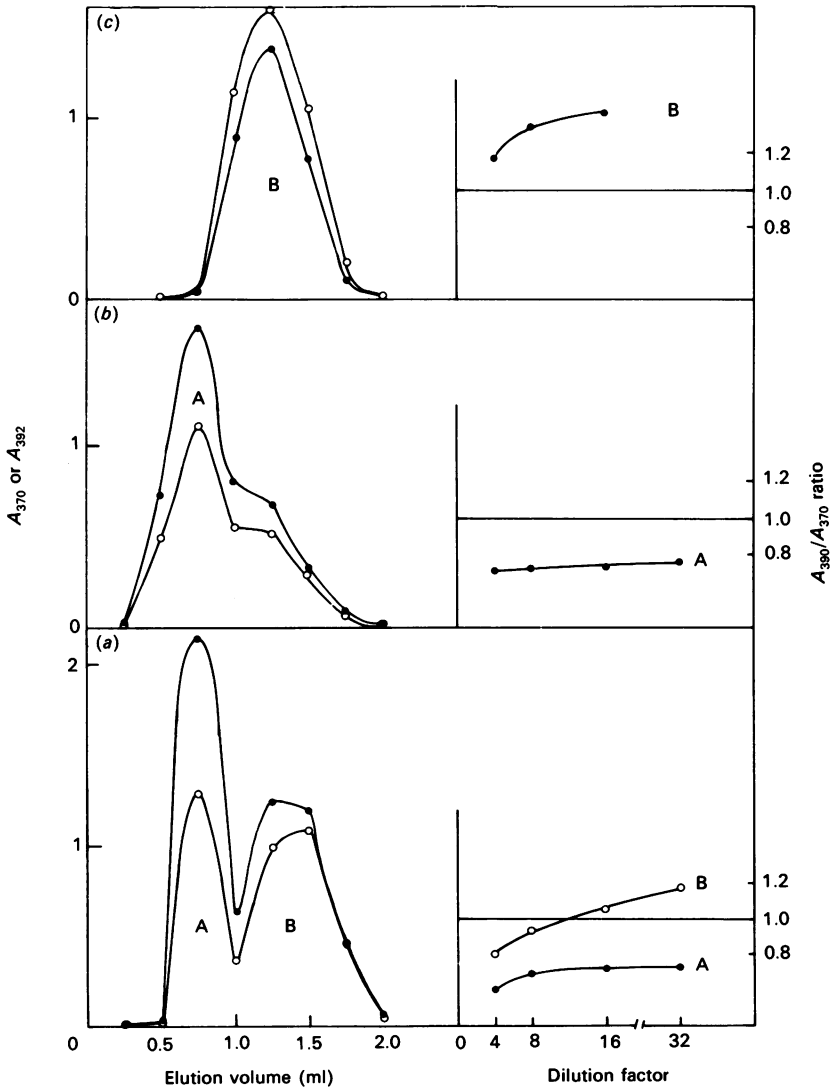

Fig. 4. Chromatography of porphyrins on mini-columns of Bio-Gel P-10

Left-hand side: elution profiles of (*a*) complete HPD, (*b*) early Sephadex LH-20 fraction and (*c*) late Sephadex LH-20 fraction. See the text and the legend to Fig. 2 for definitions of early and late fractions. ●, A_{370}; ○, A_{392}. Right-hand side: tests for dissociation by dilution, as detailed in the text: (*a*), (*b*) and (*c*) are as defined above.

independent of the source of peak B (i.e. complete HPD or late Sephadex LH-20 fractions). In contrast, the absorbance ratios of samples taken from peak A, again independently of source (i.e. complete HPD or early Sephadex LH-20 fractions) are unaffected by dilution, clearly indicating the presence of irreversible aggregates.

Using this type of gel-exclusion chromatography we could also estimate the molecular masses of the components present in the early and the late Sephadex LH-20 fractions. These, after dilution, were found to be on the order of 600 Da and 700 Da respectively, which fit with the t.l.c. data shown in Fig. 2. Thus the dominant magnitude of the irreversible aggregates isolated for the present studies is seen to correspond to ten monomeric porphyrin units.

We stress that this should be taken as an estimate, rather than a precise determination. We also argue that a precise determination of a molecular mass for irreversible porphyrin aggregates is premature. As can be seen from the chromatographic data (Fig. 4) and as shown by others (Moan & Sommer, 1981; Kessel & Chou, 1983; Dougherty *et al.*, 1984; Margalit *et al.*, 1985; Swincer *et al.*, 1985; Kessel *et al.*, 1985; Kessel, 1986*b*; Dougherty, 1987; Dougherty & Mang, 1987; Kessel *et al.*, 1987*a*,*b*; Bryne *et al.*, 1987), current preparations of such species are non-homogeneous in size. Hence our use of the term 'dominant size'. Another point to bear in mind is that the non-homogeneous nature of preparations of irreversible aggregates probably is expressed not only in chromatography but also in other phenomena, for example in membrane (Cohen & Margalit, 1985; Margalit *et al.*, 1985) and in protein binding (Moan *et al.*, 1985).

Taking, together, all the pieces of data presented above, we argue that we have isolated by chromatography on Sephadex LH-20 columns a fraction (the early one) containing the desired irreversible aggregates. Furthermore we suggest that, until complete structural resolution of these species becomes available, chromatographic and spectral (absorption) data of the type presented here will have to serve as an experimental definition, to be supplied whenever chemical and biological activities of such systems are reported.

Binding of the irreversible aggregates to HSA

A typical example of the differential absorption spectra obtained by titrating HSA with increasing concentrations of irreversible aggregates (which can be obtained from either HPD or Pf as starting materials) is shown in Fig. 5. Data obtained from such titrations, plotted according to a Langmuir isotherm, are illustrated in Fig. 6. Such data were processed according to the following one-term isotherm (i.e. for a sirgle type of binding site):

$$\frac{[B]}{[A]_T} = \frac{n \cdot K[F]}{1 + K[F]} \qquad (3)$$

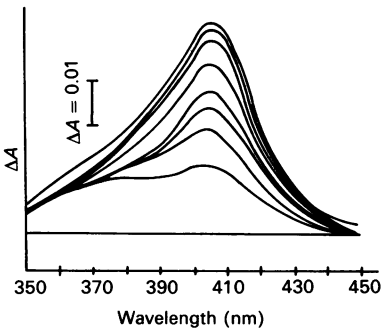

Fig. 5. Typical differential absorption spectra of the titration of HSA by irreversible porphyrin aggregates isolated from HPD and Pf

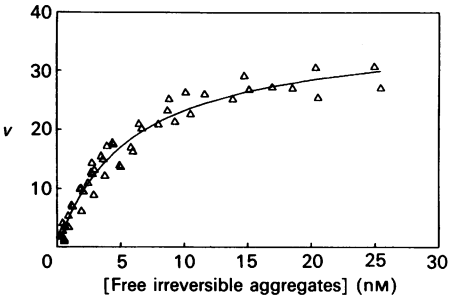

Fig. 6. Langmuir isotherm of the binding of irreversible porphyrin aggregates, isolated from HPD and Pf, to HSA

The points are experimental data and the continuous curve is the theoretical expectation, drawn according to eqn. (3) in the text for the parameters listed there.

where [F] and [B] are the concentrations of free and protein-bound irreversible porphyrin aggregates respectively (expressed in units of 600 Da), $[A]_T$ is the total protein concentration, K is the equilibrium binding constant and n is the number of porphyrin units (of 600 Da each) bound per protein molecule. We found the experimental data to fit well with the expectation for one type of binding site, yielding $K = 1.7(\pm 0.2) \times 10^4\ M^{-1}$ and $n = 38 \pm 1.4$. As can be seen from Fig. 6, the experimental data fit with the expectation according to eqn. (3) for the parameters listed above, over the entire range of concentrations of irreversible aggregates employed in this study.

DISCUSSION

Binding of porphyrin monomers to HSA

In our previous studies we have found the porphyrin–HSA association to be entropy-driven (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987), as expected for hydrophobic interactions (Kauzmann, 1959; Nemethy & Scheraga, 1962; Tanford, 1973). Such interactions are, then, substantial (although not the single) contributors to the high affinity of porphyrins for albumin.

On the basis of the data for DP (Table 1) we suggest that the porphryin core and the constant side-chain substituents account for the major free-energy gain in porphyrin binding to the protein. The substituents in the variable side chains can be seen to either add (as in the case of PP) or subtract (as in the case of HP) from that energy gain, in correlation with the hydrophobicity of the variable side chains (Hansch & Leo, 1979). This implies that the variable side chains are also involved, although in a lesser role, in the favourable hydrophobic interactions between the protein and a porphryin monomer.

The two HVD isomers can be described as 'positional isomers', as they differ from one another in the positioning of the vinyl and hydroxyethyl substituents. One isomer, denoted PI1, has the vinyl and the hydroxyethyl groups at the C-8 and the C-3 positions respectively. The other isomer, denoted PI2, has (obviously) the reverse arrangement.

We have no direct evidence upon which assignments of high and low affinity to HSA could be attempted, each to a specific positional isomer. However, several indirect findings merit consideration: (i) the experimentally observed relationship between the hydrophobicity of a given side chain and its effect on the magnitude of the binding constant (discussed above); (ii) the similarities, in terms of magnitudes of binding constants, of HVD_l and HVD_h to HP and PP respectively (Table 1); (iii) reports that the HVD PI1 is the more hydrophobic of the two (Bonnett *et al.*, 1981). Taking these findings together, we propose that HVD_h is PI1. It follows that HPV_l is PI2.

Binding of irreversible aggregates to HSA

The binding of the irreversible aggregates to the protein is quite different from that of the monomers, as well as from that of reversible dimers (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987). Although the binding remains favourable, the magnitude of the equilibrium binding constant is two to four orders of magnitude lower than the constants determined for monomers and reversible dimers. Another difference is in the number of sites. As shown in the Results section, analysis of the binding data yielded a stoichiometry of 38 ± 1.4 porphyrin molecules (i.e. 600 Da units) to 1 protein molecule. Taking into account the estimated dominant size of the present preparation of irreversible aggregates, this implies that a HSA molecule has, at the least, four binding sites for irreversible porphyrin aggregates.

These differences in HSA binding between reversible (including monomers and dimers) and irreversible aggregates seem to us quite reasonable. We did not expect similar driving forces, or the same sites for a small monomer (or dimer) and for the considerably larger aggregates.

Molecular properties of HSA as an endogenous porphyrin carrier

Four types of porphyrin–HSA complexes can be defined, on the basis of the data presented here and in our previous papers (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987): HSA_1M_1, HSA_1D_1, $HSA_1M_1D_1$ and HSA_1IA_4, where M, D and IA represent monomers, reversible dimers and irreversible aggregates respectively.

The data reported here provide two molecular factors involved in the assessment of the merits of albumin as an endogenous drug carrier: affinity and capacity. The sequence of HSA affinity for porphyrins is dimer > monomer > irreversible aggregate (Rotenberg & Margalit, 1985; Rotenberg *et al.*, 1987; the present work). In terms of HSA carrier capacity, the sequence is reversed: when carrying monomers, dimers or both, a single protein molecule can carry at the most three 600 Da units. In contrast, when carrying the irreversible aggregates, a single protein molecule can carry close to 40 600 Da units.

Thermodynamic considerations indicate that an efficient drug carrier should be capable of high payloads yet have only moderate affinity for the drug. The moderate affinity should be sufficiently high in order to make the macromolecule a carrier at all, but not too high in order to allow drug release at the target. In this respect albumin has two modes of action as a porphyrin drug carrier: a 'high-capacity–moderate-affinity' mode when carrying the irreversible aggregates and a 'low-capacity–high-affinity' mode when carrying monomers and reversible dimers.

Carrying porphyrins to the tumour (whether directly or via drug transfer to the lipoproteins) HSA should deliver more drug (per 600 Da unit) when acting in the first mode. In clearing porphyrins from the tumour, the involvement of HSA should be more extensive when acting in the second mode. Although albumin is only one of the factors involved, such discrimination among porphyrin species, with respect to delivery and clearance at the tumour sites, fits with the data obtained *in vivo*: irreversible porphyrin aggregates are retained in the tumour for prolonged periods, whereas in contrast the clearance of monomers from the tumour is rather fast (Kessel *et al.*, 1987*a*,*b*; Dougherty, 1987; Dougherty & Mang, 1987).

In conclusion, from molecular-level studies on the binding of porphyrin to albumin, we have found distinct quantitative differences upon which a beginning can be made towards the evaluation of albumin as an endogenous carrier for this group of drugs. Additional insight into this matter will come from extension of such studies to other serum proteins, in particular to the lipoproteins.

Received 7 November 1989/27 February 1990; accepted 19 March 1990

REFERENCES

Beltramini, M., Firey, P. A., Ricchelli, F., Rodgers, M. A. J. & Jori, G. (1987) Biochemistry **26**, 6852–6858

Bonnett, R., Ridge, R. J., Scourides, P. A. & Berenbaum, M. C. (1981) J. Chem. Soc. Perkin Trans. **1**, 3135–3140

Brown, S. B. & Shillcock, M. (1976) Biochem. J. **153**, 279–285

Brown, S. B., Hazikonstantinou, H. & Herries, D. (1980) Int. J. Biochem. **12**, 701–707

Bryne, C. J., Marshallsay, L. V. & Ward, A. D. (1987) Photochem. Photobiol. **46**, 575–580

Cohen, S. & Margalit, R. (1985) Biochim. Biophys. Acta **813**, 307–312

Cohen, S. & Margalit, R. (1986) Arch. Biochem. Biophys. **247**, 57–61

Dougherty, T. J. (1987) Photochem. Photobiol. **46**, 569–573

Dougherty, T. J. & Mang. T. S. (1987) Photochem. Photobiol. **46**, 67–70

Dougherty, T. J., Potter, W. R. & Weishaupt, K. R. (1984) in Porphyrin Localization and the Treatment of Tumors (Doiron, D. R. & Gomer, C. J., eds.), pp. 301–314, Alan R. Liss, New York

Grossweiner, L. & Goyal, G. (1984) Photochem. Photobiol. **40**, 1–5

Hansch, C. & Leo, A. J. (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology, John Wiley and Sons, New York

Jori, G., Beltramini, M., Reddi, E., Salvato, B., Pagnana, A., Ziron, L., Tomio, L. & Tsanov, T. (1984) Cancer Lett. **24**, 279–291

Karns, G., Gallagher, W. & Elliott, W. (1979) Bioorg. Chem. **8**, 69–81

Kauzmann, W. (1959) Adv. Protein Chem. **14**, 1–63

Kessel, D. (1982) Cancer Res. **42**, 1703–1706

Kessel, D. (ed.) (1985) Methods in Porphyrin Photosensitization, Plenum Press, New York

Kessel, D. (1986*a*) Cancer Lett. **33**, 183–188

Kessel, D. (1986*b*) Photochem. Photobiol. **44**, 489–493

Kessel, D. & Chou, T. (1983) Cancer Res. **43**, 1994–1999

Kessel, D., Chang, C. K. & Musselman, B. (1985) in Methods in Porphyrin Photosensitization (Kessel, D., ed.), pp. 213–227, Plenum Press, New York

Kessel, D., Thompson, P., Musselman, B. & Chang, C. K. (1987*a*) Cancer Res. **47**, 4642–4645

Kessel, D., Thompson, P., Musselman, B. & Chang, C. K. (1987*b*) Photochem. Photobiol. **46**, 563–568

Kongshaug, M., Moan, J. & Brown, S. B. (1989) Br. J. Cancer **59**, 184–188

Lamola, A., Asher, I., Muller-Eberhard, U. & Poh-Fitzpatrick, M. (1981) Biochem. J. **196**, 693–698

Manyak, M. J., Russo, A., Smith, P. D. & Glastein, E. (1988) J. Clin. Oncol. **6**, 380–391

Margalit, R. & Cohen, S. (1983) Biochim. Biophys. Acta **736**, 163–170

Margalit, R. & Rotenberg, M. (1984) Biochem. J. **219**, 445–450

Margalit, R., Shaklai, N. & Cohen, S. (1983) Biochem. J. **209**, 547–552

Margalit, R., Cohen, S. & Rotenberg, M. (1985) in Methods in Porphyrin Photosensitization (Kessel, D., ed.), pp. 303–313, Plenum Press, New York

Moan, J. & Sommer, S. (1981) Photobiochem. Photobiophys. **3**, 93–103

Moan, J., Rimington, C. & Western, A. (1985) Clin. Chim. Acta **145**, 227–236

Morgan, W., Smith, A. & Koskelo, P. (1980) Biochim. Biophys. Acta **624**, 271–285

Muller-Eberhard, U. & Morgan, T. (1975) Ann. N.Y. Acad. Sci. **244**, 624–649

Nemethy, G. & Scheraga, H. A. (1962) J. Chem. Phys. **36**, 3401–3417

Potter, W. R., Mang, T. S. & Dougherty, T. J. (1987) Photochem. Photobiol. **46**, 97–101

Pottier, R. & Truscott, T. G. (1986) Int. J. Radiat. Biol. **50**, 421–452

Reddi, E., Ricchelli, F. & Jori, G. (1981) Int. J. Pept. Protein Res. **18**, 402–408

Reyftmann, J. P., Morliere, P., Goldstein, S., Santus, R., Dubertret, L. & Lagrange, D. (1984) Photochem. Photobiol. **40**, 721–729

Rotenberg, M. & Margalit, R. (1985) Biochem. J. **229**, 197–203

Rotenberg, M., Cohen, S. & Margalit, R. (1987) Photochem. Photobiol. **46**, 689–693

Smith, A. & Neuschatz, T. (1983) Biochem. J. **214**, 503–509

Swincer, A. G., Ward, A. D. & Howlett, G. J. (1985) Photochem. Photobiol. **41**, 47–50

Tanford, J. (1973) The Theory of the Hydrophobic Effect, John Wiley and Sons, New York