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Note

Nucleic acid bases and derivatives: detection by Dns derivatization thin-layer chromatography

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During our studies on the chemistry of abnormal inclusions in the brain, we needed a sensitive and convenient method to analyze for CMP and related compounds. Such compounds may undergo a variety of chemical reactions[1]. Therefore we investigated the reaction of dimethylaminonaphthalene-5-sulfonyl chloride (Dns-Cl) towards nucleic acid bases and their derivatives. The reaction conditions are presented here, and thin-layer chromatography (TLC) of the fluorescent products is also described. This is apparently the first report on the detection of such molecules by the sensitive Dns derivatization method.

EXPERIMENTAL

Dns derivatization

The Dns–Cl reaction was adapted from that described by Gray[2] for amino acids and peptides. Nucleic acid bases and derivatives were purchased from Sigma (St. Louis, MO, U.S.A.) and Dns-Cl was from Pierce (Rockford, IL, U.S.A.). The stoichiometry of Dns-Cl to amino groups was modeled after the optimal conditions described by Airhart *et al.*[3] for amino acids. We used a different buffer and a higher pH than most investigators have reported for the Dns-Cl reaction. The following reaction conditions were used to prepare our Dns standards. Buffer was made with deionized water which had been boiled and flushed with nitrogen gas to remove any traces of ammonia, which if present, gives rise to Dns-amide. Each compound (0.5 mg) was dissolved in 1.25 ml of untritated 10 m*M* Na_3PO_4, pH 11.7. Then 1.25 ml of Dns-Cl in acetone (1.0 mg/ml) was added and the reaction was carried out overnight at 25°C in the dark. Subsequent steps were also protected from light[4].

TLC

An appropriate aliquot of the reaction mixture was spotted on a low-fluorescence sheet[5] of Polyamide A 1700 (Pierce), 15 cm in height. For most compounds, the amount spotted was 0.25–1.0 μl, or 50–200 ng of the individual compound. Chromatograms were subjected to ascending chromatography in various solvents. After

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the solvent had migrated nearly to the top, the sheet was air-dried. Dns spots were visualized under UV light.

RESULTS AND DISCUSSION

Dns derivatization

Some nucleic acid bases and their derivatives —especially the cytosine series— were not adequately derivatizated when sodium bicarbonate, pH 8.5 (ref. 2) was employed as the buffer. We checked the effect of the basicity on the reaction, between pH 7–13. The best results were obtained between pH 11–12.2. We then routinely used untitrated trisodium phosphate, pH 11.7.

The concentrations of reactive groups and Dns-Cl were kept at about 500 pmoles/μl and 1840 pmoles/μl, respectively, as recommended for amino acids[3,6]. However, we found that those values could be increased up to four- and two-fold, respectively, with no apparent loss in the efficiency of Dns derivatization of the nucleic acid bases and their derivatives.

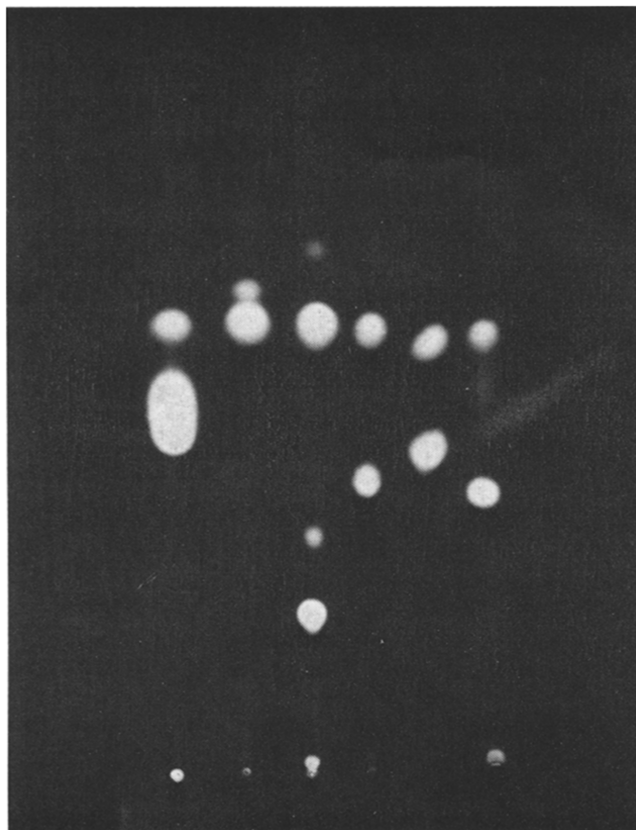

Fig. 1. TLC of Dns compounds on a sheet of Polyamide A 1700. From left to right: Dns–adenine, –cytosine, –guanine, –thymine, –uracil and –xanthine. The solvent was 6% HCOOH. The spot in common to each lane is hydrolyzed reagent (Dns-OH), a side-product always present in Dns derivatizated samples.

TLC

Fig. 1 shows the results of chromatography of the Dns derivatized nucleic acid bases adenine, cytosine, guanine, thymine, uracil and xanthine. About 200 ng of each of the compounds were spotted. These amounts were readily visualized as their Dns derivatives under UV light. Spots were still faintly detected when the amounts were 4–20 fold less.

Dns derivatization method was more sensitive for some of the compounds than for others. Minimal amounts required for visualization were: adenine and its derivatives (adenosine, AMP, and so forth), 10–20 ng; cytosine and its derivatives, 50–100 ng; others (listed in Table I), 30–60 ng. For a comparison of sensitivities based on molar amounts, 10 ng of adenine = 74 pmoles while 50 ng of cytosine = 450 pmoles.

Additional spots in the guanine sample (in the third lane, Fig. 1) were considerably weaker in intensity than the main spot presumed to Dns–guanine itself. The extra compounds were present in different batches of guanine; they are probably contaminants of the guanine standard.

Table I gives the migration data for chromatography of the Dns derivatizated

TABLE I

R_F AND R_D VALUES FOR Dns-NUCLEIC ACID BASES AND DERIVATIVES ON POLYAMIDE A 1700 SHEETS

Solvent systems: A, 6% formic acid; B, ethyl acetate–ethanol–ammonium hydroxide (20:2.5:1); C, ethyl acetate–ethanol–ammonium hydroxide (20:5:1), = solvent F of Metrione[13], for Dns–amino acids.

R_D = the distance of migration of the compound of interest, divided by the distance of migration of Dns-OH in that sample.

Dns-compound	R_F			R_D		
	A	*B*	*C*	*A*	*B*	*C*
Adenine	0.54	0.93	0.96	0.80	11.4	7.0
Cytosine	0.66	0.83	0.92	1.07	10.1	6.2
5-Methylcytosine*	—	0.88	—	—	9.0	—
Guanine	0.21	0.57	0.83	0.35	6.4	5.0
Thymine	0.40	0.91	0.94	0.66	11.4	6.8
Uracil	0.43	0.75	0.90	0.72	9.5	6.7
Xanthine	0.37	0.11	0.73	0.62	1.4	4.9
Hypoxanthine	0.36	0.50	0.82	0.61	5.6	6.0
Adenosine	0.78	0.92	0.16	1.25	7.7	0.47
Cytidine	0.89	0.87	0.09	1.43	6.9	0.26
Guanosine	0.76	0.91	0.39	1.25	9.6	1.15
Thymidine	0.45	0.69	0.13	0.72	6.7	0.37
Uridine	0.66	0.57	0.26	1.08	5.3	0.77
AMP	0.77	0.00	0.00	1.24	0.00	0.00
CMP	0.87	0.00	0.00	1.38	0.00	0.00
GMP	0.74	0.00	0.00	1.12	0.00	0.00
TMP	0.34	0.00	0.00	0.53	0.00	0.00
UMP	0.69	0.00	0.00	1.11	0.00	0.00
2'-dAMP	0.71	0.00	0.00	1.23	0.00	0.00
2',3'-cAMP	0.69	0.20	0.36	1.13	1.65	3.5
3',5'-cAMP	0.65	0.18	0.27	1.10	1.67	3.1
NAD	0.80	0.01	0.00	1.43	0.04	0.00

* A Dns spot was found for this compound only in solvent B.

compounds tested. Besides the conventional R_F value, we present R_D as defined in Table I. Comparisons made between different chromatographic runs per solvent, showed the R_D value to be highly reproducible — more so than was the R_F value.

Other results

We checked to see whether the presence of a biological tissue (brain) would interfere with Dns derivatization. Portions of normal rabbit substantia nigra were homogenized in the Dns derivatization buffer in the presence of several compounds (adenine, thymine or CMP). The weight ratios of wet brain to the solid compound were 10:1 and 100:1. Dns derivatization and TLC were carried out as described in Experimental. No interference was found in the analysis for those compounds tested. Only several additional faint spots appeared from the tissue tested at the above larger ratio.

Stability of the Dns compounds was investigated with respect to incubation in the reaction mixture, and mild acid hydrolysis. Dns derivatization at pH 11.7 for 8, 16, 24, or 48 h gave the same spot intensities. This contrasts with Dns–hexosamine spots which were markedly diminished in their intensities after 24 h, compared to several hours of Dns derivatization[7]. The Dns–nucleic acid bases and derivatives were also dried and hydrolyzed in 2 *N* HCl at 70°C for 16 h. Five Dns derivatized species were destroyed: adenine, guanine, xanthine, thymidine, and TMP. The others (see Table I) were unaffected by the acid treatment.

While the Dns method worked for those compounds listed, little success was obtained with the di- and triphosphates *e.g.* ADP and ATP. Perhaps steric hindrance is presented by the extra phosphate atoms. For example, rotary dispersion studies have indicated that in aqueous solution the pyrophosphate chain of ATP may be folded back to give bonding between the β- and γ-phosphates and the adenine amino group[8].

The present Dns method can be useful for compounds such as those in Table I, based on its simplicity and sensitivity. Some workers may also wish to prepare fluorescent derivatives (Dns) of the nucleic acid base moiety, for a variety of studies.

Other methods of analysis should also be mentioned for comparison. Post-labelling of nucleosides with [^{3}H]borohydride can detect about 25 ng of individual nucleosides[9]. Ethenylation and gas chromatography have been used for nucleosides and nucleotides of cytosine in the 500–4000 ng range[10]. High-performance liquid chromatography of nucleosides was quantitative down to 0.1 nmoles or about 25 ng[11]. Gas chromatography–mass spectrometry was used to detect as little as 1.6 pmoles (200 pg) of 5-methylcytosine[12].

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