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Fluorometric determination of *N*-terminal tryptophan-peptides after formaldehyde condensation

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TRYPTAMINE can be determined fluorometrically after condensation with formaldehyde followed by oxidation with hydrogen peroxide.[1] The method is non-specific in that also *N*-methyltryptamine and tryptophan give formaldehyde-induced fluorophores with spectral properties indistinguishable from those of the tryptamine fluorophore. Tryptamine, *N*-methyltryptamine and tryptophan are known to react with formaldehyde also on silica gel thin layer.[2–5] It was recently reported that formaldehyde is a sensitive chromatographic detection reagent also for tryptophanyl-dipeptides.[6] From this observation it appeared probable that *N*-terminal-tryptophan-peptides could be determined fluorometrically by a method similar to that described for tryptamine. This assumption was confirmed in the present study.

Experimental

Aqueous solutions of tryptamine, tryptophan and various tryptophan-containing dipeptides (see Table 1) were prepared: concentrations of 1–3 μg (free base) per ml. Aliquots of these solutions (usually 0·5 ml) were mixed with 0·1 ml formaldehyde solution (18%) in a total volume of 3 ml, made up with 0·1 N sulphuric acid. After heating the samples in boiling water for 20 min, 0·1 ml 5% hydrogen peroxide was added and heating was continued for another 20 min. The samples were cooled in a refrigerator and then analyzed in an Aminco–Bowman spectrophotofluorometer, equipped with an *x–y* recorder.

Results and comments

All the peptides with an *N*-terminal tryptophan residue gave strong fluorescence after combined treatment with formaldehyde and hydrogen peroxide; no fluorescence resulted if the hydrogen peroxide was omitted. The other tryptophan-containing dipeptides (having the tryptophan amino group engaged in peptide linkage) did not give any fluorescence with the formaldehyde-hydrogen peroxide treatment (Table 1). The spectral properties of the fluorophores of the tryptophanyl-dipeptides were without exception very similar to those of the tryptamine and tryptophan fluorophores (Fig. 1 and Table 1). For tryptophanyl-glycine the fluorescence intensity was proportional to the concentration in the range 0·1 to 3 μg/ml (Fig. 2).

The reaction of tryptamine (and tryptophan) with formaldehyde is believed to result in the formation of 1,2,3,4-tetrahydro-norharman which is subsequently dehydrogenated (by the addition of hydrogen peroxide) to 3,4-dihydro-norharman and/or norharman.[1] It appears likely that the same

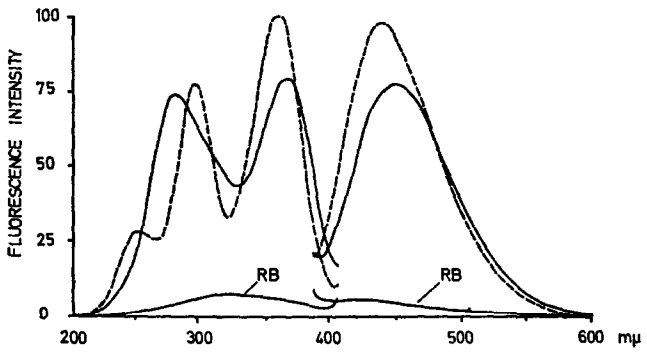


FIG. 1. Excitation (left) and emission (right) spectra of the fluorophores of tryptamine (---) and L-tryptophanyl-L-glycine (——). Equimolar concentration: 10^{-6}M. Reagent blank: RB. Spectra are uncorrected and reproduced as recorded.

TABLE 1. FORMALDEHYDE-INDUCED FLUORESCENCE OF TRYPTAMINE, TRYPTOPHAN AND TRYPTOPHAN-CONTAINING DIPEPTIDES*

Compounds	Fluorescence intensity of equimolar conc.†	Excitation/emission max. (mμ)‡
Tryptamine	100	(290) 350/440
L-Tryptophan	100	(290) 355/440
L-Tryptophanyl-L-alanine	70	(270) 360/450
L-Tryptophanyl-L-glycine	75	(270) 360/450
L-Tryptophanyl-L-tyrosine	40	(290) 370/460
L-Tryptophanyl-L-tryptophan	30	(290) 370/450
L-Glycyl-L-tryptophan	No fluorescence	
L-Phenylalanyl-L-tryptophan	No fluorescence	
L-Propyl-L-tryptophan	No fluorescence	

* All peptides were purchased from Miles Laboratories.
† The fluorescence intensity of tryptamine was given as 100.
‡ Uncorrected instrumental values. Minor peak in parenthesis.

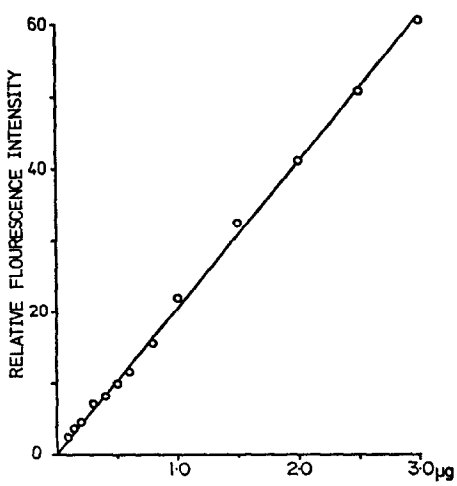
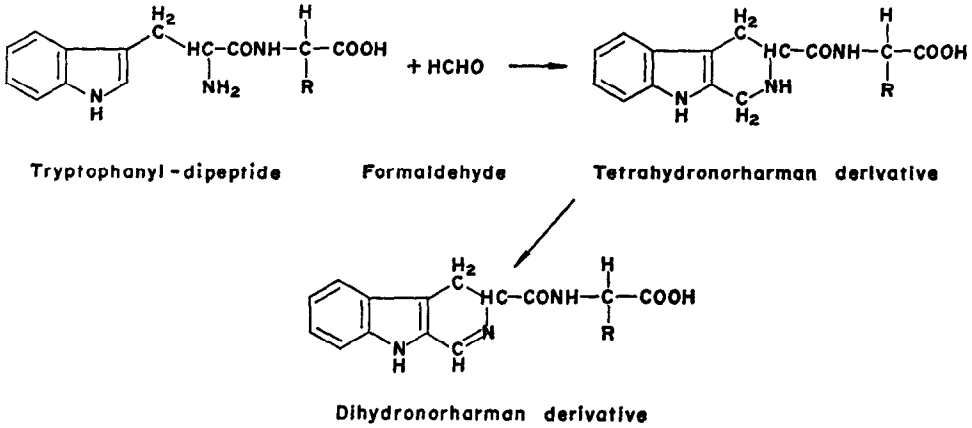


FIG. 2. Rectilinear relationship between the fluorescence intensity and the amount of L-tryptophanyl-L-glycine (in a final volume of 3 ml).

sequence of reactions takes place with the tryptophanyl-peptides according to the following reaction scheme:



Conceivably, all peptides and proteins having an *N*-terminal tryptophan residue will form highly fluorescent conjugates with formaldehyde under the reaction conditions specified. The method has been successfully adapted both for the chromatographic[6] and fluorescence histochemical detection[7] of such peptides.

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Changes in histaminase content following experimental thermal injury

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HISTAMINASE catalyses the oxidative deamination of histamine and occurs widely in many tissues. Plasma histaminase levels are remarkably increased in pregnancy.[1,2] Histaminase levels have been found, in this laboratory and others, to be raised in myocardial infarction,[3] bronchial asthma[4,5] and anaphylaxis.[6–9] Furthermore, histamine is known to be involved in many tissue challenge situations