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Note

Glyoxylic acid spray reagent for thin-layer chromatographic identification of phenylethylamines and indolylethylamines

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Glyoxylic acid (GA) forms strongly fluorescent reaction products with certain phenylethylamines and indolylethylamines equally well in solution[1], on silica gel thin layers[2] and in a dried protein matrix[2,3]. On the basis of this fluorophore-forming capacity, a fluorescence histochemical method for monoamines using GA has been devised[4–6]. In this work, the usefulness of GA as a spray reagent for the detection of phenylethylamines and indolylethylamines on silica gel thin layers has been evaluated and the spectra of the various GA-induced fluorophores have been characterized by means of microspectrofluorimetry.

EXPERIMENTAL

The experiments were performed on pre-coated silica gel plates (Merck, Darmstadt, G.F.R.). Aqueous solutions of phenylethylamines, indolylethylamines, imidazolylethylamines and some related amino acids in various amounts (up to 1 μg) were applied on to the plates in volumes of 1 μl. GA monohydrate (Fluka, Buchs, Switzerland) was dissolved in ethanol to a concentration of 0.2 or 5%. The thin-layer plates were sprayed with either of these solutions and then heated in an oven at 80° for 1 h or at 100° for 45 min. Formalin spray was used for comparison. In this case, the thin layers were first sprayed with a mixture of formalin (40% aqueous formaldehyde solution) and concentrated ammonia solution (1:1), dried in an oven at 100° for 30 min, sprayed with a mixture of formalin, 6 *M* hydrochloric acid and absolute ethanol (1:2:1), and then again dried (Procházka reagent; see refs. 7 and 8). The sensitivity of the reagents was evaluated by establishing the lowest concentration visible in the spot when examined under UV light (excitation at 365 nm).

Microspectrofluorimetric analysis of the fluorophores was performed with a modified Leitz microspectrofluorimeter[8,9]. The thin layers were placed upside-down with the silica gel facing the quartz bright-field dry condenser. The thin layer outside the spot was used to obtain blank spectra. All spectra were corrected for instrumental errors as described previously[9].

RESULTS AND DISCUSSION

The GA spray reagent induced strong fluorescence from phenylethylamines

and indolylethylamines on silica gel (Table I). Of the different procedures tested, spraying with 5% GA followed by heating at 100° for 45 min seemed to be the most sensitive. The results obtained after this treatment were similar to those after spraying with the formalin reagent (Table I). With the GA reagent, no or very little diffusion of the spots occurred, most probably due to the small amounts of water present. It should also be mentioned that the GA reagent had only a slight odour compared with the strong odour of the formalin reagent. Thus the GA spray reagent is a good alternative to the formaldehyde-based Procházka reagent. However, in some instances, the latter is to be preferred because of the weaker background fluorescence induced from the silica gel layer.

TABLE I

MINIMUM DETECTABLE AMOUNTS (μg) OF PHENYLETHYLAMINES AND INDOLYLETHYLAMINES ON SILICA GEL AFTER TREATMENT WITH THE FORMALIN OR GLYOXYLIC ACID SPRAY REAGENT

Compound	*Formalin reagent*	*Glyoxylic acid reagent**
DOPA	0.03	0.03
Dopamine	0.01–0.03	0.01
Noradrenaline	0.01–0.03	0.01
Adrenaline	0.01	0.01
p-Tyramine	0.03–0.1	—**
3-Methoxytyramine	0.01	0.01–0.03
Tryptophan	0.003	0.001–0.003
Tryptamine	0.003–0.01	0.001
5-Hydroxytryptophan	0.01	0.01
5-Hydroxytryptamine	0.003–0.01	0.003–0.01
N-Methyl-5-hydroxytryptamine	0.01	0.003–0.01
5-Methoxytryptamine	0.01	0.001–0.003
N-Acetyldopamine	—***	—**
N-Acetyl-5-hydroxytryptamine	—***	0.03
N-Acetyl-5-methoxytryptamine (melatonin)	—***	0.01–0.03
N,N-Dimethyltryptamine	—***	0.03
5-Hydroxy-N,N-dimethyltryptamine (bufotenin)	—***	0.1
5-Methoxy-N,N-dimethyltryptamine	—***	0.03
Histamine	—**	—**
GABA	—**	—**
Piperidine	—**	—**

* 5% glyoxylic acid in ethanol and heating at 100° for 45 min.
** 1.0 μg not visible.
*** Not tested.

The GA-induced fluorophores of the various substances showed reproducible and characteristic spectra, which make possible an *in situ* characterization by means of a microspectrofluorimeter. The excitation and emission maxima of the various fluorophores are given in Table II.

Both the sensitivity of the GA reagent for the various substances and the spectral characteristics of their GA-induced fluorophores are similar to those obtained when the reaction with GA is performed in a dry protein matrix[2–4,6]. Thus,

TABLE II

SPECTRAL PROPERTIES OF THE GLYOXYLIC ACID-INDUCED MONOAMINE FLUOROPHORES ON SILICA GEL

Results after spraying spots containing 1 μg of the substances with 5% GA in ethanol and heating at 100° for 45 min.

Compound	*Excitation/emission maximum (nm)**
DOPA	(330) 375 or 410**/490
Dopamine	(330) 375 or 410**/480
Noradrenaline	(330) 375 or 410**/480
Adrenaline	(330) 375 or 410**/500
3-Methoxytyramine	(325) 375/480
Tryptophan	375/500
Tryptamine	375/510
5-Hydroxytryptophan	(375) 420/500
5-Hydroxytryptamine	375 (420)/520
N-Methyl-5-hydroxytryptamine	370/510
5-Methoxytryptamine	370 (420)/560
N-Acetyl-5-hydroxytryptamine	(335) 375/480
N-Acetyl-5-methoxytryptamine (melatonin)	(335) 375/500
N,N-Dimethyltryptamine	(340) 375/460
5-Hydroxy-N,N-dimethyltryptamine (bufotenin)	(340) 370/460–480
5-Methoxy-N,N-dimethyltryptamine	(340) 370/500

* Figures in parentheses denote lower peaks or shoulders in the spectrum.

** The GA-induced fluorophores of 3-hydroxylated phenylethylamines show a pH-dependent tautomerism (excitation maximum at 370–375 nm at acid pH and at 410 nm at neutral and alkaline pH; for discussion of the chemical background see, *e.g.*, ref. 3). The pH of the solvent system used will therefore influence the spectra so that either the acid or the neutral form or mixtures of the two forms will be recorded.

GA treatment, like the Procházka reagent[7,10], also induced strong fluorescence on silica gel thin layers from N-acetylated and tertiary indolylethylamines, whereas the fluorescence yield from 4-hydroxylated phenylethylamine, *p*-tyramine and N-acetyldopamine was very low. Therefore, it seems likely that the formation of fluorophores with GA on silica gel proceeds as in the dry protein matrix[2,3]. In fact, this has been shown to be the case for tryptamine[2].

The results show that GA, which is a valuable reagent in amine histochemistry[4–6], also permits the detection and spectral characterization of minute amounts of phenylethylamines and indolylethylamines on silica gel thin layers. This is of great interest, because it makes possible a direct correlation between results obtained in fluorescence histochemistry and analytical biochemistry.

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