

# Interactions of tuftsin with bovine serum albumin

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Interactions of tuftsin (Thr-Lys-Pro-Arg) with bovine serum albumin (BSA) were analysed by fluorescence spectroscopy and circular dichroism. The data show that tuftsin interacts weakly with BSA, but this interaction is considerably enhanced by introducing an apolar substituent at the C-terminus of the tetrapeptide. It is suggested that strong binding of tuftsin to albumin in blood may enhance its macrophage-stimulating activity *in vivo*.

*Tuftsin (Blood) Peptide stability Albumin Macrophage*

## 1. INTRODUCTION

Tuftsin, Thr-Lys-Pro-Arg, is an immunologically active tetrapeptide which stimulates a number of phagocytic cells including macrophages [1–3]. We have shown that this peptide, when given to mice prior to challenge with *Plasmodium berghei*, confers partial protection against the malarial infection [4], presumably by activating macrophages. Also, it has been further demonstrated that this antiparasitic effect of the tetrapeptide is significantly enhanced by attaching an apolar residue at its C-terminus [4]. As tuftsin is known to be unstable in blood [1], we speculate that the enhanced antiparasitic action of the modified tuftsin (Thr-Lys-Pro-Arg-NH-(CH<sub>2</sub>)<sub>2</sub>-NHCOC<sub>15</sub>H<sub>31</sub>) might have resulted from its complexation with plasma proteins, especially albumin. To examine this possibility, we studied the interactions of tuftsin and modified tuftsin with BSA by using fluorescence spectroscopy and CD. Our data show

that unlike tuftsin, modified tuftsin interacts strongly with this protein.

## 2. MATERIALS AND METHODS

All the reagents used here were of the highest purity available. BSA (essentially fatty acid and globulin free) was purchased from Sigma. Tuftsin and modified tuftsin were synthesised as in [5].

### 2.1. Fluorescence measurements

Fluorescence measurements were made at 22–25°C on a Shimadzu RF 540 spectrofluorometer. An excitation wavelength of 286 nm was used to avoid the region of strong iodide absorption. Stock 1 M NaI solutions containing 0.1 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to prevent I<sub>3</sub><sup>-</sup> formation were prepared in PBS. Analysis of fluorescence quenching data was carried out by using the appropriate forms of equations proposed by Lehrer [6].

The basic equation is:

$$F_0/F - F_0 = F_0/\Delta F =$$

$$\left[ \sum_1^n f_i K_i [Q] / 1 + K_i [Q] \right]^{-1} \quad (1)$$

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**Abbreviations:** BSA, bovine serum albumin; PBS, phosphate-buffered saline; CD, circular dichroism

where  $F_0$  and  $F$  are the fluorescence values in the absence and presence of a concentration  $[Q]$  of quencher (iodide),  $f_i$  is the fraction of total fluorescing sites ( $n$ ) involved in quenching, and  $K_i$  is the quenching constant. When the distribution of fluorescent side chains in a protein molecule is heterogeneous, i.e. the side chains are under the influence of different environments such that one fraction is accessible ( $f_a$ ) and another is inaccessible ( $1 - f_a$ ) to the quencher, eqn 1 reduces to:

$$F_0/\Delta F = (1/Kf_a[Q]) + 1/f_a \quad (2)$$

where  $f_a = \sum f_i$ , the maximum accessible fraction of protein fluorescence. As  $(1/[Q]) \rightarrow 0$  on a plot of  $(F_0/\Delta F)$  vs  $(1/[Q])$ , the intercept on the  $y$ -axis yields  $F_0/\Delta F_{\max} = 1/f_a$ . Thus the inverse value of the  $y$  intercept represents the accessible fraction,  $f_a$ . When the quencher has equal access to all fluorescing sites of the protein ( $f_i = 1$  and all  $K$  terms are equal), eqn 1 simplifies to:

$$F_0/\Delta F = (1/K[Q]) + 1 \quad (3)$$

Plots of  $(F_0/\Delta F)$  vs  $(1/[Q])$  will be linear. Values of the effective quenching constant ( $K_{\text{eff}}$ ) were obtained by dividing  $f_a$  by the slope of the curve.

## 2.2. CD measurements

CD spectra were recorded at 22–25°C on a Jobin Yvon Dichrograph III, using cells with a path length of 0.05 cm.

## 3. RESULTS AND DISCUSSION

Interactions between tuftsin (or modified tuftsin) and BSA were analysed by measuring the intrinsic fluorescence of the protein in the absence as well as in the presence of these peptides. Fig.1 shows that tuftsin did not affect the emission maxima ( $\lambda_{\max}$ ), but induced a slight increase in the fluorescence intensity. However, both the  $\lambda_{\max}$  and fluorescence intensity were significantly influenced by the modified tuftsin, suggesting that, unlike tuftsin, this tetrapeptide interacts strongly with BSA. To establish further that this indeed is the case, we carried out quenching experiments by using iodide as a water-soluble quencher of tryptophan fluorescence. The modified Stern-Volmer plots ( $F_0/\Delta F$  vs  $1/[NaI]$ ) for data obtained in the absence or presence of tuftsin (or modified tuftsin)

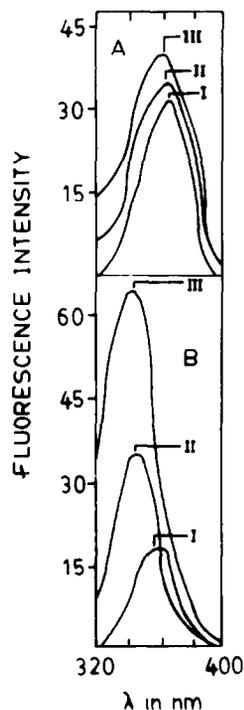


Fig.1. Fluorescence spectra of BSA (0.08 nmol) in PBS (pH 7.4) in the absence and presence of tuftsin (A) or modified tuftsin (B). I, BSA; II, BSA plus 10 nmol peptide; III, BSA plus 20 nmol peptide. Tuftsin (or modified tuftsin) alone did not fluoresce in identical conditions. Excitation at 286 nm.

are shown in fig.2. Linearity on the plots indicates that all the protein fluorescing sites accessible to the quencher lie in the same environment. Extrapolation of the curve to the  $y$ -axis showed an intercept of 1.45 [ $f_a(\text{eff})$  0.68], which suggests that about 68% of the tryptophan residues in BSA are exposed towards the aqueous environment. This fraction of the protein fluorescence was not influenced by tuftsin, but was considerably altered by the modified tuftsin (table 1). Also, the effective quenching constant ( $K_Q(\text{eff})$ ) was significantly affected by modified tuftsin as compared to tuftsin. These results clearly indicate that the interactions between modified tuftsin and BSA are strong enough to induce a conformational change in the protein such that the number of fluorescing sites exposed to aqueous environment is increased. This is quite consistent with our observation that in the

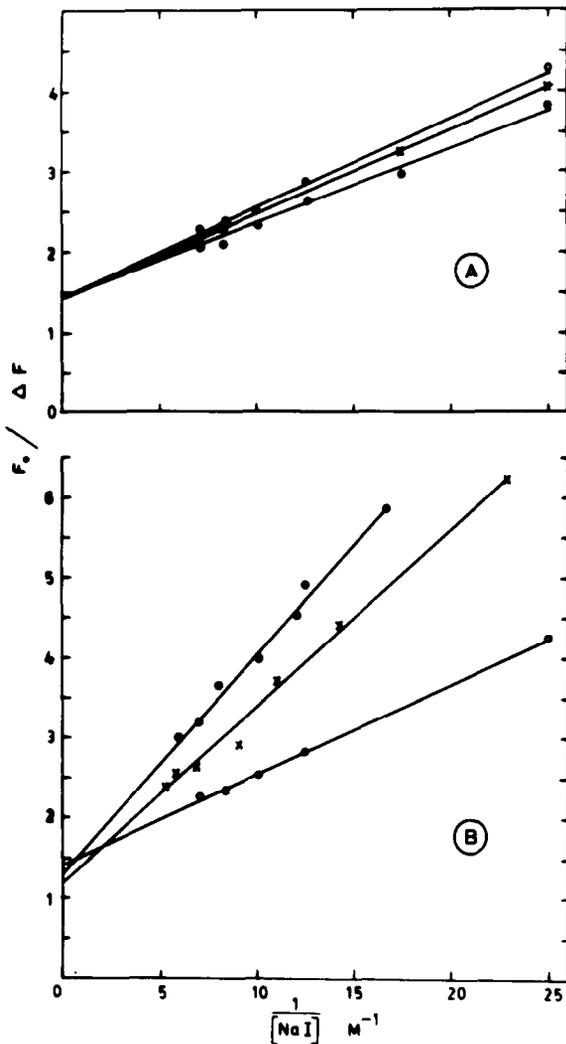


Fig.2. Modified Stern-Volmer plots of iodide quenching of BSA fluorescence in PBS (pH 7.4) in the absence and presence of tuftsin (A) or modified tuftsin (B). (○—○) BSA; (●—●) BSA plus 10 nmol peptide; (×—×) BSA plus 20 nmol peptide.

presence of modified tuftsin, the BSA emission maxima shift towards the  $\lambda_{max}$  of L-tryptophan [7].

To confirm that modified tuftsin-BSA interactions lead to unfolding of the protein, we analysed these interactions by CD. As shown in fig.3, BSA gave two CD bands centered at 208 and 220 nm. The intensity of these bands was slightly increased by adding 20 nmol tuftsin to the protein solution,

Table 1

Effect of tuftsin and its derivative on quenching parameters of BSA

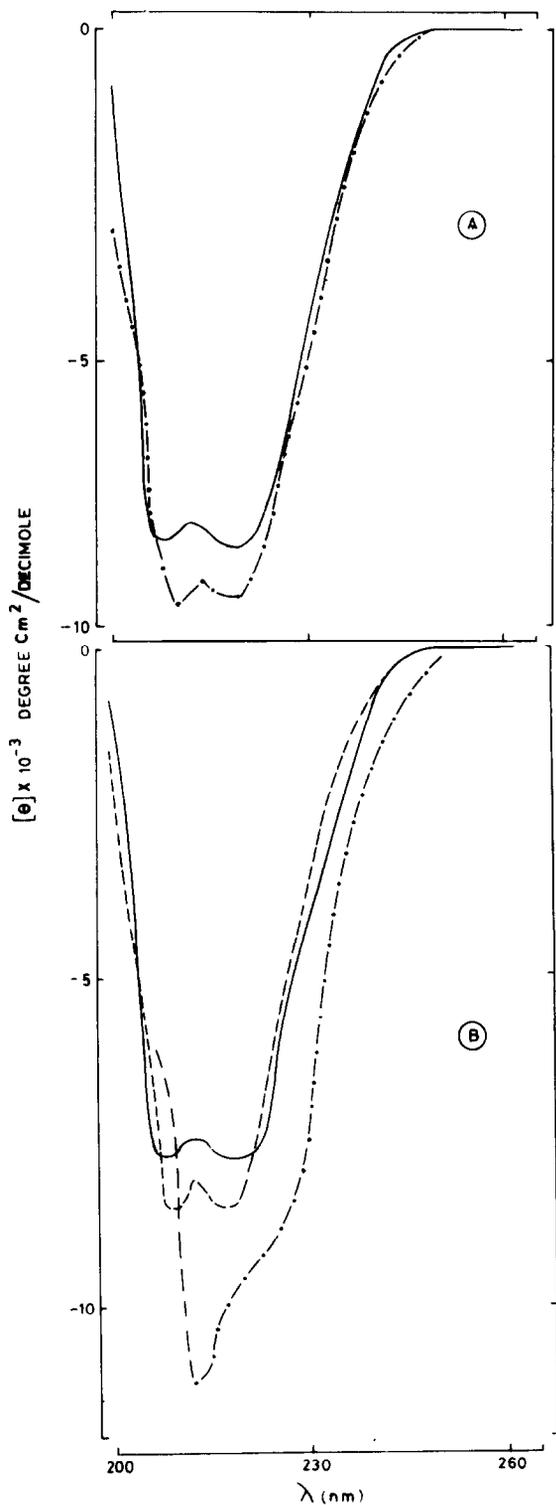
Sample	Peptide concentration (nmol)	$f_a(\text{eff})$	$K_Q(\text{eff}) (M^{-1})$
BSA	0	0.68	1.36
BSA + tuftsin	10	0.68	1.59
BSA + tuftsin	20	0.68	1.47
BSA + modified tuftsin	10	0.71	0.73
BSA + modified tuftsin	20	0.83	1.00

The concentration of BSA was 0.08 nmol in all experiments

indicating that this tetrapeptide induces a slight increase in the  $\alpha$ -helical content of the protein. This is consistent with our finding that the effective quenching constant of BSA is enhanced in the presence of tuftsin (table 1). Unlike these observations, modified tuftsin markedly altered the CD spectrum of the protein under identical conditions. The bands at 220 and 208 nm disappeared and a new prominent band appeared at 212 nm, suggesting that the modified tuftsin-BSA interactions transform the protein conformation from the predominant  $\alpha$ -helix to mainly  $\beta$ -structure.

The present study demonstrates that tuftsin interacts weakly with albumin. These interactions are, however, significantly enhanced by introducing an appropriate apolar substituent at the C-terminus of the tetrapeptide. This enhancement in the interaction is probably due to the presence of fatty acyl residue in the modified tuftsin, for serum albumin is known to contain binding sites for fatty acids [8,9]. The binding efficiency of modified tuftsin is about 50% in comparison to that of palmitic acid, as judged from the fluorescence quenching data (not shown) obtained using this fatty acid.

Strong binding between tuftsin and albumin in vivo would serve two useful purposes. On the one hand, it should protect this tetrapeptide from degradation by the blood enzymes [1] while on the other, it will ensure delivery of tuftsin to macrophages [10]. We therefore suggest that the biological activity of tuftsin may increase



significantly by promoting its binding with albumin.

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Fig. 3. CD spectra of BSA (0.08 nmol) in PBS (pH 7.4) in the absence and presence of tuftsin (A) or modified tuftsin (B). (—) BSA; (---) BSA plus 10 nmol peptide; (-·-·-) BSA plus 20 nmol peptide. Tuftsin (or modified tuftsin) alone gave a baseline CD spectrum, similar to that of PBS, in identical conditions.