Drug-binding properties of human α -foetoprotein

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The drug-binding properties of human α -foetoprotein (α FP) were investigated by a fluorescence-spectral method. Human α FP was shown to bind to albumin's site I marker (warfarin, phenylbutazone), site II marker (L-tryptophan), but not site III marker (cholic acid, digoxin). The binding of human α FP towards lower alcohols was examined, and this binding seems to depend partly on the hydrophobicity of the ligands. The binding of human α FP is discussed in comparison with human serum albumin or rat α FP.

INTRODUCTION

It is well known that human serum albumin (HSA) binds various drugs, and the distribution and pharmacological actions of drugs can be largely attributed to this. Hence the examination of the binding of a drug to albumin is a significant factor in pharmacokinetic analysis. Furthermore, the displacement of one drug by another from the albumin binding sites is thought to contribute to drug interaction, and the side effects caused by the liberated drug raise a serious problem (Christensen *et al.*, 1963; Aggeler *et al.*, 1967). A clear understanding of albumin binding would therefore be helpful for predicting binding phenomena such as displacement reactions. Since the primary structure of HSA is known, the location and composition of the drug-binding sites in the HSA molecule can be investigated (Fehske *et al.*, 1981).

During pregnancy, when drugs are given to the mother, a proportion of them passes into the foetus, an event that may have an undesirable effect on its development (Niebel, 1982; Berkowitz *et al.*, 1983). Consequently the serum transport of drugs into the foetus and the newborn is an important problem. Thus it seemed worth while to determine the binding properties of the individual serum proteins in regard to a particular drug.

Among serum proteins, α FP is predominantly a foetal glycoprotein. α FP shares many similarities with HSA, including sequence homology (Gorin *et al.*, 1981; Morinaga *et al.*, 1983), immunological cross-reactivity (Ruoslahti & Engvall, 1976) and physiological properties (Ruoslahti & Terry, 1976; Luft & Lorscheider, 1983). Although the biological role of α FP remains to be elucidated, the proposed functions of α FP include the binding of substances such as oestrogen (Uriel *et al.*, 1976; Vallette *et al.*, 1980), fatty acids (Berde *et al.*, 1979; Savu *et al.*, 1981), dyes (Berde *et al.*, 1979) and the maintenance of osmotic pressure in the foetal body. α FP may therefore function as a foetal albumin.

In a previous paper (Hirano *et al.*, 1984a) we examined the drug-binding properties of human α FP and reported that the binding was undetectable by the peroxidation method. Thereafter the binding properties of rat α FP towards a series of compounds was investigated by equilibrium dialysis, and it was found that rat α FP bound albumin's high-affinity site I markers (warfarin, phenyl-

butazone, azapropazone), but neither site II (diazepam) nor site III (digoxin, cholic acid) markers (Herve *et al.*, 1984). In view of the differences between human and rat α FP, it seemed to us worth while to re-examine the drug binding of human α FP by more sensitive methods. In the present study we have investigated the drug binding of human α FP by the fluorescence-spectral method, and the drug-binding abilities of HSA and human α FP are compared.

MATERIALS AND METHODS

α FP was purified from human umbilical-cord serum as described previously (Watanabe *et al.*, 1982). HSA was purchased from Miles Laboratories. α FP and HSA were confirmed to be homogeneous by disc and SDS/polyacrylamide-gel electrophoresis. The protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The ligands, namely phenylbutazone, acetazolamide, warfarin, amethopterin, tetracycline, cholic acid, digoxin, phenytoin and streptomycin, were obtained from Sigma; L-tryptophan was from Wako Pure Chemical Industries, Osaka, Japan. β -Oestradiol was purchased from Tokyo Kasei Industries, Tokyo, Japan.

Delipidation by treatment with charcoal

α FP and HSA were delipidated with activated charcoal (Chen, 1967). Protein solution (0.1 mg/ml) was incubated with Norit A charcoal (0.05 mg/ml) for 60 min at 4 °C and at pH 3.0. The mixture was then centrifuged for 10 min at 4 °C and 10000 g, and the supernatants was collected and adjusted to pH 7.0.

Fluorescence-spectral method

The binding of ligand to protein was examined by the method of Maes *et al.* (1978). A 1.5 μ M solution of α FP or HSA was prepared in 10 mM-sodium phosphate buffer, pH 7.4, containing 0.9% NaCl. Then 100 μ M concentrations of each ligand solution were prepared in buffer containing 1.5 μ M- α FP or -HSA. Portions of ligand solution were added to 2 ml of α FP or HSA solution. After each addition of ligand, the fluorescence spectrum was excited at 290 nm and emission was recorded at 335 nm at room temperature. Bandwidths of excitation and emission were both 10 nm.

Abbreviations used: α FP, α -foetoprotein; HSA, human serum albumin.

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Table 1. Binding of ligands to HSA

The affinity (K) and capacity (n) of the binding sites were determined at a protein concentration of $1.5 \mu\text{M}$. K and n denote the apparent association constant and the number of the binding sites respectively.

Ligand	n	$10^6 \times K \text{ (M}^{-1}\text{)}$
Cholic acid	1.9 ± 0.28	0.78 ± 0.12
Digoxin	2.5 ± 0.12	0.60 ± 0.080
Phenylbutazone	1.3 ± 0.22	2.7 ± 0.13
L-Tryptophan	3.4 ± 0.29	0.32 ± 0.025
Warfarin	2.8 ± 0.25	0.54 ± 0.16

Correction for the quenching due to the inner filter was calculated (Parker, 1968). After this correction for self-absorption, the actual quenching of the fluorescence by the bound drug was obtained. Quenching (Q) was defined as:

$$Q = 1 - (F/F_0)$$

where F_0 and F are the corrected fluorescence values of protein solution and the protein/ligand mixture respectively. Maximum quenching values were determined by extrapolating the mean corrected values (Wallach *et al.*, 1970). The binding data were plotted as described by Scatchard (1949), and the apparent association constants for proteins were calculated. Each value is the mean for three duplicate experiments.

RESULTS AND DISCUSSION

Delipidation of α FP and HSA

Both human α FP and HSA were known to show charge heterogeneities, which came from the binding with endogenous or exogenous ligands, such as fatty acids and hormones. However, by treatment with charcoal, human α FP and HSA were confirmed as having pI values of 4.7 and 4.8 respectively.

Binding of ligands to HSA

The binding of ligands that were already known to bind to HSA was investigated. As Table 1 shows, typical site I markers (warfarin, phenylbutazone), site II marker (L-tryptophan) and site III markers (cholic acid, digoxin) bound to HSA. The binding constant of HSA with phenylbutazone is well consistent with that given by Maes *et al.* (1978). However, the binding constants of some of these ligands were higher than those previously reported (Hirano *et al.*, 1984a). This would seem to result from the use of a different data-analysis technique: in the previous report (Hirano *et al.*, 1984a) Q/Q_m was used instead of the ligand/protein ratio (Q and Q_m denote respectively the quenching of the protein/ligand mixture and maximal quenching for the protein solution). In the present method the ratio of ligand to protein added to the reaction mixture was employed.

Binding of ligands to human α FP

The binding of ligands to human α FP was investigated. As Table 2 shows, human α FP bound warfarin and phenylbutazone as site I markers and L-tryptophan as a site II marker. However, α FP could not be shown to bind cholic acid and digoxin as site III markers even if the concentrations of drug and ligand were changed. These

Table 2. Binding of ligands to α FP

For details, see Table 1. Abbreviation used: ANS, anilino-naphthalene sulphonate.

Ligand	n	$10^6 \times K \text{ (M}^{-1}\text{)}$
Phenylbutazone	3.4 ± 0.41	0.37 ± 0.067
Warfarin	1.6 ± 0.043	1.4 ± 0.13
L-Tryptophan	2.2 ± 0.45	0.91 ± 0.20
Streptomycin	1.3 ± 0.12	6.9 ± 0.16
Phenytoin	3.9 ± 0.13	0.31 ± 0.019
ANS	1.4 ± 0.043	5.1 ± 1.2
Methanol	2.6 ± 0.12	0.66 ± 0.039
Ethanol	2.3 ± 0.31	0.71 ± 0.12
Propanol	1.2 ± 0.14	7.2 ± 0.49

results suggested that human α FP had a similar structure to HSA on binding sites I and II and the absence of drug-binding site III within human α FP. Also, these results may explain why this region showed the least homology (30% amino acid identity in the domain I) and only a 16% identity at N-terminal residues 1–86 between human α FP and HSA. The findings that human α FP bound site I markers but not site III markers were well in accord with those for rat α FP. In contrast with rat α FP, however, human α FP bound L-tryptophan as site II marker. As for oestrogen, both mouse and rat α FP bound it, but human α FP did not, although rat α FP had 67% primary-structure homology with human α FP. In the present experiments, human α FP was confirmed not to bind β -oestradiol (results not shown).

Next, drugs that are known to cause unexpected effects on the foetus during pregnancy were examined for binding to α FP. As shown in Table 2, human α FP could bind streptomycin and phenytoin. But acetazolamide, tetracycline and amethopterin could not be shown by the present method to bind to human α FP.

The binding of lower alcohols to human α FP was also investigated. As shown in Table 2, the apparent association constants increased in proportion to the increase in the number of carbon atoms in the chain. The results confirmed that the binding properties of human α FP partly depend on the hydrophobicity of the ligands. Furthermore, anilino-naphthalene sulphonate, which is known to bind to the hydrophobic region on the surface of proteins, was examined for its binding to human α FP. Its apparent association constant was lower than those previously reported (Hirano *et al.*, 1984b), and this would seem to result from differences in the techniques used. In the previous paper (Hirano *et al.*, 1984b) the amount of bilirubin liberated from protein–bilirubin complex by binding of ligand was measured, whereas, in the present experiment the fluorescence quenching resulting from the protein–ligand binding was measured.

Concluding remarks

In the adult, HSA serves as a depot protein and a transport protein for low- M_r endogenous and exogenous compounds. α FP was also shown to bind bilirubin, fatty acids, anilino-naphthalene sulphonate and oestrogen. The present study confirmed that human α FP had a similar structure, except for the digitoxin-binding site, to that of HSA. The low-affinity drug-binding ability of human α FP and the low HSA in foetal plasma indicated that

when drugs are given to pregnant women, considerable care needs to be exercised.

It will therefore be necessary to study the ability of human α FP to bind to other classes of drug, since a clarification of the role of human α FP in the foetus as a drug-transporting protein would give us information about the effects of administering drugs to pregnant women.

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