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Effect of Semicarbazones on Endogenous Protein Hydrolysis in Liver Homogenate

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Semicarbazones of substituted benzaldehydes were synthesized and their effect on endogenous protein hydrolysis in liver homogenate was studied. It was found that *p*-nitrobenzaldehyde semicarbazone exerted maximum effect.

Key Words: Semicarbazones, Proteolysis, Liver homogenate, Endogenous protein substrates.

INTRODUCTION

Semicarbazones constitute an important class of compounds for new drug development. These compounds possessing an azomethine -NH-N=CH- have been synthesized as target structures, which possess varied biological activites such as anticonvulsant[1,2], antitumoral[3], antihypertensive[4-6], antimicrobial[7], antifungal[8-10] and antitrypanosomal[11] activities. For example 3-chloro-2-methylphenyl substituted semicarbazones are found to possess anticonvulsant activity[12], 4-bromophenyl substituted arylsemicarbazones and thiosemicarbazones derivatives showed better activity against *Toxoplasma gondii*[13]. 4-Substituted semicarbazone derivatives of *o*- and *p*-chloro as well as 2,6-dichlorobenzaldehyde were synthesized and investigated for antihypertensive activity[14]. The present work is focused on the effect of semicarbazones on endogenous protein hydrolysis in liver. Proteolysis is the directed degradation of proteins by cellular enzymes called proteases. Many diseased conditions involve altered protease expression and substrate proteolysis. In particular, excessive protease activity is often observed, offering targets for therapeutic inhibition. At present an estimated 5-10 % of all pharmaceutical targets are proteases. The literature survey reveals that work is reported on proteases acting at highly acidic pH[15], to proteases acting at near neutral pHs[16] and to a few neutral and alkaline proteases[17]. The present work provides a view on *in vitro* proteolysis of endogenous substrates by liver proteases in presence of substituted benzaldehyde semicarbazones.

EXPERIMENTAL

Reactions were monitored by thin-layer chromatography. TLC plates were coated with silica gel G (suspended in $CHCl_3$-MeOH) and iodine vapours were used as visualising agent. Melting points were determined in open capillaries and

thus are uncorrected. Yields were determined from isolated products. ELISA reader was used for measuring absorbance in the visible range. Goat liver was purchased freshly from the local slaughter house. Microfuge was used for centrifugation purposes.

General method for the synthesis of semicarbazones: The semicarbazones were synthesized by the usual method[18]. Ethanolic solution of substituted benzaldehyde (1 mmol) was mixed with aqueous solution of semicarbazide hydrochloride (1 mmol) and sodium acetate (1 mmol). The reaction mixture was heated to obtain a clear solution. It was then cooled. The precipitates obtained were filtered, washed with cold water, dried and recrystallized with ethanol.

Preparation of liver homogenate: The fresh goat liver was first washed with cold isotonic saline solution. The tissue was then homogenized in 0.1 M sodium acetate buffer pH 5.3 containing 0.2 M NaCl, 1 mM EDTA and 0.1 % Triton X-100 in a mixer-cum-blender to obtain 10 % (w/v) homogenate. It was then stored at 4 ºC.

Assay for proteolytic activity: The proteolysis was carried out over a pH range 2.0-9.5 at 37 ºC using 0.12 M universal buffer consisting of boric acid, citric acid, 5,5'-diethyl barbituric acid and Na_2HPO_4 as the incubation medium. The enzyme homogenate was mixed with universal buffer at different pHs separately and was incubated at 37 ºC for 3 h. The reaction was stopped by the addition of TCA and the resulting solution was centrifuged to precipitate proteins. The acid soluble proteins were quantitated in the supernatant using Bradford method[19,20]. The experiment was conducted in triplicate and the results are presented in Table-1.

TABLE-1
PROTEASE ACTIVITY OF LIVER TISSUE AT VARIOUS pH

pH	2.0	3.5	5.0	6.5	8.0	9.5
Protease activity (h/mL) at 630 nm	0.560	1.212	1.398	0.130	0.224	0.012

Proteolytic activity of liver homogenate at various pHs: after proteolysis for 3 h at 37 ºC, TCA soluble peptides were estimated at 630 nm using bradford method and the results are the mean of one of the experiment conducted in triplicate and are calculated as protease activity (h/mL) in 1 % liver homogenate.

Similarly, the proteolytic studies on endogenous protein substrates were carried out in presence of substituted benzaldehyde semicarbazones at pH 5.0 and their results are presented in Table-2.

RESULTS AND DISCUSSION

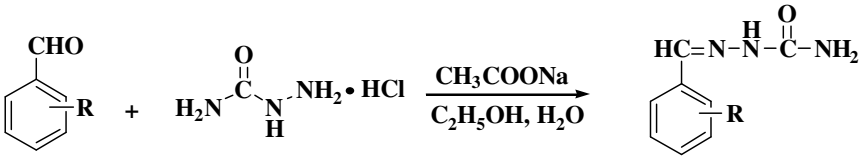
The semicarbazones were synthesized by condensing the semicarbazide hydrochloride with substituted benzaldehydes (**Scheme-I**) in presence of ethanol by the method reported earlier[18]. The products synthesized were confirmed by comparing the melting points with literature.

Protease activity profile at various pHs: Enzyme homogenate was mixed with universal buffer at different pHs (2.0, 3.5, 5.0, 6.5, 8.0 and 9.5). This assay mixture was incubated immediately at 37 ºC for 3 h. The reaction was stopped by

TABLE-2
EFFECT OF SEMICARBAZONES ON ENDOGENOUS PROTEIN HYDROLYSIS IN LIVER HOMOGENATE

Semicarbazones	m.p. (ºC) (lit. m.p.)	Effective conc. (mM)	Absorbance at 630 nm pH 5.0
–	–	–	1.260 ± 0.010
o-Chloro benzaldehyde	222-225 (225)	1.0	0.700 ± 0.005
m-Chloro benzaldehyde	230-232 (228)	1.0	0.532 ± 0.004
p-Chloro benzaldehyde	234-236 (230)	1.0	0.000 ± 0.000
		0.1	0.826 ± 0.006
o-Methoxy benzaldehyde	206-208 (215)	1.0	1.274 ± 0.017
m-Methoxy benzaldehyde	224-226 (233)	1.0	0.854 ± 0.013
p-Methoxy benzaldehyde	206-208 (210)	1.0	0.000 ± 0.000
		0.1	1.106 ± 0.016
o-Nitro benzaldehyde	250-252 (256)	1.0	1.134 ± 0.006
m-Nitro benzaldehyde	238-240 (246)	1.0	1.022 ± 0.020
p-Nitro benzaldehyde	210-212 (221)	1.0	0.000 ± 0.000
		0.1	0.000 ± 0.000
Benzaldehyde	222-224 (222)	1.0	0.280 ± 0.007
		0.1	0.434 ± 0.002

Proteolytic activity of the liver homogenate at 5.0; after proteolysis for 3 h at 37 ºC in presence of different conc. of substituted benzaldehyde semicarbazones. The values in paranthesis denote the protease activity (h/mL) in respective controls which contained equal amount of solvent instead of the compound. The TCA soluble peptides were estimated at 630 nm using bradford method and the results are the mean and SD of one of the experiment conducted in triplicate and are calculated as protease activity (h/mL) in 1.0 % liver homogenate.



R= H, *o*-Cl, *m*-Cl, *p*-Cl, *o*-OCH_3 *m*-OCH_3 , *p*-OCH_3, *o*-NO_2, *m*-NO_2, *p*-NO_2

Scheme-I

addition of TCA and the proteolytic activity was measured as described in experimental section. Fig. 1 shows the results of proteolysis in liver homogenate at different pHs ranging from 2.0-9.5. It can be observed that liver homogenate contains protease activity over the entire pH range 2.0-8.0, but at pH 9.5 there was no activity. Although the liver proteases are active over the acidic and slight alkaline pH range, the maximum activity is present arround pH 3.5-5.0. The results correlate well with earlier studies done with brain homogenate[21].

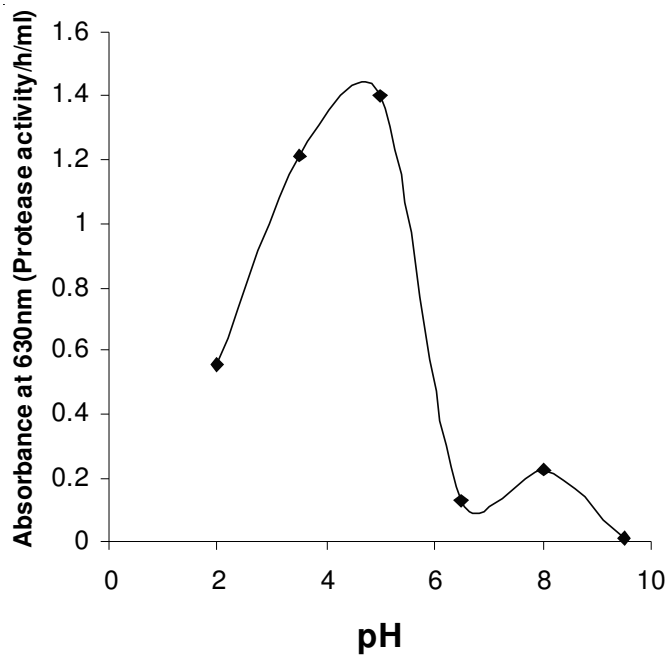


Fig. 1. Proteolytic activity of the liver homogenate at different pHs; after autolysis for 3 h at 37 ºC. The TCA soluble peptides were estimated at 630 nm and the results are the mean of one of the experiment conducted in triplicate and are calculated as protease activity (h/mL) in 1 % liver homogenate

Further studies were carried out at pHs 3.5 and 5.0, because maximum protease activity was found at these pHs, where the effect of semicarbazones was evaluted at 1 mM final concentration on liver endogenous protein substrates[22-24]. It was reported earlier that inhibition of proteolytic activity in liver homogenate at pH 3.5 was less as compared to the effect at pH 5.0. Therefore, the present work was undertaken to evaluate comparative effect of substituted benzaldehyde semicarbazones on endogenous protein hydrolysis at pH 5.0. It can be observed from Table-2 that proteolytic activity was completely inhibited at pH 5.0 in presence of *p*-substituted benzaldehyde semicarbazones at 1 mM final concentration. The effect of *o*- and *m*-substituted benzaldehyde semicarbazones was less significant and could not lead to effective inhibition, suggesting thereby involvement of some steric factors at the interacting site. Further, the effect was evaluated at 0.1 mM final concentration of *p*-substituted semicarbazones and it was found that the order of inhibition was as follows: $-NO_3 > -H > -Cl > -OCH_3$. At 0.1 mM final concentration proteolytic activity was completely inhibited in presence of *p*-nitrobenzaldehyde semicarbazone whereas it was inhibited to *ca.* 65, 34 and 12 % in presence of unsubstituted, *p*-chloro and *p*-methoxy substituted benzaldehyde semicarbazones.

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REFERENCES

1. J.R. Dimmock, R.N. Puthueode, J. Tuchek, G.B. Baker, C.N. Hinko, C.L. Steinmiller and J.P. Stables, *Drug Dev Res.*, **46**, 112 (1999).
2. S.K. Sridhar, S.N. Pandeya, J.P. Stables and A. Ramesh, *Eur. J. Pharm. Sci.*, **16**, 129 (2002).
3. M.H. Yousef, O. Oyebola, R.J. Butcher and J.P. Jasinski, *Acta Cryst.*, **E65**, 01111 (2009).
4. J.D. Warren, D.L. Woodward and R.T. Hargreaves, *J. Med. Chem.*, **20**, 1520 (1977).
5. R.V. Heinzman, *Ann. Rep. Med. Chem.*, **10**, 61 (1975).
6. P.S. Chan, D. Poorvin, M.A. Ronsberg, G. Vice, F. Beyer and P. Scully, *Pharmacologist*, **17**, 253 (1975).
7. S.N. Pandeya, A.S. Raja and G. Nath, *Indian J. Chem.*, **45B**, 494 (2006).
8. S.K. Sengupta, O.P. Pandey, G.P. Rao and P. Singh, *Metal Based Drugs*, **8**, 293 (2002).
9. K.K. Arya and S. Ahmad, *Orient. J. Chem.*, **23**, 239 (2007).
10. B.K. Rai, P. Choudhary, P. Sahi and S. Rana, *Orient. J. Chem.*, **23**, 291 (2007).
11. H. Cerecetto, R. Di Maio, M. Gonzalez, G. Ibarruri, G. Seoane, A. Denicola, G. Paleefo, C. Quijano, M. Paulino, C. Olea-Azar and M.A. Bosombrio, *Farmaco*, **53**, 89 (1998).
12. P. Yogeshwari, R. Thirumurugan, R. Karya, J.S. Samuel, J.P. Stables and D. Sriram, *Farmaco*, **59**, 609 (2004).
13. S.N. Pandeya, P. Yogeshwari and J.P. Sables, *Pharmazie*, **48**, 659 (1993).
14. J.D. Warren, D.L. Woodward and R.T. Hargreaves, *J. Med. Chem.*, **20**, 1520 (1977).
15. M. Benuck and N. Marks, *Brain Res.*, **148**, 181 (1978).
16. A. Suhar and N. Marks, *Eur. J. Biochem.*, **101**, 23 (1979).
17. S. Wilk and M. Orlowski, *Biochem. Biophys. Res. Commun.*, **90**, 1 (1979).
18. A.I. Vogel, Textbook of Practical Org. Chem., ELBS and Longman, London, edn. 4 (1978).
19. M.M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
20. C.M. Stoschek, *Methods Enzymol.*, **182**, 50 (1990).
21. R.C. Kamboj, S. Pal, N. Raghav and H. Singh, *J. Indian Chem. Soc.*, **69**, 270 (1992).
22. N. Raghav, R. Kaur and M. Singh, *Proc. Recent Adv. Chem. Environ. Sci.*, (2010) (accepted).
23. N. Raghav, R. Kaur and M. Singh, *Proc. Recent Adv. Chem. Environ. Sci.*, (2010) (accepted).
24. N. Raghav, R. Kaur and M. Singh, Proc. New Science Horizons, pp. 27-28 (2010).

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