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Gaseous formaldehyde: A sensitive chromatographic detection reagent for tryptophanyl-dipeptides

A few years ago, formaldehyde gas was introduced as a sensitive and specific chromatographic detection reagent for catecholamines and indoleamines[1–8]. After condensation with formaldehyde, these amines, or rather their condensation products, were found to emit strong fluorescence in UV light. The condensation procedure used for the chromatographic analysis was essentially the same as that described earlier for the fluorescence microscopic localization of these biogenic arylethylamines[9–12]. The chemical reactions that result in fluorophore formation have been investigated in some detail[10–13]. Briefly, the concept is as follows: in an initial reaction step, the catecholamines and indoleamines are condensed with formaldehyde, forming tetrahydroisoquinolines and tetrahydro-β-carbolines, respectively. In a second step, these derivatives are oxidized (by dehydrogenation) to highly fluorescent dihydroisoquinolines and dihydro-β-carbolines. The precise chemical identities of the resulting fluorophores are unknown. Recent evidence suggests that a number of different formaldehyde-induced fluorophores may result from each individual amine[14].

Apart from catecholamines and indoleamines, certain amino acids with a catechol or indole group also emit strong fluorescence upon formaldehyde treatment. Tryptophan is one of the indoleamino acids which readily form a fluorophore with formaldehyde. Also tryptophanyl-peptides (with a free tryptophan amino group) might be expected to react with formaldehyde, giving rise to fluorescent conjugates. This assumption was confirmed in the present investigation. Certain tryptophan-containing dipeptides were found to give intense fluorescence on silica gel thin layers after formaldehyde treatment, permitting the chromatographic detection of small amounts of these compounds.

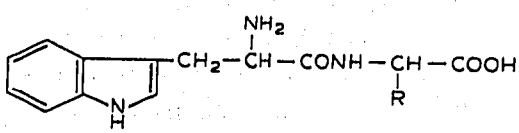
Experimental

Silica gel thin layers were prepared by coating glass cover slips (24 × 32 mm, for routine histology) with approximately 100-μm Kieselgel H (Merck, Darmstadt). The layer was applied as a slurry consisting of 20 g silica gel suspended in 50 ml glass-distilled water. The plates were dried at room temperature before use. Aqueous solutions of tryptamine hydrochloride, tryptophan and tryptophan-containing peptides (see Table I) in various concentrations were applied to the thin layers in volumes of 0.5 μl. The silica gel thin layers were exposed to formaldehyde gas generated from paraformaldehyde (equilibrated in an atmosphere of about 50% relative humidity) at 100° for 30–60 min. The thin layers were examined in UV light (Sterisol UV-lamp, Original Hanau, equipped with a UG 1 filter) and the fluorescence was analyzed in a modified Leitz microspectrograph[15]. The fluorescence intensities of the various spots were visually evaluated in the UV light; the minimum detectable amount was used as a measure of the fluorescence intensity. For the microspectrofluorometric analysis, the optical system for the exciting light consisted of quartz components, and the glass cover slips were placed upside down with the thin layer facing the condenser. The thin layer outside the fluorescent spot was used to obtain blank spectra. All spectra were corrected for instrumental errors according to procedures previously

described[15]. All values given for excitation and emission maxima are the mean values of at least four separate determinations.

Results and discussion

All the tryptophanyl-peptides tested gave strong greenish-yellow fluorescence upon formaldehyde treatment. As a chromatographic detection reagent formaldehyde gas had a similar sensitivity for tryptophanyl-dipeptides as for tryptamine and tryptophan. The minimum detectable amount varied from 0.3 to 0.1 μg for the different tryptophanyl-dipeptides (Table I). None of the tryptophan-containing peptides having the tryptophan amino group engaged in peptide linkage, gave visible fluorescence with formaldehyde. Thus, the dipeptides that gave strong formaldehyde-induced fluorescence had the following general formula:



The excitation and emission maxima of the various tryptophanyl-dipeptides were remarkably similar: excitation at 375–380 mμ, emission at approximately 500 mμ (Table I). The second amino acid in the dipeptide appeared to have only a minor influence on the spectral properties of the fluorophore. It should be noted that the spectral properties of the fluorophores of the tryptophanyl-dipeptides showed a fairly close resemblance to those of the tryptamine fluorophore while differing from those of the tryptophan fluorophore (Fig. 1).

TABLE I

FORMALDEHYDE-INDUCED FLUORESCENCE OF TRYPTAMINE, TRYPTOPHAN AND SOME TRYPTOPHAN-CONTAINING DIPEPTIDES ON SILICA GEL

All peptides were purchased from Miles Laboratories, Ill., U.S.A.

Compounds	*Minimum detectable amount (μg)*	*Excitation/emission max. (mμ)*
Tryptamine	0.01	370/500
L-Tryptophan	0.03	375/450
L-Tryptophanyl-L-alanine	0.1	375/500
L-Tryptophanyl-L-glycine	0.1	375/500
L-Tryptophanyl-L-tyrosine	0.3	380/500
L-Tryptophanyl-L-tryptophan	0.3	380/505
L-Glycyl-L-tryptophan	Non-detectable	
L-Phenylalanyl-L-tryptophan	Non-detectable	
L-Propyl-L-tryptophan	Non-detectable	

Conceivably, all peptides with N-terminal tryptophan residues give fluorophores upon formaldehyde condensation. The results strongly suggest that also peptides with N-terminal 3,4-dihydroxyphenylalanine or 5-hydroxytryptophan residues can be expected to emit formaldehyde-induced fluorescence.

The method has been adapted also for the fluorescence histochemical demonstration of peptides and proteins with N-terminal tryptophan residues[16].

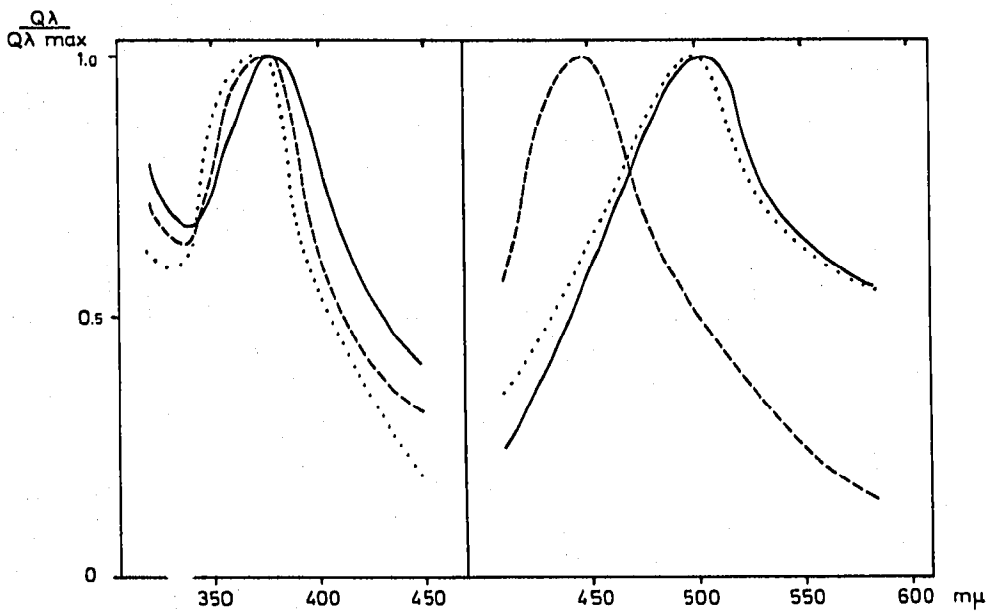


Fig. 1. Excitation (left) and emission (right) spectra of the formaldehyde-induced fluorophores of tryptamine (. . .), L-tryptophan (- - -) and L-tryptophanyl-L-glycine (——). Spectra were recorded from spots containing 0.3 μg/cm^2 of the indole and are expressed as relative quanta *versus* wavelength.

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