Gossypol binds to a high-affinity binding site on human serum albumin

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The triterpene gossypol competes with bilirubin for a high-affinity binding site on human serum albumin. Similar competition between bilirubin and gossypol occurs in the binding of these ligands to the glutathione *S*-transferases from human liver and placenta. In each case, gossypol and bilirubin exhibit similar binding constants. The binding properties of gossypol may generally mimic those of bilirubin.

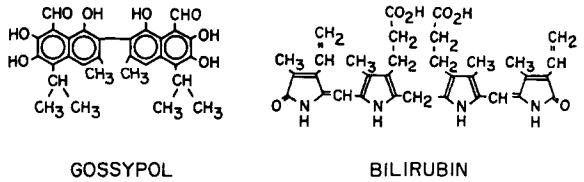
Albumin *Glutathione S-transferase* *Gossypol* *Bilirubin*

1. INTRODUCTION

Recent reports from the People's Republic of China on the antifertility properties of gossypol have prompted a renewed interest in this abundant polyphenolic triterpene obtained from cottonseed oil [1]. Clinical trials in China suggest that gossypol is an effective, non-steroidal, antifertility drug for men [2,3]. In addition, reports have appeared recently on the potential usefulness of gossypol as a drug against herpes simplex virus [4], Chagas disease [5] and malaria [6]. In view of the potential broad spectrum activity of gossypol as a drug, knowledge of the transport and metabolism of gossypol will be useful. In general, it appears from studies with a variety of animals that gossypol is metabolized primarily in the liver where glucuronides and sulfate esters of gossypol are produced and subsequently excreted into the bile [7]. There are no data on the mode of transport of gossypol in the circulation or in the liver. Here, we demonstrate that gossypol binds to a high-affinity binding site on human serum albumin with an affinity comparable to that for the binding of bilirubin.

2. MATERIALS AND METHODS

Bilirubin, gossypol-acetic acid (scheme 1) and human serum albumin were obtained from Sigma. Stock solutions of bilirubin were prepared fresh in 0.1 M KOH and kept in the dark. Stock solutions of gossypol were prepared in 95% ethanol. The binding of gossypol to albumin was determined by following the quenching of the intrinsic protein fluorescence upon addition of gossypol; pH 7.4, 0.02 M potassium phosphate, 0.1 M NaCl, 25°C, λ_{ex} = 280 nm, λ_{em} = 320 nm, slits = 3 nm. The binding of bilirubin to albumin and subsequent displacement of the bilirubin by gossypol was followed by circular dichroism, using the same buffer system as was used in the fluorescence studies.



GOSSYPOL BILIRUBIN

SCHEME I

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3. RESULTS

3.1. *Fluorescence titration of the binding of gossypol to human serum albumin*

Human serum albumin contains a single tryptophan residue, located in domain II [8]. The fluorescence emission spectrum of albumin, due mainly to tryptophan emission, overlaps with the absorption spectrum of gossypol. The addition of gossypol to solutions of albumin results in quenching of the intrinsic fluorescence, as shown in fig.1, when gossypol was added to 1 μM albumin. Quenching of the fluorescence is essentially stoichiometric for the first equivalent of gossypol, suggesting that binding is taking place at a high-affinity site.

3.2. *Competition between bilirubin and gossypol for binding to human serum albumin*

Fig.2 (curve a) shows the circular dichroic spectrum of the bilirubin–albumin complex formed by treating 50 μM albumin with one equivalent of bilirubin. Addition of up to 5 equivalents of gossypol to this solution results in a progressive displacement of bilirubin, as monitored by loss of the circular dichroic spectrum. The complex of gossypol and albumin produces a very weak spectrum which does not interfere. Three equivalents of gossypol can displace 50% of the bilirubin. Previously we determined the dissociation constant for bilirubin under the same experimental conditions used in the present study, $K_d = 3 \times 10^{-8}$ M [9]. Thus we can estimate the dissociation constant for gossypol, $K_d = 9 \times 10^{-8}$ M, based upon the competition in fig.2.

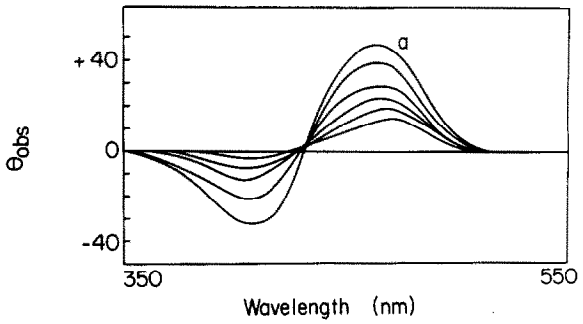

Fig.2. Circular dichroic spectrum of the bilirubin–albumin complex (curve a) and displacement of bilirubin upon addition of 1–5 equivalents of gossypol, pH 7.4; [albumin] = 50 μM. Ellipticities are in millidegrees.

In order to demonstrate that gossypol displaces bilirubin, as suggested by the data in fig.2, bilirubin was added to albumin and the resulting mixture was treated with various amounts of gossypol. The free ligands were separated from albumin-bound ligands by chromatography on Sephadex LH20. Albumin transports 3 equivalents of bilirubin through the column. Gossypol is able to displace 1 equivalent. Albumin transports 4 equivalents of gossypol through the column. Bilirubin is able to displace 1 equivalent. Thus there is a single site where bilirubin and gossypol compete. The circular dichroism data suggest that this is the high-affinity bilirubin-binding site.

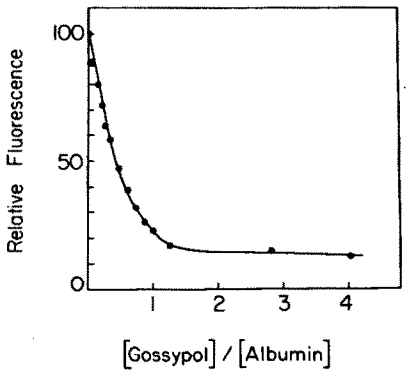

Fig.1. Fluorescence quenching of human serum albumin upon addition of gossypol (pH 7.4) 25°C; [albumin] = 1 μM.

3.3. *Binding of gossypol to other bilirubin-binding proteins*

The glutathione *S*-transferases from human liver [9] and placenta [10] have unique bilirubin-binding sites. Fig.3 shows the circular dichroic spectrum of the bilirubin–transferase complex formed by adding two equivalents of bilirubin to 22 μM placenta transferase. Gossypol can displace about 50% of the bilirubin when the gossypol and bilirubin concentrations are equal, suggesting comparable binding constants for bilirubin and gossypol. Previously, the dissociation constant for bilirubin was determined to be 14 μM [10]. A similar experiment with liver transferase (not shown) again showed that gossypol can displace bilirubin from the bilirubin–transferase complex. In this case

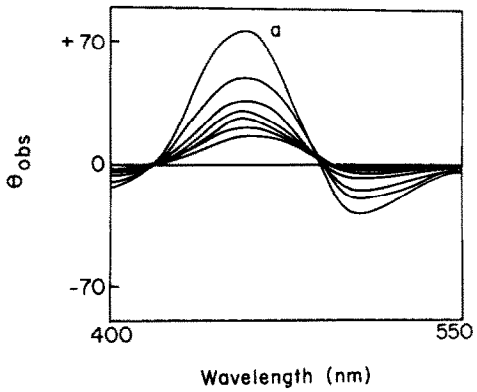

Fig.3. Circular dichroic spectrum of the bilirubin complex with placenta glutathione *S*-transferase (curve a) and displacement of bilirubin by gossypol, pH 7.4. Two equivalents of bilirubin were added to 22 μM transferase, followed by addition of 1–6 equivalents of gossypol.

also, the binding of gossypol or bilirubin showed similar affinities. Thus it appears that, for a variety of proteins which are known to bind bilirubin, the binding of gossypol occurs at the bilirubin-binding site with affinities which are similar to those for the binding of bilirubin.

4. DISCUSSION

Many drugs bind to serum albumin in competition with bilirubin [11]. However, the binding of these drugs is generally not very tight compared with the binding of bilirubin. For example, sodium salicylate shows a $K_d = 1.1 \times 10^{-4}$ M; most known drugs which compete with bilirubin show $K_d = 10^{-2}$ to 10^{-4} M [11]. Gossypol appears to be unique compared with other drugs which can displace bilirubin, in that the binding of gossypol to albumin is almost as tight as the binding of bilirubin and is 10^3- to 10^5-times better than the binding of other drugs. The fact that this same pattern is observed with liver glutathione *S*-transferase and placenta glutathione *S*-transferase suggests that gossypol may generally mimic bilirubin. Gossypol should be a useful compound for studies of bilirubin metabolism. There remain many unanswered questions concerning transport, hepatic uptake and biliary excretion of bilirubin [12].

ACKNOWLEDGEMENT

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