Affinity chromatography and affinity partition of human serum pre-albumin using immobilized Remazol Yellow GGL

Evidence that albumin increases binding of pre-albumin to the dye

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Pre-albumin was isolated from human serum or plasma by ammonium sulphate fractionation and by chromatography on dye-substituted Sephadex G-100 to a high degree of purity. The interaction of the pre-albumin with the dye Remazol Yellow GGL was studied in more detail by means of affinity partitioning in an aqueous two-phase system composed of dextran and poly(ethylene glycol). In the presence of dye-substituted poly(ethylene glycol) the partition coefficient of pre-albumin increases, indicating an interaction with the dye. Human serum albumin was found to enhance this interaction considerably. A similar effect was also shown by bovine albumin but not by egg albumin

Affinity chromatography *Affinity partitioning* *Albumin* *Pre-albumin* *Reactive dye*

1. INTRODUCTION

Various plasma proteins were found to interact with synthetic dyes. The triazine dye Cibacron Blue F3G-A was applied for chromatographic purification of blood coagulation factors [1], complement factors [2], albumin [3], vitamin D-binding protein [4] and other proteins [5]. Recently, authors in [6] reported the binding of subclasses of immunoglobulins and pre-albumin to the dye Remazol Yellow GGL. Based on their findings the present work describes the purification of pre-albumin from serum or plasma by dye-affinity chromatography. In addition, a more detailed study of this dye–protein interaction was performed by means of affinity partitioning in an aqueous two-phase system [7]. The results clearly demonstrate that the binding of pre-albumin to Remazol Yellow GGL is influenced by serum albumin.

2. MATERIALS AND METHODS

Dextran T 500 (M_r = 500000), poly(ethylene glycol) 6000 (M_r = 6000) (PEG), bovine serum albumin and egg albumin were products of Serva, Heidelberg, FRG. Sephadex G-100 and Cibacron Blue F3G-A were purchased from Pharmacia, Uppsala, Sweden. Remazol Yellow GGL was a gift from Hoechst, Frankfurt, FRG. All other chemicals used were of analytical reagent grade. Antibodies against plasma proteins were obtained from Behringwerke AG, Marburg, FRG. Human serum and citrated plasma were obtained from blood of healthy blood donors.

2.1. *Preparation of albumin and albumin-depleted plasma or serum*

Human albumin was prepared by passing 2 ml plasma or serum dialysed against 10 mM sodium phosphate buffer, pH 7.4 containing 2 mM β-

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mercaptoethanol (buffer I) through a column (1.5 × 15 cm) filled with Cibacron Blue F3G-A-substituted Sephadex G-100 [8]. The break-through fraction, free of albumin, was concentrated to a volume of 2 ml by ultrafiltration and was designated in the following experiments as albumin-depleted plasma. The bound albumin was eluted from the column in a highly purified form by 1 M NaCl.

2.2. *Preparation of pre-albumin and pre-albumin-depleted plasma*

500 ml serum or citrated plasma were precipitated at 4°C with solid ammonium sulphate up to a final concentration of 50% saturation. The supernatant obtained after centrifugation (30 min at 10000 × *g*) was exhaustively dialysed against buffer I. The protein solution was then mixed with 250 ml of Remazol Yellow GGL–Sephadex G-100 gel and stirred in a beaker for 30 min at 4°C. After washing the gel on a Büchner funnel with one liter of buffer I the gel was transferred into a column (3.5 × 40 cm) and washed at 30 ml/h with the same buffer until constant absorbance was reached. Elution of pre-albumin was achieved by 10 mM sodium barbiturate added to buffer I. The fractions of the emerging protein peak containing pre-albumin were pooled, concentrated to 10 mM and directly passed through a second column (2.5 × 30 cm) containing Cibacron Blue F3G-A-substituted Sephadex G-100, which was previously equilibrated with 10 mM sodium phosphate, 50 mM sodium chloride, pH 7.4 (buffer II). After washing the column with buffer II at 30 ml/h the emerging break-through fraction containing pre-albumin was finally concentrated by ultrafiltration.

Pre-albumin-depleted plasma was prepared by passing 1 ml of plasma dialysed against buffer I through a column (1 × 10 cm) containing Remazol Yellow GGL-substituted gel. The break-through fraction obtained after washing the column with the same buffer, was concentrated to the original volume by ultrafiltration. This sample was completely depleted of pre-albumin as checked by immunological analysis.

The preparation of the dye–gel conjugates was performed according to [9].

The polyacrylamide gradient gel electrophoresis was performed as described previously [10].

The protein content of solutions was determined according to [11].

2.3. *Affinity partitioning*

The two-phase systems were prepared by mixing aqueous stock solution of dextran (20%, w/w), PEG (40%, w/w), dye–PEG, buffer and protein solution previously dialysed against the buffer of the respective system. All partition experiments were carried out at 0°C. The partition coefficient, K, is defined as the ratio of the concentration of the protein in the upper and lower phases. The term $\Delta \log K$ expresses the difference between the $\log K$ value in the presence and the absence of the dye–PEG. Pre-albumin and albumin in the two phases were determined by rocket electroimmunodiffusion [12].

The PEG derivative was prepared by the covalent coupling of the dye Remazol Yellow GGL via its reactive vinyl sulphone residue to the polymer according to the procedure as described in [7].

3. RESULTS

3.1. *Purification of pre-albumin*

Starting from 500 ml serum or plasma 36 mg of pre-albumin were obtained as demonstrated in table 1. Remazol Yellow GGL-substituted gel extracts pre-albumin from the fractionated original material. By elution with 10 mM sodium barbiturate albumin as well as pre-albumin were eluted from the column in comparable quantities, pointing to an interaction of these proteins with the dye (fig.1). In a second chromatographic step albumin was quantitatively removed from this solution owing to its high affinity to Cibacron Blue F3G-A. The latter does not bind pre-albumin. The final product of pre-albumin as obtained after only two chromatographic steps still contained a small proteic impurity in very low concentration. This contaminating protein did not react immunologically with antisera against the following serum proteins: α_1-antichymotrypsin, albumin, group-specific component, thyroxine-binding globulin, IgG, IgM, transferrin and coeruloplasmin.

Table 1

Purification of pre-albumin

Fraction	Total protein[a] (mg)	Pre-albumin[b] (mg)	Yield (%)
Serum[c] (500 ml)	33750	105	100
Ammonium sulphate fractionation	17550	52	49
Remazol Yellow GGL chromatography	76	41	39
Cibacron Blue F3G-A chromatography	34	36	34

[a] Measured according to [11]
[b] Measured by rocket electroimmunodiffusion [12]
[c] When plasma was used similar results were obtained

3.2. *Affinity partition*

The interaction of the dye Remazol Yellow GGL with human pre-albumin was studied in a two-phase system composed of 10% dextran, 7.5% PEG including dye–PEG, and 10 mM sodium phosphate buffer, pH 7.0.

In the absence of dye-liganded PEG, pre-albumin partitioned in favour of the lower dextran-rich phase, yielding a K value of 0.41 which corresponds to a log$K$ value of -0.38. When the PEG was stepwise replaced by dye-liganded PEG, pre-albumin was increasingly transferred into the dye–PEG-rich upper phase, giving rise to an increase of the partition coefficient (fig.2). The maximum change in partition can be used as a measure for the extraction power of the dye. In addition, fig.2 shows that pre-albumin exhibits a significantly higher partition coefficient in presence of other plasma proteins. This gives evidence of the presence of a plasma constituent capable of in-

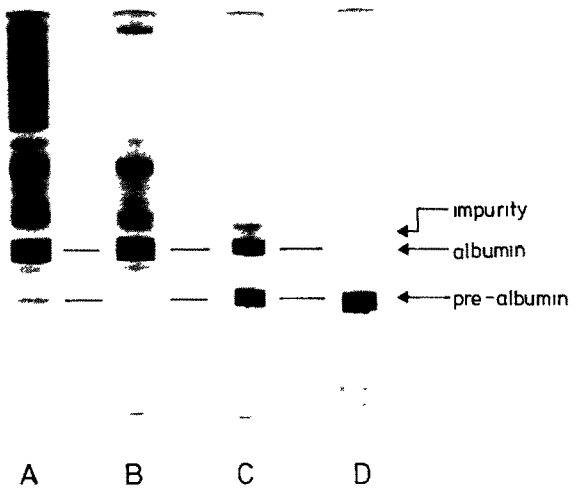

Fig.1. Disk-electrophoresis of pre-albumin at different stages of purification. The electrophoretic runs were performed in polyacrylamide gel gradients of 3% to 15% for 90 min at 3 mA per tube. A, human serum (400 μg); B, supernatant after ammonium sulphate precipitation (200 μg); C, after Remazol Yellow GGL–Sephadex G-100 chromatography (200 μg); D, after Cibacron Blue F3G-A–Sephadex G-100 chromatography (80 μg).

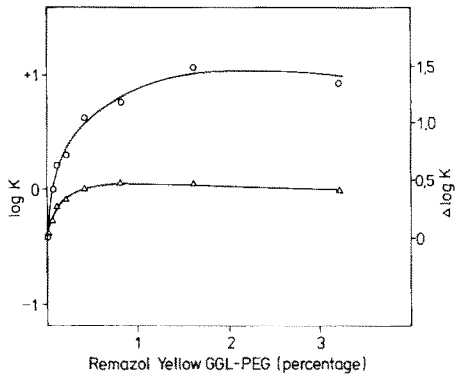

Fig.2. Partition of pre-albumin in dependence on increasing concentration of Remazol Yellow GGL–PEG. Pure pre-albumin (0.17 mg) and plasma (6 mg) were partitioned at 0°C in systems of 2 g containing 10% (w/w) dextran (M_r = 500000), 7.5% (w/w) PEG (M_r = 6000) including different amounts of Remazol Yellow GGL–PEG, and 10 mM sodium phosphate buffer, pH 7.0. The percentage of dye–PEG refers to the portion of total PEG in the system which is replaced by substituted PEG. The partition of dye–PEG in the system was found to correspond to a log$K$ value of 1.47. (△) Pure pre-albumin; (○) pre-albumin of plasma.

creasing binding of pre-albumin to the dye-liganded PEG.

In order to study this phenomenon in more detail the effects of various plasma protein fractions on the partition of purified pre-albumin were tested. In the absence of dye–PEG the partition coefficient of pre-albumin remained nearly unchanged upon addition of plasma proteins (fig.3). As expected from fig.2, a high log$K$ value of pre-albumin was obtained when whole plasma (fig.3A) or a mixture of purified pre-albumin and pre-albumin-depleted plasma (fig.3B) was partitioned in the presence of 1.6% dye–PEG. On the other hand, however, only a small change in partition was observed when pre-albumin was distributed in the presence of albumin-depleted plasma (fig.3C). No difference exists between fig.3C and fig.3D, the latter of which shows the partition of pure pre-albumin. Apparently, the plasma effect on the binding of pre-albumin to the dye is due to its albumin content.

In fig.4 the effects of albumin from three different sources on partition of human pre-albumin is presented. In these experiments 3.2 mg of pure albumin were actually added to the standard system. This corresponds to the albumin content of whole plasma as used in fig.2. The addition of

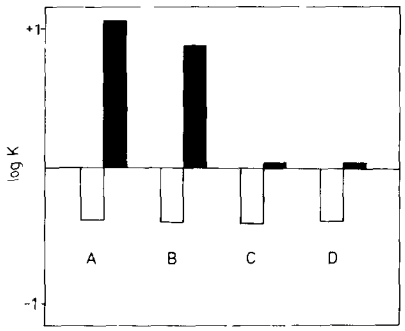

Fig.3. Effect of plasma proteins on the partition of pre-albumin in absence and presence of Remazol Yellow GGL–PEG. Systems of 2 g composed of 10% (w/w) dextran, 7.5% (w/w) PEG including dye–PEG and 10 mM sodium phosphate buffer, pH 7.0, were used. White bars, systems without dye–PEG; black bars, systems with 1.6% dye–PEG. Additions were: A, whole plasma (6 mg); B, pure pre-albumin (0.17 mg) + plasma depleted of pre-albumin (6 mg); C, pure pre-albumin (0.17 mg) + plasma depleted of pre-albumin and albumin (3 mg); D, pure pre-albumin (0.17 mg).

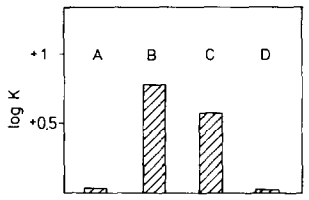

Fig.4. Effect of human, bovine and egg albumin on partition of pre-albumin in presence of Remazol Yellow GGL–PEG. Systems containing 1.6% dye–PEG were prepared as described in fig.2. The proteins included into the systems were: A, pure pre-albumin (0.17 mg); B, pure pre-albumin (0.17 mg) + human serum albumin (3.2 mg); C, pure pre-albumin (0.17 mg) + bovine serum albumin (3.2 mg); D, pure pre-albumin (0.17 mg) + egg albumin (3.2 mg).

human albumin caused a significant increase of the partition coefficient of pre-albumin reaching a log$K$ value of 0.78 at 1.6% dye–PEG (compare fig.4B with the control in fig.4A). Similarly, a significant change in the partition of pre-albumin was observed when bovine albumin was applied (fig.4C). In contrast, egg albumin did not produce this effect (fig.4D).

In view of the albumin-mediated increase of the binding of pre-albumin to the dye, it was interesting to determine the actual affinities of the different types of albumin to Remazol Yellow GGL. Table 2 presents their relative affinities to the dye expressed in terms of $\Delta\log K$ values. Human albumin and bovine albumin undergo a similar change in partition which amounts to about 0.5 units of $\Delta\log K$, whereas egg albumin has only a minimal affinity to the dye.

Table 2

Effect of Remazol Yellow GGL–PEG on partition of human, bovine and egg albumin

Albumin	log$K$ without dye–PEG	$\Delta\log K$
Human serum albumin	−1.3	0.45
Bovine serum albumin	−1.3	0.4
Egg albumin	−0.75	0.05

Albumin (0.6 mg) was partitioned in systems in absence and presence of 1.6% dye–PEG as described in fig.2. $\Delta\log K$ refers to the change of log$K$ caused by replacing 1.6% of PEG by dye–PEG

4. DISCUSSION

One possibility to purify pre-albumin by affinity chromatography is based on its property to form a complex with the retinol-binding protein at high ionic strength [13]. In the present work pre-albumin was isolated from serum or plasma in a faster and simpler way by dye-affinity chromatography taking advantage of the strong interaction with the dye Remazol Yellow GGL. The isolated protein was of high purity as judged from the electrophoretic analysis. The protein impurities amount to approximately 5%. The ultraviolet absorption spectrum of pre-albumin did not reveal an absorption at 330 nm (not shown). It is therefore evident that complete dissociation of pre-albumin and retinol-binding protein had occurred at the low ionic strength of the buffer during the steps of dye-affinity chromatography.

Attempts to measure the binding capacity of Remazol Yellow GGL-substituted gels for pre-albumin revealed that, in contrast to pre-albumin in presence of plasma proteins, the isolated protein was rather weakly bound to the dye (not shown). In order to investigate this phenomenon the method of affinity partition was applied, which is an excellent method for studying dye–protein interactions [14,15]. The differences in the $\Delta \log K$ values obtained for pure pre-albumin and pre-albumin in the presence of plasma proteins agreed very well with the results of the column experiments mentioned above. Our findings that albumin is able to influence the binding of pre-albumin to the dye have not yet been published. The promoting action of albumin on the partition of pre-albumin seems to be specific, because albumin-depleted plasma was found to be without effect. Apparently, a certain affinity of albumin to the dye is necessary to enhance the pre-albumin–dye interaction. Assuming an interaction between albumin and pre-albumin, this would occur only in the presence of the dye, otherwise a change in the partition coefficient of pre-albumin would also have been noticed in the absence of the dye–PEG.

According to the theory of affinity partitioning [16], a variation in the $\Delta \log K$ value of a protein is caused by a change in the number of its binding sites for a specific ligand. Consequently, the effect of albumin on the dye-binding properties of pre-albumin might be mediated either by providing additional binding sites in the pre-albumin or by an induction of pre-albumin aggregation.

According to authors in [6], Remazol Yellow GGL may act as an analogue of thyroxine in respect to its binding to pre-albumin. It is known that pre-albumin and albumin are involved in binding and transport of this hormone [17,18]. In the light of these findings one is tempted to ask whether the observed effect of albumin on the pre-albumin–dye interaction may have a biological significance.

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