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Short communication

Comparison of the thin-layer chromatographic properties of sulfur-containing amino acids and their aminophosphonic analogues

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Dedicated to Prof. Dr. B. Zwanenburg on the occasion of his retirement from the Department of Organic Chemistry, University of Nijmegen

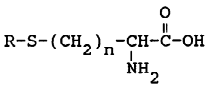
Abstract

The thin-layer chromatographic behavior of the natural, sulfur-containing amino acids (Cys, Hcys, Met) and their phosphonic analogues (Cys^P, $Hcys^P$, Met^P) has been studied. Their detection limits using iodine and molybdate, ninhydrine and iodine–azide reagents were determined, and the R_F values of these two classes of amino acids in acidic, neutral and mild basic solvent systems were compared.

Keywords: Amino acids; Aminophosphonic acids; Sulfur compounds

1. Introduction

Sulfur-containing amino acids (SAA) play an essential role in the metabolism of living organisms [1]. Their phosphonic analogues (SAPA) synthesized and explored in two last decades [2,3] were found to exhibit strong biological activity and some of them therefore find application in the area of pharmacology [4–6].

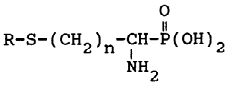


SAA

(R=H, n=1) [Cys]

(R=H, n=2) [Hcys]

(R=Me, n=2) [Met]



SAPA

(R=H, n=1) [Cys^P]

(R=H, n=2) [$Hcys^P$]

(R=Me, n=2) [Met^P]

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Replacement of the carboxylic function of the SAA by the phosphonic group strongly influence the physico-chemical properties, especially acidity and polarity, thus changing also their chromatographic mobility. For these reasons, a comparative study of the thin-layer chromatographic (TLC) behavior of the SAA and SAPA is of some relevance. Pertinent data are presented in this paper.

2. Experimental

2.1. Materials

1-Aminoalkanephosphonic acids (Cys^P, $[Cys^P]_2$, $Hcys^P$, $[Hcys^P]_2$ and Met^P) were prepared according to Ref. [2,3]. Other amino acids, reagents and chemicals were purchased from Aldrich (Milwaukee, WI, USA).

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2.2. Solutions and reagents

Aqueous solutions of amino acids were used (concentration from 5×10^{-2} *M* to 1×10^{-3} *M*).

2.3. Solutions for the TLC detection

A 1 *M* aqueous solution of sodium azide, acidified to pH ca. 6 before use and a 1 *M* solution of iodine (in a 1 *M* aqueous solution of potassium iodide) were employed for the TLC detection [8].

Molybdate reagent was prepared by dissolving 1 g of ammonium molybdate in 40 ml of water, followed by addition of 3 ml of concentrated hydrochloric acid and 5 ml of a 70% perchloric acid. This solution was finally diluted with 100 ml of cold acetone [9].

2.4. Determination of the inductive effect on the iodine–azide reaction

The efficiency of amino acids as inductors has been characterized and compared on the basis of their induction factors (F_i), defined by Eq. (1)

$$F_i = n_I/n_i \quad (1)$$

where n_I equals the amount of iodine consumed in the induced iodine–azide reaction and n_i equals the amount of the inductor (both given in millimoles).

The consumption of iodine in the induced iodine–azide reaction was determined by iodometric titration according to Ref. [7].

2.5. Thin-layer chromatography

Precoated cellulose DC plates (10 cm×5 cm, 0.1 mm thick layer) (Merck, Darmstadt, FRG), were used for TLC experiments. The plates were spotted with an appropriate amount of compound (deposition area ca. 0.2 cm^2) developed for a distance of 8 cm with the eluent (at 20°C), air dried and detected with the appropriate detection system (for details see Tables 1 and 2).

2.6. Detection of amino acids by the iodine–azide procedure

Indirect detection by means of the iodine–azide reagent was carried out using a freshly prepared 1:1 (v/v) mixture of sodium azide and iodine solutions. The amino acids — inductors of the iodine–azide reaction appeared as white spots on an yellow background, and were stable for more than 0.5 h.

Table 1
Comparison of the TLC chromatographic properties of amino acids and their aminophosphonic analogues

Amino acid[a] Abbreviation	TLC [R_F] TLC system[b]							
	A	B	C1	C2	D1	D2	D3	E
Gly	0.20	0.15	0.32	0.48	0.41	0.37	0.42	0.27
Cys	0.19	0.15	0.17	0.39	0.49	0.43	0.50	0.25
$(Cys)_2$	0.07	0.03	0.05	0.20	0.23	0.15	0.24	0.11
Hcys	0.45	0.36	0.51	0.64	0.56	0.56	0.58	0.57
$(Hcys)_2$	0.24	0.14	0.17	0.40	0.45	0.33	0.44	0.25
Met	0.51	0.44	0.62	0.71	0.68	0.68	0.69	0.65
Gly^P	0.18	0.11	0.12	0.29	0.29	0.25	0.32	0.30
Cys^P	0.25	0.21	0.18	0.33	0.31	0.27	0.36	0.52
$(Cys^P)_2$	0.08	0.03	0.00	0.00	0.07	0.04	0.10	0.18
$Hcys^P$	0.40	0.30	0.20	0.43	0.45	0.41	0.49	0.61
$(Hcys^P)_2$	0.15	0.08	0.00	0.00	0.00	0.00	0.00	0.36
Met^P	0.46	0.41	0.36	0.52	0.58	0.54	0.59	0.68

[a] Amino acids were taken in amounts ca. 20 nmol and after development (at 20°C) were localized by means of the ninhydrine reagent.

[b] Following TLC systems were applied: A: cellulose plates DC vs. n-BuOH/$MeCO_2H$/water (12:3:5). B: cellulose plates DC vs. n-BuOH/$EtCO_2H$/water (12:4:4). Cl: cellulose plates DC vs. t-BuOH/HCO_2H/water (14:3:3). C2: cellulose plates DC vs. t-BuOH/HCO_2H/water (12:3:5). D1: cellulose plates DC vs. t-BuOH/TFA/water (12:3:5). D2: cellulose plates DC vs. t-BuOH/TFA/water (12:4:4). D3: cellulose plates DC vs. t-BuOH/TFA/water (11:4:5). E:cellulose plates DC vs. pyridine/MeOH/water (10:7:3).

Table 2
Comparison of the TLC detection properties of acids and their phosphonic analogues

Amino acid		F_i[a]	TLC				R_F[g]
Nr	Abbrev.		Detection limits [nmol] per spot][i] Detection procedure				
			I_2–N_3[b]	I_2[c]	Ninh.[d]	Mo[e]	
1a	Cys	325	20	20	20	30[f]	0.19
1b	Hcys	95	30	5	5	30[f]	0.45
1c	Met	–[h]	20	5	20	–[j]	0.51
2a	Cys^P	138	20	20	20	20	0.25
2b	$Hcys^P$	60	30	5	5	30	0.40
2c	Met^P	–[h]	20	5	1	5	0.46

[a] Induction factor (F_i) of amino acids determined in solution.
[b] Iodo–azide procedure: white spots on yellow background.
[c] Iodine procedure: brown spots on yellow background.
[d] Ninhydrine procedure: blue spots.
[e] Molybdate procedure: blue spots.
[f] Spots appeared after 15 min of exposition time.
[g] TLC system: cellulose plates DC vs. n-BuOH/$MeCO_2H$/water (12:3:5).
[h] Does not induce the iodine–azide reaction in solution.
[i] Not detectable in UV up to the level of 100 nmol of amino acid.
[j] Not detectable up to the level of 50 nmol of amino acid.

2.7. Detection of amino acids by the molybdate procedure

The chromatographic plates were air dried in a fume hood (approximately 30 min), sprayed with the molybdate reagent and while still wet, were irradiated using a 254 nm ultraviolet source for 3 to 5 mm. The plate was then further exposed to light for 1 to 2 h to assure complete color development. Amino acids appeared as blue spots on a white background.

3. Results and discussion

3.1. Separation

The R_F data (TLC/cellulose) of (investigated amino presented in Table 1.

The corresponding R_F values in the examined series of phonic acids illustrate an inverse order in respect chain size of these amino acids:

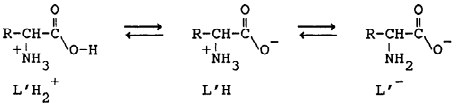
$$R_F[(Cys^P)_2] < R_F[(Hcys^P)_2] < R_F(Gly^P) < R_F(Cys^P) < R_F(Hcys^P) < R_F(Met^P);$$

which is generally in accordance with an earlier Neuzil and Cassaigne [10] on a similar topic.

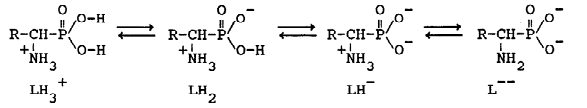
The distinct separation between SAA and respective SAPA appears evident from the chromatograms. These data show a difference in the chromatographic behavior of the amino acids in acidic (A, B, C and D) and mild basic (E) development systems. Thus, in the acidic systems aminophosphonic acids have, generally, lower R_F values than their carboxylic analogues. However, in neutral or mild basic development system (E) the opposite occurs with aminophosphonic acids being retained less than the corresponding carboxylic amino acids. This phenomenon can be explained on the basis of the structural differences between the phosphonic and the carboxylic functions, which influence the dissociation (protonation) equilibria of the examined amino acids.

Thus, $pK_{1(Ala)} = 2.34$, $pK_{2(Ala)} = 9.60$ [11], when $pK_{1(Ala^P)} < 1$, $pK_{2(Ala^P)} = 5.6$ and $pK_{3(Ala^P)} = 10.2$ [12]. (The corresponding pK data of Cys, Hcys and Met are given in Refs. [3] and [13].)

As a result, for difunctional amino acids (Gly and Met) and corresponding aminophosphonic acids (Gly^P and Met^P) the dissociation equilibria can be illustrated by Eqs. (2) and (3) [13], respectively.



(2)



(3)

Correspondingly, fully protonated thiolic amino acids exist in the forms LH_4^+ (Cys^P and $Hcys^P$) or LH_3^+ (Cys and Hcys), and respectively their disulfides in the forms LH_6^{++} [$(Cys^P)_2$ and $(Hcys^P)_2$] or LH_4^{++} [$(Cys)_2$ and $(Hcys)_2$], and undergo the dissociation in the similar way.

Taking into account the strong acidic character (pK_1) of the α-carboxylic and/or the α-phosphonic group both types of examined amino acids exist as zwitterions (L′H and LH_2) in weak acidic systems

(A, B and C) and/or as cations (LH_3^+ and $L'H_2^+$) in the D systems.

In neutral or mild basic development systems (e.g. E) amino acids exist still as zwitterions (L′H) whereas aminophosphonic acids (with diprotic phosphonic function) exist as anions (LH^-), what causes higher chromatographic mobilities than those exhibited by corresponding zwitterion forms.

4. Detection

The detection limits (DL) for cysteines (Cys and Cys^P), homocysteines (Hcys and $Hcys^P$) and methionines (Met and Met^P) are summarized in Table 2.

Thus, these amino acids are transparent in UV light up to 100 nmol per spot. The amino acids exhibit a similar trend in the detection sensitivities by means of the ninhydrine reagent. Thus, the highest detection sensitivity is fixed for Met^P (DL = 1 nmol), lower homocysteines ($Hcys^P$ and Hcys; DL = 5 nmol) and the lowest for the cysteines (Cys and Cys^P) and Met (DL = 20 nmol). This detection behavior reveals a characteristic structural influence of examined amino acids on their detection reaction with ninhydrine, namely the influence of the distance between the sulfur atom (in the SH or MeS function) the aminomethine moiety and also of the type of acidic group (carboxylic or phosphonic).

The reaction of the examined amino acids with iodine exhibits a similar detection sensitivity for methionines and homocysteines (Met and Met^P, Hcys and $Hcys^P$; DL = 5 nmol) and lower sensitivity for cysteines (Cys and Cys^P; DL = 20 nmol).

All examined aminophosphonic acids, as well as the thiolic amino acids, can be detected by means of the molybdate detection reagent (the so called phosphate detection reagent [9,14]). Thus, for Met^P the detection limit was as the order as 5 nmol, and for Cys^P and $Hcys^P$ of the order of 20 nmol. Surprisingly, for Cys and Hcys (amino acids without phosphonic moiety) the detection limit was 30 nmol, whereas Met and gly are not detectable up to a level of 50 nmol per spot. This phenomenon can be explained by the interaction of the sulphydryl function of these amino acids with the molybdate reagent. The results on the detection of aminophosphonic acids are in contradiction with the earlier report of Lepri and co-workers [15] who claimed that only β-Ala from the broad spectrum of various aminophosphonic acids can be detected by the molybdate reagent.

The results of the reaction of the examined amino acids with the iodine–azide reagent, carried out both in solution and on a TLC plate, generally presented poor correlation. Thus, Cys ($F_i = 325$), Cys^P ($F_i = 138$) and $Hcys^P$ ($F_i = 60$) exhibit a DL = ca. 20 nmol, whereas Hcys ($F_i = 95$) is detectable at DL = 30 nmol per spot. Both methionines (Met and Met^P) have not been characterized by induction coefficients due to their reaction with iodine alone, occurring in a solution [16]. However, their treatment with the iodine–azide reagent on TLC plates gave typical positive test results by formation of characteristic white spots on a yellow background with the DL = ca. 20 nmol per spot. This specific behavior of the examined amino acids resembles the unusual induction exhibited by some no-sulphur-containing phosphoroorganic derivatives during their detection by this reagent on silica oxide TLC plates, which we have reported recently [17].

References

[1] F. Challenger, in: Aspects of the Organic Chemistry of Sulphur, Butterworths Scientific Publications, London, 1959.
[2] Z.H. Kudzin and W.J. Stec, Synthesis (1980) 645.
[3] Z.H. Kudzin and W.J. Stec, Synthesis (1983) 814.
[4] V.M. Shumyantseva, I.A. Gandurina, T.R. Osipova, E.V. Kugaevskaya, Vopr. Med. Khim., 36 (1990) 74; Chem. Abstr.; 113: 144; 878.
[5] Y. Morita, Y. Hoshiide, S. Taniguchi, R. Andoi, Y. Takashiha, JPN Kokai Tokkyo Koho Jp. 62, 215, 557; Chem. Abstr.; 108 (1988) P 221 893.
[6] E.W. Chavel, C. Llorens-Cortes, P. Goric, S. Wilk, B. Roques, M.C. Formie-Zalewski, J. Med. Chem. 37 (1994) 2590.
[7] W. Ciesielski, Z.H. Kudzin, P. Kiełbasiński, Talanta 41 (1994) 1493.
[8] Z.H. Kudzin, A. Kotyński, P. Kiełbasiński, J. Chromatogr. 588 (1991) 307.
[9] B.R. Bochner, D.M. Maron, B.W. Ames, Analytical Biochemistry 117 (1981) 81.
[10] E. Neuzil, A. Cassaigne, Bull. Soc. Chim. Fr., (1967) 3994.
[11] H. Jakubke, H. Jeschkeit, in: Aininosauren, Peptiden und Proteinen, Academie-Verlag-Berlin (1982); second Polish edition, PWN-Warsow, 1989, p. 32.

[12] M. Wozniak, G. Nawrocki, Talanta 26 (1979) 1135.
[13] Z.H. Kudzin, G. Andrijewski, J. Drabowicz, J. Heteroatom Chem. 5 (1994) 1.
[14] C.S. Haves, F.A. Isherwood, Nature (London) 164 (1949) 1107.
[15] L. Lepri, P.G. Desideri, V. Coats, J. Chromatogr. 52 (1970) 421.
[16] Z. Kurzawa, Z. Lemieszek, Chem. Anal. (Warsow) 20 (1975) 147.
[17] A. Kotyński, Z.H. Kudzin, W. Ciesielski, J. Drabowicz, J. Chromatogr. A 831 (1999) 321.