UDC 547.963.3

THE THIN-LAYER CHROMATOGRAPHY OF DINUCLEOTIDES

K. A. Averburg, E. N. Morozova, and N. S. Tikhomirova-Sidorova

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 4, pp. 287–291, 1969

We have begun a study of the chromatographic behavior of the dinucleotides of a ribonuclease hydrolysate in a thin layer of sorbent.

The products of the hydrolysis of RNA and DNA have been analyzed until recently by paper chromatography [1, 2], paper electrophoresis [3], or ion-exchange chromatography [4, 5]. Since 1962, after the work of Randerath [6], thin-layer chromatography has also been introduced into the chemistry of the nucleic acids and their derivatives

The chromatographic analysis of the mononucleotide fraction of hydrolyzates of RNA by thin-layer chromatography is known [7–11]. Only with the successive use of two solvent systems, n-propanol–ammonia–water (60 : 30 : 10) and 0.24 M acetic acid, are the isomers of adenylic acid—the 2'- and 3'-phosphates— separated satisfactorily [7] The isomers of adenylic and guanylic acids have been separated satisfactorily with a combination of electrophoresis and chromatography in a thin layer on one and the same cellulose plate [10].

Recently the thin-layer chromatography of the pyrimidine oligonucleotides of a DNA hydrolysate has been described [12]. Two-dimensional chromatography in a thin layer of Kodak ECS silica gel with a fluorescing indicator was used.

We have obtained the dinucleotide fraction by the ion-exchange separation of an RNA hydrolysate on DEAE-Sephadex A-25 (Cl' form) in Tomlinson's system (7 M urea, NaCl gradient) [13] with subsequent rechromatography on Dowex 1 × 4 resin (HCOO' form) in a gradient of formic acid and ammonium formate [14].

Tables 1–3 give the results of chromatography of the dinucleotides of a ribonuclease hydrolysate in thin layers of ECTEOLA- and DEAE-celluloses and on powdered celluloses in various solvent systems.

In a thin layer of ECTEOLA-cellulose (see Table 1), all four of the dinucleotides studied had very similar relative mobilities (R_f). This prevented the separation of the mixture of dinucleotides. Stepwise elution in dilute solutions of hydrochloric acid showed the presence of an impurity in the dinucleotide G_pC_p (two spots on the chromatogram).

Authentic mixtures of mononucleotides and dinucleotides were not separated in a layer of DEAE-cellulose (see Table 2) or a layer of cellulose powder (see Table 3) in spite of the fact that the components of the mixture had different R_f values. This phenomenon is explained by the fact that in the solvent systems studied (apart from system 1 in Table 2), the nucleotides tend to form diffuse elongated spots on the chromatograms.

Only in system 1 (stepwise chromatography in dilute solutions of HCl) was it possible to separate authentic mixtures of dinucleotides and mixtures of mono- and dinucleotides. Because of the similar relative mobilities of the components of the mixtures, $A_p C_p - A_p$, $A_p C_p - C_p$ and $A_p C_p - C_p - A_p$ (see Table 2, system 1) were not separated in a thin layer of DEAE-cellulose. On this chromatogram these mixtures each gave a single spot with R_f values of 0.77, 0.78, and 0.75, respectively

Table 1

Dinucleotides	Solvent systems*							
	1	2	3	4	5	6	7	8
$A_p C_p$	0.58	0.03	0.08	0.89	0.92	0.30	0.54	0.79
$G_p C_p$	0.53 and 0.49	0.04	0.10	0.90	0.90	0.26	0.55	0.83
$A_p U_p$	0.47	0.00	0.06	0.93	0.93	0.27	0.72	0.96
$G_p U_p$	0.27	0.00	0.04	0.90	0.91	0.45	0.62	0.78

*1) Stepwise chromatography in dilute solutions of HCl: 0.01 N HCl–2 min; 0.02 N HCl–3 min; 0.04 N HCl–4 min; 0.06 N HCl–5 min; 2) 0.60-0.80 N NaCl [19]. 3) 1 M LiCl. 4) 1 M LiCl–2M HCOOH (1 : 1) [20]. 5) 1.5 M LiCl–2 M HCOOH (1 : 1) [20]. 6) 0.1 N NaCl–C_2H_5OH (65 : 35) [21]; 7) 0.6 N NaCl–C_2H_5OH (65 : 35). 8) Continuous elution with solutions of NaCl in dil NH_3 and phosphate buffer [22].

Table 2

Mono- and dinucleotides	Solvent systems*					
	1	2	3	4	5	6
$A_p C_p$	0.78	0.09	0.01	0.30	0.87	0.61
$G_p C_p$	0.28	0.06	0.00	0.45	0.68	0.58
$A_p U_p$	0.21	0.05	0.12	0.56	0.56	0.40
$G_p U_p$	0.09 and 0.29	0.07	0.00	0.78	0.39	0.39
A_p	0.80	0.18	0.02	0.53	0.81	0.62
U_p	0.20	—	—	—	—	—
G_p	0.32	—	—	—	—	—
C_p	0.79	0.23	0.01	0.52	0.83	0.60

*1) Stepwise chromatography in dilute solutions of HCl. 2) 0-0.125-0.25 N NH_4Cl. 3) Propan-2-ol-NH_3-H_2O (17 : 4.4 : 3.6). 5) 0.06-0.125-0.25-0.50-1.0 N-2.0N HCOOH; 6) 0.1 N NH_4Cl in 7 M urea.

Table 3

Mono- and dinucleotides	Solvent systems*		
	1	2	3
$A_p C_p$	0.55	0.31	0.58
$G_p C_p$	0.26	0.05	0.44
$A_p U_p$	0.43	0.10	0.60
$G_p U_p$	0.40	0.06	0.52
A_p	—	0.27	0.58
U_p	—	0.14 and 0.59	0.91
G_p	—	0.06 and 0.13	0.52 and 0.76
C_p	—	0.04	0.65

*1) 0.1 N NH_4Cl, 2 and 3) See legend to Table 2. Chromatography was carried out on a plate 13 × 18 cm the length of the run being 15 cm and the time 3 hr.

The mixture A_pU_p–A_p gave two spots with R_f 0.23 and 0.78, the mixture A_pU_p–U_p only one spot with R_f 0.20 (the R_f values of the components coincided and the mixture was not separated) and the mixture $ApUp$–Ap–Up two spots with R_f 0.21 and 0.77.

In the chromatography of authentic mixtures in a thin layer of DEAE-cellulose, the length of the solvent run was 8–9 cm and the time of chromatography 15 min. Under these conditions it was possible to separate authentic combinations of dinucleotides in a thin layer of DEAE-cellulose.

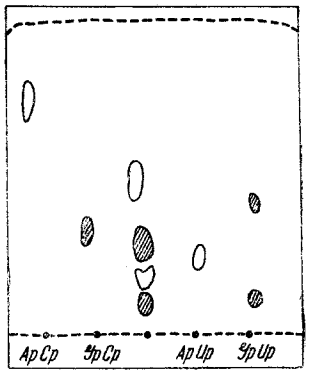
1. G_pC_p–G_pU_p, R_f 0.29 and 0.08 (the dinucleotide G_pU_p contained an impurity with R_f 0.29, apparently G_p. The spot of the G_p coincided in R_f value with the component of the impure G_pC_p.

2. A_pC_p–A_pU_p, R_f 0.76 and 0.20.

3. A_pC_p–G_pC_p, R_f 0.78 and 0.27.

4. A_pU_p–G_pU_p, R_f 0.32, 0.21 and 0.09 (spot with R_f 0.32–G_p as impurity in the dinucleotide G_pU_p).

In a thin layer of DEAE-cellulose in dilute solutions (0.01–0.06 N) of hydrochloric acid, the dinucleotide fraction of a ribonuclease hydrolysate was readily separated into the dinucleotides A_pC_p, G_pC_p, A_pU_p and G_pU_p (figure) in 15 min. The R_f values of the dinucleotides differ somewhat from the values obtained for the individual substances (see Table 2, system 1): $ApCp$–0.42, $GpCp$–0.22, $ApUp$–0.13 and $GpUp$–0.07.



Chromatogram of the dinucleotide fraction of a hydrolysate of RNA on DEAE-cellulose (with reference samples).

Experimental

Preparation of the cellulose. A) ECTEOLA-cellulose (Serva cellulose with a capacity of 0.43 meq/g was used). The commercial preparation was shaken with double-distilled water (1000 ml of water was necessary per 100 g of cellulose). The aqueous layer was decanted off and the cellulose was treated with acetone and dried at 105° C for 3 hr [15].

B) DEAE-cellulose. A powder of DEAE cellulose (capacity 0.57 meg/g) was sieved through a 150-mesh sieve and subjected to alkaline and acid treatment [16, 17].

C) Cellulose powder. For the thin-layer chromatography we used powdered cellulose supplied to us by S. M. Zhenodarova (Institute of Biophysics, AS USSR, Pushchino-na-Oke). The powder was obtained from "Leningrad rapid" filter paper by treatment with 10% nitric acid [18].

Preparation of the plates. The air-dry cellulose powder was suspended in water or acetone and was carefully triturated to give a homogeneous mass which was deposited on 9 × 12 cm plates. The ratios of the sorbents and diluents were as follows: A) 3 g of ECTEOLA-cellulose and 23 ml of distilled water; B) 3 g of DEAE-cellulose and 45 ml of water; C) 3 g of powdered cellulose and 25 ml of acetone.

The plates were dried at room temperature for 12–18 hr.

Chromatography. The substances—the dinucleotides of a ribonuclease hydrolysate, A_pU_p, G_pU_p, A_pC_p, G_pU_p, or mixtures of them in an amount of 6×10^{-8}–1×10^{-8} mole (0.003–0.005 ml)—were deposited on the starting line 1 cm from the edge of the plate. The distance between the neighboring substances on the chromatogram was 1 cm. The size of the initial spots was 0.3–0.5 cm.

The substances under investigation were chromatographed by the ascending method with a depth of immersion of the plate of 0.3 cm.

The chromatography in solutions of hydrochloric acid, ammonium chloride, sodium chloride, and lithium chloride was carried out in an open vessel and that in solutions of sorbic acid, ethanol, propan-2-ol, and ammonia in closed vessels with previous saturation. The time of chromatography was from 15 min to 1 hr. The plates were dried at 105° C for 5–7 min, and the spots were revealed in UV light on the ultrachemiscope.

The visual detection of the substances on the chromatogram in UV light was facilitated by the fact that in a thin layer of DEAE cellulose in the hydrochloric acid system the spots had different colors: the dinucleotides A_pC_p and A_pU_p appeared in the form of dark lilac spots and the dinucleotides G_pC_p and G_pU_p fluoresced.

Conclusions

1. The chromatographic behavior of the dinucleotides of an RNA hydrolysate in thin layers of ECTEOLA- and DEAE-cellulose and on a cellulose powder has been studied. The R_f values of the four dinucleotides A_pC_p, A_pU_p, G_pC_p and G_pU_p in various solvent systems have been determined. It has been shown that in a thin layer of DEAE cellulose the dinucleotide fraction of a ribonuclease hydrolysate is separated into the four individual components.

2. Conditions for the separation of authentic mixtures of dinucleotides and of mixtures of mono- and dinucleotides have been found.

REFERENCES

1. R. Markham and A. J. D. Smith, Biochem. J., **49**, 401, 1951.
2. G. W. Rushizsky and C. A. Knight, Proc. Natl. Acad. Sci., US, **46**, 945, 1960.
3. J. N. Davidson and A. R. Smellie, Biochem. J., **52**, 599, 1952.
4. W. E. Cohn , J. Am. Chem. Soc., **72**, 1471, 1950.
5. W. E. Cohn and E. Volkin, Biochim. Biophys. Acta, **24**, 359, 1957.
6. K. Randerath, Dünschichtchromatography, Verlag Chemie, Weinheim, 1962.
7. T. A. Dyer, J. Chromatog., **11**, 414, 1963.
8. J. L. Starr and B. Ramberg, Nature, **211**, 414, 1966.
9. C. Ratapongs, Naturw., **53**, 251, 1966.
10. W. Hiby and H. Kröger, J. Chromatog., **26**, 545, 1967.
11. G. R. Björk and J. Svensson, Biochim. Biophys. Acta, **138**, 430, 1967.
12. D. Lando, J. De Rudder, and M. P. De Garilhe, J. Chromatog., **30**, 143, 1967.
13. R. V. Tomlinson and G. M. Tener, Biochem., **2**, 697, 1963.
14. V. D. Aksel'rod, T. V. Venkstern, and A. A. Baev, Biokihm., **30**, 999, 1965.
15. N. S. Panteleeva, Vestn. LGU, biol. ser., no. 9, **73**, 1964.
16. N. S. Tolmacheva and L. N. Nikolenko, ZhAKh, **22**, 298, 1967.
17. P. Grippo, M. Faccarino, M. Rossi, and E. Scarano, Biochim. Biophys. Acta, **95**, 1, 1965.
18. M. I. Khabarova and S. M. Zhenodarova, KhPS [Chemistry of Natural Compounds], **2**, 48, 1966.
19. K. Randerath, Biochim. Biophys. Acta, **61**, 852, 1962.
20. K. Banderath, J. Chromatog., **16**, 111, 1964.
21. R. Barber, Biochim. Biophys. Acta, **114**, 422, 1966.
22. N. V. Nikolaeva, L. P. Semenova, and K. E. Kruglyakova, DAN SSSR, **169**, 1203, 1966.

1 April 1968

Institute of High-Molecular-Weight Compounds AS USSR