Polyamide Thin-Layer Chromatography of Phosphorylated Tyrosine, Threonine, and Serine

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One-dimensional thin-layer chromatography on polyamide plates offers an easy and rapid identification of *O*-phosphotyrosine. The thin-layer plate is developed for 30 min in 5% propionic acid containing 0.013%–0.025% sodium dodecyl sulfate. *O*-Phosphotyrosine, with $R_f = 0.54$, can be well separated from *O*-phosphothreonine and *O*-phosphoserine, which comigrate at $R_f = 0.72$.

KEY WORDS: polyamide; TLC; phosphoamino acids.

Phosphorylation of tyrosine residues has been reported in polyoma T antigens (1), the Abelson murine leukemia virus protein (2), pp60^{src} of Rous sarcoma virus (3), the insulin receptor (4), and a membrane protein in Swiss mouse 3T3 cells stimulated by platelet-derived growth factor and epidermal growth factor (5). It appears that phosphorylation of the tyrosine side chain might be a very early step in the transformation by tumor viruses as well as in the expression of some hormonal functions. The phosphotyrosine has been detected by high-voltage electrophoresis on paper (4) or cellulose thin layers (1,3,5), both requiring special apparatus and taking hours to complete an analysis. Here we describe a simple and rapid thin-layer chromatography for the analysis of phosphotyrosine.

MATERIALS AND METHODS

O-Phosphorylated threonine, serine, and tyrosine, 5'-UMP, and 3'-UMP were purchased from Sigma Chemical Company (St. Louis, Mo.). Polyamide thin-layer plates were the

product of Cheng-Chin Company (Taiwan, R. O. C.). Propionic acid and sodium dodecyl sulfate (SDS)² were from E. Merck (Darmstadt, West Germany). [γ -³²P]ATP was purchased from New England Nuclear Company (Boston, Mass.).

Polyamide plates, 5 × 15 cm, were loaded with 0.4 μ g each of these three phosphoamino acids and developed in 5% propionic acid containing various amounts of SDS for 30 min. After drying, the plates were sprayed with 0.2% ninhydrin solution and dried in an oven (approx 50°C) for 1 min. The spots of amino acids should be located within 5–10 min; otherwise these spots cannot be differentiated from the background color.

RESULTS AND DISCUSSION

The chromatogram of authentic compounds is shown in Fig. 1. Phosphotyrosine could be easily separated from the other two phosphorylated amino acids. However, phosphothreonine and phosphoserine comigrated in this simple system. Several solvents containing formic, acetic, and butyric acids in various proportions have been tried and did not offer better results.

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² Abbreviation used: SDS, sodium dodecyl sulfate.

FIG. 1. Thin-layer chromatogram of phosphoamino acids in 5% propionic acid containing 0.025% SDS. (A) P-Tyr; (B) P-Thr; (C) P-Ser; (D) mixture of the three.

In order to check the potential effect of residual acid and the separation of phosphotyrosine from other phosphorylated compounds commonly found in the hydrolyzed phosphoprotein samples, ATP, hydrolyzed phosphotyrosine, phosphothreonine, phosphothreonine, 5'-UMP, and 3'-UMP were run in this system. The results are summarized in Fig. 2. Furthermore, we have isolated a phosphotyrosine-containing polypeptide from mouse thymoma cell EL-4 (S.-C. Lee, manuscript in preparation). The phosphotyrosine from the hydrolyzed sample could be detected in this simple system (Fig. 2). It is clear that phosphotyrosine could be separated from many potentially interfering substances in this system. Inorganic phosphate ran with the solvent front (data not shown). Within 30 min, the solvent should reach 1–2 cm from the end of the plate in our system.

The presence of SDS significantly improves the resolution of phosphotyrosine from other

FIG. 2. Thin-layer chromatogram of hydrolyzed phosphotyrosine, phosphoserine, and phosphoserine (lane 1), 3'-UMP (lane 2), 5'-UMP (lane 3), hydrolyzed 3'-UMP (lane 4), hydrolyzed 5'-UMP (lane 5), [γ - 32 P]ATP (lane 6), and hydrolyzed phosphotyrosine-containing phosphoprotein from EL-4 (lane 7). Lanes 2–5 were visualized by short-wavelength uv while lanes 6–7 were detected by autoradiography (Kodak XAR-2 X-ray film, -70°C , 20 h exposure). S.F. is solvent front. Ori is origin.

phosphoamino acids in polyamide thin-layer chromatography. The optimal concentration was 0.013–0.025%, as shown in Table 1. Two other detergents, Tween 20 (E. Merck) and

TABLE 1

THE EFFECT OF SDS CONCENTRATION ON RESOLUTION OF PHOSPHOAMINO ACIDS

SDS (%)	R_f values (average of two experiments)		
	P-Tyr	P-Thr	P-Ser
0	0.66	0.75	0.77
0.0063	0.55	0.71	0.71
0.013	0.53	0.70	0.71
0.025	0.56	0.73	0.75
0.05	0.59	0.75	0.73

Note. SDS was dissolved in 5% propionic acid as the developing solvent. See Materials and Methods for details. P-Tyr, phosphotyrosine; P-Thr, phosphothreonine; P-Ser, phosphoserine.

NP-40 (Sigma Chemical Co.), were found to cause extensive tailing of the amino acid spots (data not shown). We believe that the ionic detergent nature of SDS makes it useful in this system. However, we have not investigated this point further. Ninhydrin (0.2%) was used for the localization of phosphoamino acids. The amino acid spots could easily be located within 5 min of spraying. Phosphotyrosine appeared to be a light yellowish-brown spot on a blue background even after several days.

Polyamide thin-layer chromatography offers a simple, fast, economical, and easy-to-perform method for the detection of phos-

photyrosine. Moreover, the study of tyrosine kinase could be facilitated by using this technique.

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