PERTURBATION OF TRYPTOPHANYL FLUORESCENCE OF BOVINE GROWTH HORMONE

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1. Introduction

Bovine growth hormone (BGH) has been found [1] to have about 61% of its amino acid sequence homologous with that of human growth hormone (HGH) [2, 3]. BGH does not stimulate growth in man but HGH is active in most non-primates in which it has been tested. No significant differences in their secondary structure have been reported. However, from hydrogen-exchange studies Cambiaso et al. [4] have concluded that HGH was 2–10 times more permeable to the solvent than BGH and other growth hormones. We [5] have recently measured the effect of non-polar solvents and tyrosyl ionization on the fluorescence of the single tryptophanyl residue of HGH. These and other chemical studies [6, 7] suggest that the tryptophanyl residue of HGH may be exposed to the outside and be accessible to solvent molecules. In order to compare the environment around the single tryptophanyl residue in the two growth hormones, fluorescence perturbation studies have been extended to BGH.

2. Experimental

BGH (Preparation B16, mean relative potency of 0.93 units/mg) was a gift from the Endocrinology Study Section, National Institutes of Health (USA). The hormone was further purified at 0–4° on a column of Sephadex G-100 by elution with 0.05 M Tris-HCl and 0.1 M KCl, pH 7.5. Two protein (absorption at 280 nm) peaks were obtained. The material of peak I (19% of total) which was at the void volume was rejected. Material from the center of peak II (81% of total) which came out at twice the void volume was used for these studies. A similar preparation has been found to be homogeneous by ultracentrifugation and by precipitation with antisera [8].

Protein concentration was determined by the optical density at 280 nm ($A^{0.1\%}_{1\ cm} = 0.70$) [8]. Details of excitation and determination of fluorescence intensity of protein and model compound solutions were as described [5]. When guanidine hydrochloride (Ultrapure, Schwartz Co., USA) was used, solutions were allowed to stand in its presence for 2 hr before fluorescence intensity was measured. Fresh solution of potassium iodide (A.C.S. Reagent Grade) was prepared where needed.

3. Results and discussion

Accessibility of tryptophanyl and tyrosyl residues of BGH to solvent molecules was measured by the effect of ethanol on the fluorescence intensity of the two residues and comparing with those of the corresponding model compounds (fig. 1). Rate of increase of protein fluorescence was linear up to about 20% ethanol (v/v). Rate of initial increase (up to 20% ethanol, by volume) of tryptophanyl fluorescence (350 nm) was only 36% of that of *N*-acetyl-L-trypto-

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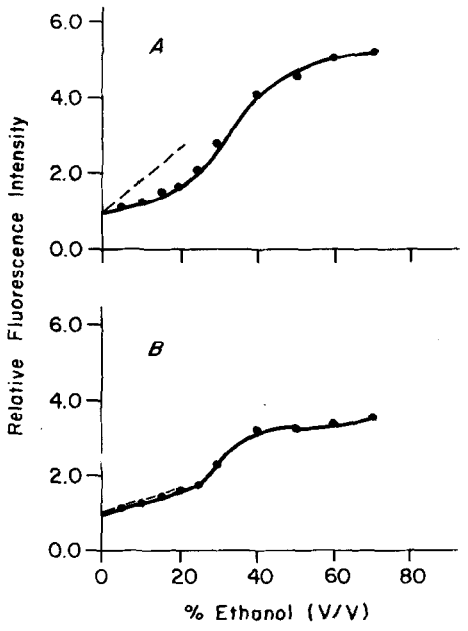

Fig. 1. Effect of ethanol (v/v) on the relative fluorescence intensity of bovine growth hormone (0.01%) excited at 280 nm in 0.01 M Tris-HCl and 0.15 M NaCl, pH 7.5. A) Tryptophanyl emission at 350 nm; (– – – –): *N*-acetyl-L-tryptophanamide [5]. B) Tyrosyl emission at 300 nm; (– – – –): *N*-acetyl-L-tyrosinamide [5].

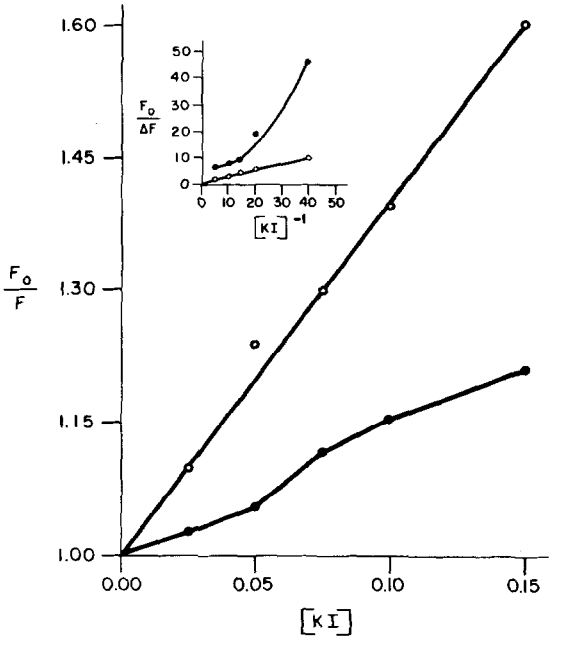

Fig. 2. Effect of KI (molar concentration) on tryptophanyl fluorescence (350 nm) of bovine growth hormone (0.0026%) excited at 280 nm in 0.01 M Tris-HCl and 0.15 M NaCl, pH 7.5, with (○—○—○) and without (●—●—●) 5 M guanidine hydrochloride. ΔF is the difference between the relative fluorescence intensities in the absence (F°) and presence (F) of KI.

phanamide (fig. 1A) [5], which suggests that the tryptophanyl residue is only partially accessible to ethanol molecules. On the other hand, the rate of initial increase of tyrosyl fluorescence (300 nm) was identical to that of *N*-acetyl-L-tyrosinamide [5] suggesting that all or some of the tyrosyl residues are exposed to solvent.

Accessibility of the tryptophanyl residue in BGH was further studied by quenching of its fluorescence with low concentration of iodide [9]. Quenching of tryptophanyl fluorescence of model compounds and of lysozyme has been found [9] to follow predominantly a collision mechanism as it obeyed the Stern–Volmer law [10] given by equation (1) where

$$\frac{F^0}{F} = 1 + K[I^-] \qquad (1)$$

F^0 and F are fluorescence intensities in the absence and presence of I^-, K is the quenching constant, and $[I^-]$ is molar concentration of I^-. For model tryptophanyl compounds and polymers with only one tryptophanyl residue per molecule equation (1) can be rearranged to equation (2), from which the extent of quenching at infinite concentration of I^- could be assessed. An intercept value of 1 for the polymer would be suggestive of complete accessibility of the tryptophanyl residue.

$$\frac{F^0}{F^0 - F} = 1 + \frac{1}{K[I^-]} \qquad (2)$$

A plot of tryptophanyl fluorescence intensity ratios of BGH in aqueous buffer and in the presence of 5 M guanidine hydrochloride against iodide concentration is shown in fig. 2. Quenching curve of the hormone in aqueous solution by I^- was non-linear, indicating that the low quenching pattern observed may not follow the collisional mechanism [9]. This may be partly due to lack of accessibility or exposure of the tryptophanyl residue, in which case the iodide radicals could not

diffuse and hit the excited fluorophore. A similar non-linear but a higher rate of quenching at low concentrations of KI and protein has been observed with HGH in aqueous solution, and the mechanism will be discussed elsewhere (results to be published). On the other hand, quenching in the presence of 5 M guanidine was completely linear with I^- concentration and a value of 4.06 M^{-1} for the quenching constant could be obtained from the slope of the straight line. The conclusion that the tryptophanyl fluorophore may have become completely accessible to iodide radicals in 5 M guanidine solution is further strengthened when the data are plotted according to equation (2) as shown in the inset of fig. 2. Again the quenching curve in the aqueous buffer was non-linear, but in 5 M guanidine it was linear and gave an intercept value of 1.

A comparison of the present and other fluorescence data of BGH [11] with that of HGH [5] suggests that the single tryptophanyl residue is located in different environments in these hormones. In HGH [5] it appears to be in a polar environment and situated close (13.4 Å) to the tyrosyl residue(s). As tryptophanyl fluorescence of BGH was found [11] to increase in the pH range of tyrosyl ionization, the inter-residue distance and orientation of the tryptophanyl and ionized tyrosyl residues may not be optimal for radiationless transfer of excitation energy to occur [12]. Some of these factors may be contributing to the difference between the two hormones with regard to tertiary structure [4] and biological specificity.

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