0273-2289/94/4802—0057$04.60

Degradation and Disposal of Some Enzyme Inhibitors

Scientific Note

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Received October 4, 1993; Accepted November 12, 1993

ABSTRACT

Five enzyme inhibitors (phenylmethylsulfonyl fluoride, 4-amidinophenylmethanesulfonyl fluoride, 4-(2-aminoethyl)benzenesulfonyl fluoride, Nα-*p*-tosyl-L-lysine chloromethyl ketone, and *N*-tosyl-L-phenylalanine chloromethyl ketone) in buffer, DMSO, or stock solutions were completely degraded by adding 1*M* NaOH and the final reaction mixtures were not mutagenic. The stability of these compounds decreased as the pH increased.

Index Entries: Safety; degradation; stability; enzyme inhibitors; PMSF; APMSF; AEBSF; TLCK; TPCK; HPLC.

INTRODUCTION

The degradation of five enzyme inhibitors in buffers, DMSO, and stock solutions was investigated. These inhibitors were: phenylmethylsulfonyl fluoride (*1,2*) (PMSF), 4-amidinophenylmethanesulfonyl fluoride.HCl (*3*) (APMSF), 4-(2-aminoethyl)benzenesulfonyl fluoride (*4*) (AEBSF), Nα-*p*-tosyl-L-lysine chloromethyl ketone.HCl (*5*) (TLCK), and *N*-tosyl-L-phenylalanine chloromethyl ketone (*5,6*) (TPCK).

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EXPERIMENTAL

Stock solutions were 100 m*M* PMSF, 20 m*M* AEBSF, and 1 m*M* TPCK in isopropanol; 100 m*M* APMSF and 5 m*M* TLCK in water, and 25 m*M* APMSF in 1:1 isopropanol:pH 3 buffer. DMSO solutions were at the same concentrations.

Buffers were pH 5 50 m*M* phthalate, Hanks' balanced salts (Hanks') (pH 6.4), 50 m*M* pH 7 phosphate, Dulbecco's phosphate buffered saline (Dulbecco's) (pH 7.2), 10 m*M* phosphate buffered saline (PBS) (pH 7.2), 50 m*M* HEPES containing 500 m*M* NaCl (pH 7.5), and 50 m*M* Tris-Borate-EDTA (TBE) (pH 8.0). Inhibitor concentrations were 1 m*M* except for PMSF (10 m*M*) and APMSF (2.5 m*M*).

The reaction mixtures were neutralized with acetic acid before mutagenicity testing (*7*) and analysis by reverse phase HPLC. The mobile phases (1 mL/min), UV detection wavelengths (nm), and limits of detection (μg/mL) were: PMSF acetonitrile:water 50:50, 220, 0.9; AEBSF acetonitrile:0.1% trifluoroacetic acid (TFA) 40:60, 225, 0.1; APMSF acetonitrile: 0.1% TFA 40:60, 232, 0.5; TLCK acetonitrile:0.1% TFA 40:60, 228, 0.37; and TPCK acetonitrile:10 m*M* phosphate buffer (pH 7) 48:52, 228, 2.

Destruction of Inhibitors in Buffer

For each 10 mL of solution add 1 mL of 1*M* NaOH. Check to ensure that the pH is ≥ 12 and allow to stand for 1 h (PMSF, APMSF, and AEBSF) or 18 h (TPCK and TLCK).

Destruction of Inhibitors in Stock Solutions and DMSO

For each 1 mL of PMSF or APMSF solution add 5 mL of 1*M* NaOH. For each 1 mL of AEBSF solution add 10 mL of 1*M* NaOH. For each 10 mL of TPCK or TLCK solution add 1 mL of 1*M* NaOH (50 mL for TLCK in DMSO). Shake to ensure complete mixing and allow to stand for 24 h (PMSF, APMSF, or AEBSF) or 18 h (TPCK or TLCK).

RESULTS

When the enzyme inhibitors were degraded using the above procedures destruction was >99% in each case, except for TPCK in TBE where analytical problems increased the limit of detection (destruction >98.3%). None of the reaction mixtures was mutagenic.

Although degradation of PMSF in stock solution was instantaneous, other peaks were seen by HPLC and the reaction mixture was mutagenic after 1 h. After 24 h these peaks decayed to a very low level (~1 ppm) and the reaction mixture was not mutagenic. Accordingly, degradation of the sulfonyl fluorides was allowed to proceed for 24 h.

The mutagenicity of the inhibitors was determined. AEBSF and TPCK in DMSO and TLCK in aqueous solution were mutagenic.

Table 1
Stability of Inhibitors in Aqueous Solution[a]

Buffer	Half-life				
	AEBSF	PMSF	APMSF	TLCK	TPCK
pH 5.0	6 d	6 d	285 min	41 h	103 h
pH 6.4 Hanks'	11 d	6 h	90 h[b]	>28 d[c]	74 h
pH 7.0	35 h	62 min	2.6 min	106 min	33 h
pH 7.3 Dulbecco's	34 h	23 min	3.5 min[d]	93 min	53 h
pH 7.4 PBS	33 h	23 min	3.5 min[d]	98 min[e]	45 h
pH 7.5 HEPES	17 h	11 min	<1 min[f]	84 min[f]	29 h
pH 8.0 Tris	136 min	7 min	<1 min	8 min	12 h

[a]The pH of the reaction mixtures was within ±0.2 U of that shown, except as indicated.
[b]Actual pH 3.7.
[c]Actual pH 3.8.
[d]Actual pH 6.9.
[e]Actual pH 7.0.
[f]Actual pH 7.2

In buffer solution the stability of these inhibitors decreased as the pH increased (Table 1). In Hanks' some of the half-lives were longer than expected, perhaps because the addition of the stock solution of the inhibitor affected the pH of the buffer.

PMSF, AEBSF, and TPCK were stable in isopropanol or DMSO over 3 wk. APMSF in DMSO was stable over 3 wk and in 1:1 isopropanol:pH 3 buffer it had a half-life of 26 d. After 22 d the concentration of TLCK in DMSO was 79% of the original concentration.

ACKNOWLEDGMENT

We thank Hazleton Washington and A. W. Andrews (NCI-FCRDC) for the mutagenicity testing. Work was supported by the Division of Safety, NIH through NCI Contract NO1-CO-74102.

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