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Binding Capacity of Human Serum Albumin with Estrogen and Other Ligands

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Abstract

This study was trying to find what physical changes occurred to albumin when it reacted with estrogen and other ligands. Each concentration of human serum albumin with 100μl estradiol reacted at the highest binding capacity of 280nm. In addition, 1hr of reaction time showed the highest binding rate. Conformational changes in human serum albumin with diethylstilbestrol and N-ethyl-maleimide produced strong binding capacites. The changes were immediate and they did not increase or decrease over time. Effects of human serum albumin with estriol induced no interaction each other. The binding capacity of human serum albumin with vitamin D_2 was lower than estradiol, and the highest binding rate showed 1hr of reaction time. Vitamin D_2 was very similar to the binding capacity of estradiol.

Key words : human serum albumin, estradiol, diethylstilbestrol, vitamin D_2

INTRODUCTION

The major protein component in the serum of the developing fetus is the α-globulin, α-fetoprotein, (AFP) which is synthesized in the embryonic liver, and yolk sac[1]. After birth, the serum concentrations of AFP decrease drastically to levels which are barely detectable in nonpregnant adults. The fall in serum AFP level results from a gradual decrese of its rate of synthesis by the liver and, in the case of rodents, the loss of the yolk sac[2]. In contrast, serum albumin, which is the major serum protein synthesized by the adult liver, increases from low levels early development, to high, relatively constant levels after birth and in adult life. However, the synthesis of AFP is resumed in adult liver during liver regeneration, and in specific tumors such as hepatomas and teratocarcinomas[3,4]. There are several striking structural and functional similarities between AFP and albumin, which have led to the suggestion that AFP serves as a fetal albumin, and that their genes arise in evolution as the consequence of a duplication of an ancestral gene, followed by divergence. The high concentrations of AFP and albumin in plasma help control the osmotic pressure of the intravascular fluid. Albumin is also involved in the binding and transporting of metabolites and metabolic effectors, and such functions have, more recently, been proposed for AFP as well. In addition, AFP has been implicated in suppression of the immunoresponse of the mother and protection of the rodent fetus from the effects of maternal estrogens. The two proteins are very similar in size 68,000 daltons for albumin ; 70,000 daltons for AFP[5,6]. Among serum protein, AFP is predominantly a fetal glycoprotein. AFP shares many similarities with albumin including sequence homology, immunological cross-reactivity and physiological properties. Although the biological role of AFP remains to be elucidated, the proposed functions of AFP include the binding of substances such as estrogen, fatty acids and dyes, and the maintenance of osmotic pressure in the fetal body. The AFP may have function as a fetal albumin[7-9]. Binding specificity studies of AFP with various steroids are necessary because they provide a better insight in the biological role of this calcino-fetal globulin, particularly in comparison with the rat uterus estradiol receptor[10,11]. A large number of steroids have been investigated by studying the diminution of the fluorescence of a dye bound to the protein[12].

Most of the recent estrogen binding experiments with rat AFP were considered and realized in relation to the well-known microheterogenicity of this pro-

tein. Some differences were found in binding capacity of the varoius forms. While essentially identical binding properties for the iso AFP were reported[13-16]. However, AFP is very hard to be isolated because the purification procedure need very long time and can be obtained very small quantities. Due to the lack of AFP, bovin serum albumin and human serum albumin was used as standard protein.

MATERIALS AND METHODS

Chemicals

The ligands, 17-β-estradiol, diethylstilbestrol (DES), estriol, vitamin D_2 and N-ethyl-malemide (NEM) were obtained from Sigma Chemical Co.. Standard protein was human serum albumin (HSA) and bovin serum albumin (BSA).

Determination of reactivity of albumin with ligands

Absorbance of ligands to protein was determined by difference spectroscopy as described Fig.1 (Perkin-Elmer Model 320). Difference spectroscopy is a technique in which light is passed through a solution and the amount of light absorbed by the solution is measured by using specially designed cuvettes, each consisting of two chambers were used. To the reference and sample cuvettes, 900μl of albumin solution (concentration of 1mg/ml) in trizma buffer (pH 7.5, concentration of 0.02mol/L) was added to the first chamber, and 900μl of trizma buffer was added to the second chamber. This was scanned on the spectrophotometer to create a baseline upon which the changes would be measured. Since albumin was in both cuvettes, there was no difference in the amount of light absorbed.

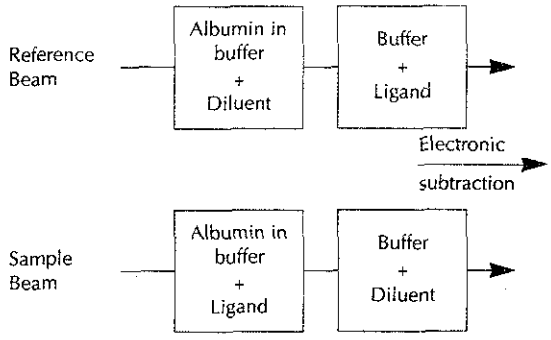

Fig. 1. Experimental design for difference spectroscopy.

Estradiol (E_2) was dissolved in the 1 : 1 mixture ethanol and trizma buffer (pH 7.5, concentration of 0.02mol/L) to make the concentration of 250μg/ml. One hundred μg of E_2 solution was added to the buffer solution in the reference cuvette and to the albumin solution in the sample cuvette, while 100μl of ethanol/buffer (diluent without E_2) was added to the albumin solution of the reference cuvette and buffer solution of the sample cuvette. Spectra was recorded between 340nm and 230nm, before and after the addition of E_2. In the sample cuvette E_2 has the opportunity to interact with albumin and this leads to a greater absorbance of light than in the reference cuvette.

RESULTS AND DISCUSSION

Effects of HSA and BSA with estradiol

Physical identification methods involve difference spectroscopic studies to monitor changes in the structure of albumin when incubated with various ligands. Spectroscopy is a technique in which light is passed through a solution and the amount of light absorbed by the solution is measured. In difference spectroscopy, there is a sample solution and a reference solution, and what is being measured is the difference of absorption between the sample and the reference solution. Absorbance of albumin with estradiol conducted some changes in the spectra, between 290nm and 260nm, with a peak at 280nm (Fig. 2, Fig. 3). The absorbing capacity of HSA at 280nm after a 1 hour incubation was AU 0.026, which was higher than that of BSA and also similar responses were found after a 2 hour incubation.

Effects of reaction time on estradiol absorption with HSA and BSA

The responses of the BSA and E_2 at each concentration were higher absorbing capacity in according to incubation time, and the hightest absorbance was found at 1 hour incubation (Table 1, 2). Higher absorbing capacity occurred, lower Molar ratio (E_2/BSA). The responses of HSA and E_2 at each concentration

time were similar changes with those responses of BSA and E2.

Binding of human serum albumin with diethylstilbestrol

This was done in order to find out if the change in spectra occurred only with E2 or it also occurred with other ligands. For the experiments with other ligands, the concentration of the solution were kept the same, and the volume of the solutions were also unchanged.

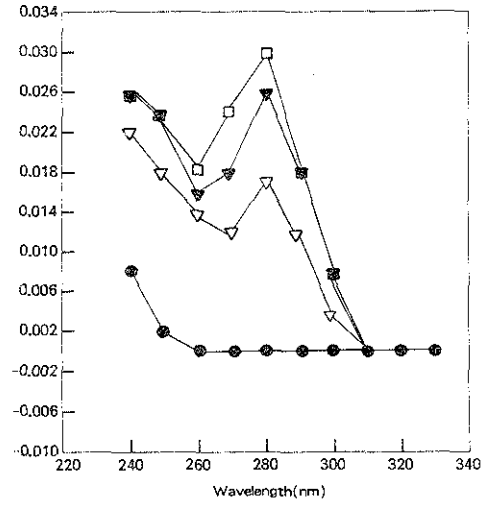

Fig. 2. Absorbance of human serum albumin solution after addition of estradiol.
●—● : 0 hour ▽—▽ : 0.5 hour
▼—▼ : 1 hour □—□ : 2 hours
Concentration of HSA (1mg/ml)
Concentration of estradiol (250μg/ml)

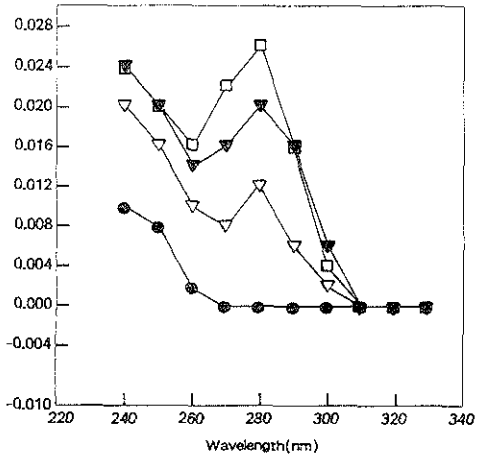

Fig. 3. Absorbance of bovine serum albumin solution after *addition of estradiol.*
●—● : 0 hour ▽—▽ : 0.5 hour
▼—▼ : 1 hour □—□ : 2 hours

The reaction that occurred between albumin and DES produced a very large change in the spectra (Fig. 4) and this change was not time dependant. This result is somewhat different from other studies[17]. They established that the serum fetoestrogen binding protein of the rat did not as strongly bind testosterone, 11-β-methoxyethynylestradiol, or diethylstilbestrol as it estradiol. The change was immediate and it did not increase or decrease with time. This may suggest that a different type of interaction occurs when albumin is incubated with DES rather than with E2.

Binding of estriol and vitamin D2 with human serum albumin

Absorption capacities of estriol and vitamin D2 sh-

Table 1. Effects of reaction time on estradiol absorption with BSA

Concentration*		Ratio	Absorption**		
BSA	E2	$\frac{E_2}{BSA}$	0.5hr	1hr	1.5hr
1.2×10^{-5}M	9.2×10^{-5}M	7.97	0.015	0.022	0.024
7.6×10^{-6}M	9.2×10^{-5}M	12.07	0.010	0.018	0.020
5.1×10^{-6}M	9.2×10^{-5}M	17.98	0.009	0.014	0.015
2.6×10^{-6}M	9.2×10^{-5}M	35.27	0.007	0.010	0.012
6.4×10^{-7}M	9.2×10^{-5}M	143.28	0.004	0.005	0.005

*Concentration of BSA (1mg/ml)
Concentration of E2 (250μg/ml)
**Absorbance units at 280mm

Table 2. Effects of reaction time on estradiol absorption with HSA

Concentration*		Ratio	Absorption**		
BSA	E2	$\frac{E_2}{BSA}$	0.5hr	1hr	1.5hr
1.0×10^{-5}M	9.2×10^{-5}M	8.82	0.017	0.028	0.030
6.9×10^{-6}M	9.2×10^{-5}M	13.29	0.016	0.024	0.024
5.0×10^{-6}M	9.2×10^{-5}M	19.93	0.011	0.020	0.022
2.5×10^{-6}M	9.2×10^{-5}M	39.87	0.007	0.015	0.017
6.3×10^{-13}M	9.2×10^{-5}M	142.90	0.005	0.007	0.007

* Concentration of HSA (1mg/ml)
Concentration of E2 (250μg/ml)
** Absorbance units at 280mm

own in Fig. 5 and Fig. 6. The reaction that occurred between albumin and estriol produced no change in the spectra. This result was consistent with other studies[18] and may suggest that there was little or no interaction between albumin and estriol. According to the well known estrogenic binding properties of albumin, the importance of an aromatic A ring among the factors intervening in steroid recognition was emphasized[10].

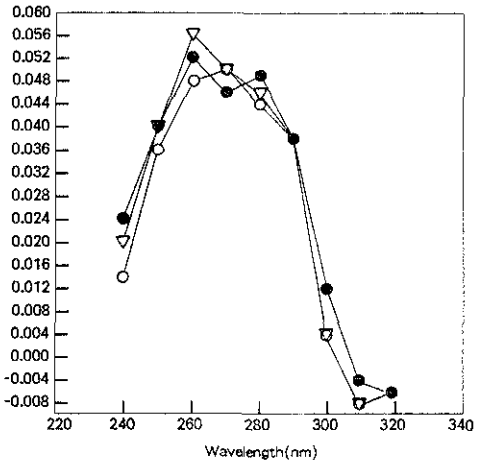

Fig. 4. Binding of human serum albumin solution after addition of diethylstibestrol.
○—○ : 0 hour ●—● : 0.5 hour
▽—▽ : 1 hour
Concentration of HSA (1mg/ml)
Concentration of estradiol (250μg/ml)

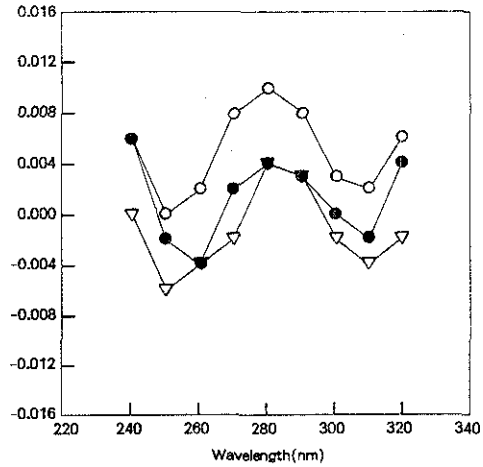

Fig. 5. Binding of human serum albumin solution after addition of estriol.
○—○ : 0 hour ●—● : 0.5 hour ▽—▽ : 1 hour

Vitamin D_2 has a structure that is very similar to that of estradiol. The changes that were recorded with this ligand were very similar to the changes that occurred with E_2 ; the reaction was time dependant, and the peaks in the albumin/vitamin D_2 were similar to the peaks in the albumin/E_2 curve, even though they were not exactly alike. This evidence may imply that albumin interacts in a similar way with E_2 and with vitamin D_2. Based on these results it can be concluded that the changes occur when albumin is incubated with another ligand, the only other ligand that can create changes that are similar to that of E_2 is vitamin D_2, and even though in this case the changes are not identical.

Effect of albumin with N-ethyl-maleimide and then estradiol

Fig. 7 showed that the results of absorption capacities of albumin with NEM and estradiol. 20μl of a solution of NEM (concentration of 13.75μg/ml) was added to 1ml of albumin solution (concentration of 1mg/ml), and after this solution had incubated for an hour, 100μl of E_2 was added to it. This experiment was done in order to have a better idea of where estradiol might bind to albumin. When NEM was added to the albumin solution, a large immediate change in spectra was recorded. This change probably meant that NEM had incubated in the albumin

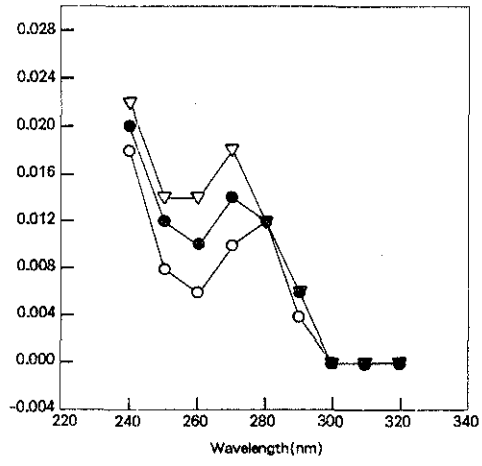

Fig. 6. Binding of human serum albumin solution after addition of vitamin D_2.
○—○ : 0 hour ●—● : 0.5 hour ▽—▽ : 1 hour

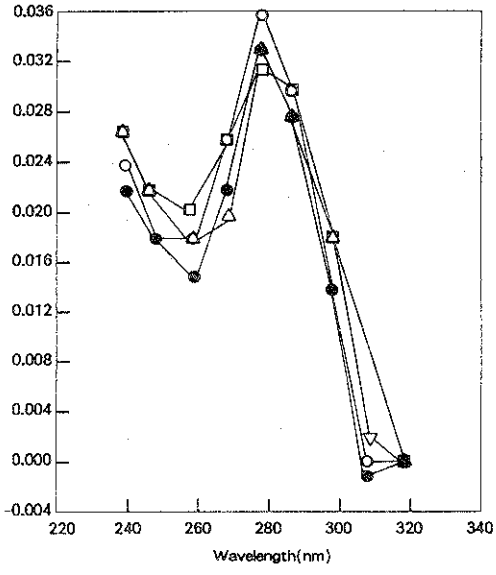

Fig. 7. Effect of human serum albumin solution after addition of N-ethyl-maleimide and another addition of Estradiol.
●—● : 0 hour ○—○ : 0.5 hour ▽—▽ : 1 hour
□—□ : addition of estradiol after 1 hours

solution for an hour when the E_2 was added, and causing no change in the spectra. These results could lead to the conclusion that E_2 binds to albumin near its sulfhydryl group, but because the NEM added earlier had already blocked the sulfhydryl group, E_2 could not bild itself to albumin and that is why no changes in spectra were recorded.

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Human Serum Albumin이 Estrogen과 기타 Ligands와의 결합력에 관한 연구

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요 약

본 실험은 human serum albumin이 estradiol 및 기타 ligands와 반응하였을 때 일어나는 물리적인 변화를 관찰하여 α-fetoprotein의 연구를 위한 control로서 사용할 수 있는지에 대한 기초적인 자료를 얻고자 실행하였다. 각 농도별 human serum albumin이 100μl의 estradiol과 반응했을 때 280nm에서 최고의 흡수도를 나타내었다. 각농도별 시간의 경과에 따른 변화는 1시간에서 가장 높은 흡수율을 나타내었다. Diethystilbestrol과 N-ethyl-maleimide는 human serum albumin과 반응 즉시 결합력이 강하게 나타났으며 시간에 따른 변화가 없었다. Estriol은 human serum albumin과 반응 시켰을 때 서로간에 아무런 작용이 일어나지 않았다. Vitamin D_2와 albumin의 결합력은 estradiol보다 조금 낮았으나 시간의 경과함에 따라 반응력도 증가하여 반응 1시간 후에 최고의 반응력를 나타내었다. 사용한 ligands 중에서 vitamine D_2가 유일하게 estradiol과 비슷한 결과를 나타내었다.