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INTRINSIC FLUORESCENCE OF A PROTEIN DEVOID OF TYROSINE AND TRYPTOPHAN: HORSE HEPATOCUPREIN

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1. Introduction

The study of intrinsic fluorescence of the protein has recently received much attention. The intrinsic fluorescence is due to the aromatic amino acids phenylalanine, tyrosine and tryptophan. The quantum yield of these amino acids linked in a polypeptide chain is very different. In fact tryptophan is about one order of magnitude more fluorescent than tyrosine, and two orders of magnitude more fluorescent than phenylalanine. Thus proteins containing both tryptophan and tyrosine show almost exclusively the fluorescence of the former, although often it is possible to observe a contribution from tyrosine in the short wavelength side of the spectrum.

In the present communication we report the fluorescence spectra of horse hepatocuprein, a cytocuprein with superoxide dismutase activity, which is a metalloprotein devoid of tyrosine and tryptophan. In this protein in the intrinsic fluorescence is solely due to the phenylalanine. This fluorescence is different from that of phenylalanine monomer.

2. Materials and methods

Hepatocuprein was isolated from horse liver as previously described [1]. The purity was checked by electrophoresis on cellulose acetate at pH 5, 7.1, and 9.7 (μ 0.05), and by disc electrophoresis at pH 9. A single band is always observed, which reacts also with alizarine blue. The concentration of the protein was determined by absorbance at 258 nm (E_M = 10 400). Optical spectra were measured with a Beckman DK2A ratio recording spectrophotometer. Fluorescence spectra were measured with a FICA L55 spectrofluorimeter which gives absolute values of fluorescence.

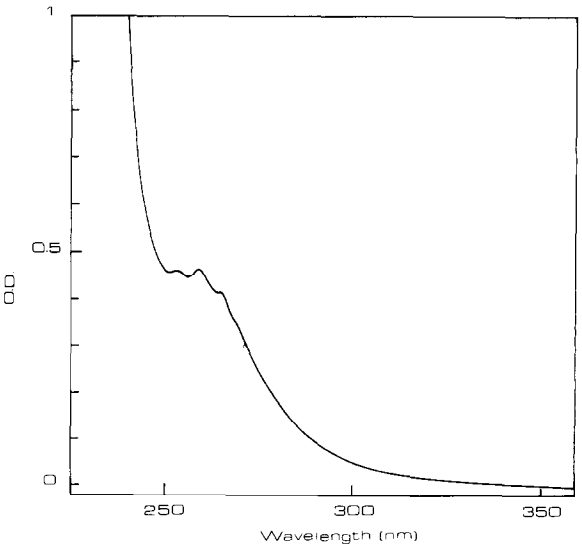

Fig. 1. Ultraviolet absorption spectrum of horse hepatocuprein. 4 × 10^{-5} M hepatocuprein dissolved in water. Optical path: 1 cm.

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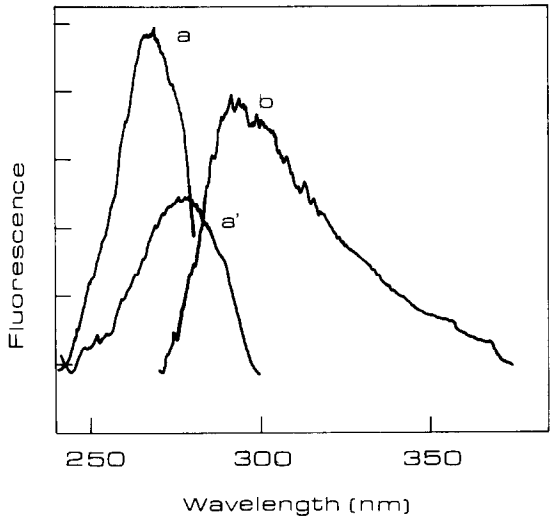

Fig. 2. Fluorescence excitation and emission spectra of native hepatocuprein. The spectra were obtained using an aqueous solution of hepatocuprein (2×10^{-5} M). Excitation 260 nm for the spectrum (b); emission 290 nm for the spectrum (a) and 340 nm for the spectrum (a'). The bandwidths were 2.5 nm (excitation) and 7.5 nm (emission) for the excitation spectra and 2.5 nm (both) for the emission spectrum.

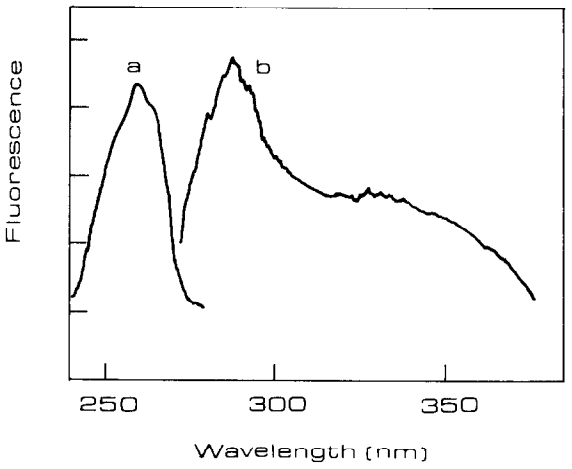

Fig. 3. Fluorescence of denatured hepatocuprein. 2×10^{-5} M hepatocuprein was dissolved in water containing 6 M guanide hydrochloride and 10^{-2} M EDTA. Then the pH of the solution was brought to pH 0.5. Excitation 260 nm (curve b); emission 290 nm (curve a). Other experimental details as in fig. 2.

3. Results and discussion

The U.V. absorption spectrum of hepatocuprein is reported in fig. 1. The position of the absorption maximum and the presence of a typical fine structure seem to indicate that the absorption is due to phenylalanines residues. However the extinction coefficient is much higher than expected. In fact the protein contains ten phenylalanines and neither tryptophan nor tyrosine [1]. Being the extinction coefficient at 258 nm per phenylalanine residue equal to about 200, other chromophores should be present under the phenylalanine absorbance, which contribute to the overall absorbance. A similar observation was made by Fee [2] who found that the removal of metals from a bovine superoxide dismutase, a protein homologous to this hepatocuprein, reduced the extinction coefficient from about 10 000 to 2900.

The fluorescence spectra of hepatocuprein are presented in fig. 2. The protein shows a maximum at 292 nm with poorly resolved fine structure and a long tail on the red edge of the peak. As shown in the same figure it is possible to obtain two different excitation spectra with maxima at 258 nm and 282 nm by setting the emission at 290 nm or 340 nm respectively. This fact indicates that probably a contamination of a tryptophan-containing protein is present. On the basis of the quantum yield of phenylalanine and tryptophan it is possible to estimate the contamination to about 1% which is in good agreement with the chemical determination previously reported [1].

Horse hepatocuprein is the first relatively large protein the fluorescence of which is reported to be due only to phenylalanine. Therefore reference can be only made either to phenylalanine or to simple phenylalanine-containing peptides. It has been reported that the fluorescence of phenylalanine monomer is sensitive to the environment. Furthermore it appears that the fluorescence of phenylalanine in dipeptides is influenced by the nature of the partner amino acid [3]. In any case the fluorescence spectra show a significant amount of fine structure. The absence of fine structure in the fluorescence emission has been already reported in the case of the polypeptide antibiotic gramicidin [3]. However the structural basis of this behaviour is yet unsolved, since no correlation has been made with the steric arrangement of the amino acid side chain. The absence of fine struc-

ture in the fluorescence spectra of hepatocuprein is not associated with a tertiary folding of the protein. This is indicated by the small change of the fluorescence spectra of hepatocuprein treated with 6 M guanidine hydrochloride at pH values lower than one (fig. 3). Probably in these conditions the Cu and Zn atoms contained in the protein are removed and any tertiary structure is lost. All phenylalanine residues in the protein seem to be equivalent as far as the fluorescence is concerned. This would indicate that they are located in equivalent segments of the polypeptide chain.

References

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