Vol. 82, No. 1, 1978
May 15, 1978

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 342-347

IDENTIFICATION OF TRYPTOPHAN PYRROLASE IN LIVER LYSOSOMES AFTER TREATMENT OF RATS WITH HYDROCORTISONE AND CHLOROQUINE

Donna E. Rudek, Philip Y. Dien and Donald L. Schneider

Department of Biochemistry, Dartmouth Medical School,

Hanover, N.H. 03755, USA

Received April 5,1978

SUMMARY

Liver lysosomes isolated from rats treated with hydrocortisone and chloroquine were found to contain an increased amount of protein including 3% of the tryptophan pyrrolase enzyme activity and 5% of the antigenic activity. Immunoprecipitates were obtained by incubating lysosomes with antibody to tryptophan pyrrolase. Analysis of these immunoprecipitates by sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed tryptophan pyrrolase subunits (MW 45,000) and smaller polypeptides, presumably proteolytic degradation products of intact subunits.

Lysosomes contain an assortment of hydrolytic enzymes and are known to be sites of degradation of internalized plasma proteins (1,2); it has been suggested that intracellular proteins are also degraded in lysosomes (3-6). In this communication we demonstrate the presence of tryptophan pyrrolase, an inducible and rapidly degraded enzyme of the cytosol (7), in liver lysosomes of rats which have been treated with hydrocortisone and chloroquine.

METHODS

Albino male rats from Charles River, Wilmington, Mass., were kept on a daily cycle of 12 h each, light and dark, and were fed food and water *ad libitum*.

Lysosomes were prepared after treatment of rats with Triton WR-1339 by differential flotation gradient centrifugation (8).

Glucose 6-phosphatase and 5'-nucleotidase were measured by release of inorganic phosphate from glucose 6-phosphate (9) and adenosine 5'-monophosphate (10), respectively; the liberated phosphate was determined colorimetrically (11). N-Acetylglucosaminidase activity and protein were determined as described previously (12).

Tryptophan pyrrolase was measured after activation in the presence of hematin and excess tryptophan (13). The assay included formylase which

0006-291X/78/0821-0342$01.00/0

TABLE I

Effect of Hydrocortisone and Chloroquine

on the Protein Content of Liver Lysosomes

Rats were treated with Triton WR-1339 4 d prior to isolation of lysosomes (8). Hydrocortisone 21-phosphate (5 mg per 200 g body weight) and chloroquine (10 mg per 200 g body weight) were injected intraperitoneally 7 h prior to killing. Lysosomes were isolated (8), and purity was calculated from the specific activities of N-acetylglucosaminidase of the lysosomes relative to homogenate (E+N) (9). The amount of lysosomal protein was determined by the Lowry assay (17) of trichloroacetic acid precipitable material and was corrected for yield.

Treatment of Rats	Properties of Isolated Liver Lysosomes		
	Purity	Protein	
	(fold)	(mg per g liver)	(% of total)
None	85	1.6	1.2
Hydrocortisone	89	1.5	1.2
Chloroquine	83	1.5	1.2
Hydrocortisone + Chloroquine	35	4.1	3.1

converted the intermediate N-formylkynurenine to kynurenine. The latter was measured colorimetrically after diazotization (14).

Cytochrome oxidase was measured spectrophotometrically with reduced cytochrome c as substrate (10).

RESULTS AND DISCUSSION

Effect of Chloroquine on the Protein Content of Liver Lysosomes. Seven h after treatment of rats with chloroquine and hydrocortisone, an increase in the amount of lysosomal protein and a corresponding decrease in the apparent purity of the lysosomes were observed (Table I). This could be due to the mode of action of chloroquine, a lysosomotropic agent (15) and inhibitor of protein degradation (16); chloroquine presumably inhibited proteolysis, thereby promoting the protein accumulation in the lysosomes. However, the increase in protein in the lysosomal fraction could have resulted from an increase in contamination. Therefore, the extent of microsomal, mitochondrial and plasma membrane contamination was assessed by measuring glucose 6-phosphatase, cytochrome oxidase and 5'-nucleotidase, respectively. As

TABLE II

Enzymatic Identification of Proteins which Accumulate in Liver Lysosomes of Hydrocortisone-Chloroquine Treated Rats

Treatment of Rat	Enzyme Activity										
	Tryptophan pyrrolase		Glucose-6-phosphatase			5'-Nucleotidase			Cytochrome Oxidase		
	Total (mU/g liver)	Lysosomal (%)	Total (U/g liver)	Lysosomal (RSA)	(%)	Total (U/g liver)	Lysosomal (RSA)	(%)	Total (U/g liver)	Lysosomal (RSA)	(%)
Hydrocortisone	41	0.6	8.5	6.5	9.1	1.1	2.0	4.2	770	0.2	0.2
Hydrocortisone + Chloroquine	51	3.1	9.5	6.8	7.4	1.3	3.2	8.2	940	0.1	0.3

Rats were treated with hydrocortisone and chloroquine and lysosomes were isolated as in Table I. Enzyme activities were measured as described in Methods. Total enzyme activity is that of E+N; lysosomal values have been corrected for yield of N-acetylglucosaminidase. Relative specific activity (RSA) is a measure of enrichment (9).

TABLE III

Tryptophan Pyrrolase Content of Rat Liver Lysosomes as Determined by Double Antibody Immunoprecipitation

Tryptophan pyrrolase was isolated from rat liver as described (18) and injected into rabbits to obtain antisera (19). Rats were treated as described in Table I except that 2 h prior to sacrifice each received 0.5 mCi of ^{3}H-leucine intraperitoneally. Lysosomes were prepared as in Table I. Aliquots of the post-mitochondrial supernates and of the lysosomes were incubated with either antisera or control sera in phosphate-buffered saline, 0.2% Triton X-100. Goat anti-rabbit gamma-globin was added and immunoprecipitation was allowed to proceed overnight in the cold. Precipitates were collected by centrifugation at 2000 x g for 15 min and were washed three times with phosphate-buffered saline, Triton X-100. Washed precipitates were dissolved in 0.2N NaOH, 2% sodium deoxycholate and counted in Triton-toluene scintillant (20). The radioactivity precipitated with control sera was about 20% of experimental and was subtracted to give net radioactivity.

Treatment of Rat	Tryptophan Pyrrolase Antigen		
	Post-mitochondrial Supernate (net cpm/g liver)	Lysosomes (net cpm/g liver)	(%)
Control	4,600	130	3
Hydrocortisone + Chloroquine	8,600	430	5

Table II indicates, microsomal and mitochondrial contamination was slight and was unaffected by chloroquine. Plasma membrane (5'-nucleotidase) contamination increased; however this increase is insufficient to account for the increase in lysosomal protein. The increase in 5'-nucleotidase may very well reflect increased autophagy because invagination of plasma membrane, which may be the mechanism for the formation of autophagic vacuoles, would direct more plasma membrane to the lysosomes.

Identification of Tryptophan Pyrrolase in Lysosomes. While membrane contamination was not responsible for the increase in lysosomal protein, the activity of tryptophan pyrrolase, a soluble protein, was found to increase 5-fold in lysosomes after chloroquine treatment (Table II). In order to verify the results obtained by the assay of tryptophan pyrrolase activity, rats were injected with hydrocortisone and chloroquine and then pulse-labeled

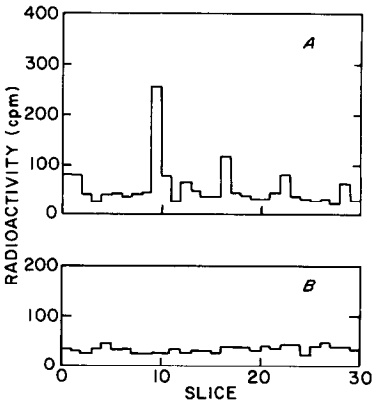

Figure 1. Analysis by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of Tryptophan Pyrrolase Immunoprecipitated from Rat Liver Lysosomes.

Immunoprecipitates were prepared from lysosomes isolated from hydrocortisone-chloroquine treated rats as in Table III, with the exception that goat antibody was omitted in order to avoid overloading the gels. Immunoprecipitates were dissolved in sodium dodecyl sulfate, heat denatured and electrophoresed as described (21). Gels were cut into 2 mm slices which were dissolved in H_2O_2 and counted (22). With bovine serum albumin, ovalbumin and hemoglobin as standards, the major peak was found to have an apparent molecular weight of 45,000. The bands of smaller molecular weight are presumably tryptophan pyrrolase fragments. Panel A antiserum; panel B control serum.

with ^{3}H-leucine. Lysosomes were prepared and analyzed by immunoprecipitation with antibody to tryptophan pyrrolase. Table III shows that tryptophan pyrrolase antigen was found in lysosomes whether or not chloroquine was administered. The amount of tryptophan pyrrolase antigen in lysosomes was always greater than the amount of enzyme activity. This suggests that the antibody may precipitate inactive enzyme, or even fragments of the partially degraded enzyme. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the immunoprecipitate showed that the antibody precipitated radiolabeled tryptophan pyrrolase subunits (MW 45,000) (Figure 1), as well as smaller polypeptides. The presence of these polypeptides is very suggestive evidence that lysosomes are the site of proteolysis of tryptophan pyrrolase. Examination of the peptides by tryptic peptide mapping may lend further support to the hypothesis that these are proteolytic digestion products of an intracellular enzyme.

ACKNOWLEDGEMENTS

The technical assistance of Bruce MacDonald is appreciated. The authors are grateful to Joan Eaton for preparation of the manuscript, to Joan Burnside and Kenneth Gerard whose criticisms were helpful, to C. de Duve and B. Poole who introduced us to tryptophan pyrrolase, and to NIH (Grant GM 24459) and NSF (Grant PCM 77-02635) for support.

REFERENCES

1. Mego, J. L., Farb, R. M. and Barnes, J. (1972) Biochem. J. 128, 763-769.
2. Gregoriadis, G. (1975) in Lysosomes in Biology and Pathology (Dingle, J. T. and Dean, R. T., eds.) Vol. 4, pp. 265-294, North-Holland, Oxford.
3. Neely, A. N. and Mortimore, G. E. (1974) Biochem. Biophys. Res. Commun. 59,680-687.
4. Segal, H. L. (1975) in Lysosomes in Biology and Pathology (Dingle, J. T. and Dean, R. T., eds.) Vol. 4, pp. 295-302, North-Holland, Oxford.
5. Dean, R. T. (1975) Nature 257, 414-416.
6. Ray, D., Cornell, E. and Schneider, D. (1976) Biochem. Biophys. Res. Commun. 71, 1246-1250.
7. Schimke, R. T., Sweeney, E. W. and Berlin, C. M. (1965) J. Biol. Chem. 240, 322-331.
8. Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J. W., Fowler, S. and de Duve, C. (1968) J. Cell Biol. 37, 482-513.
9. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604-617.
10. Schneider, D. L. (1977) J. Membrane Biol. 34, 247-261.
11. Chen, P. S., Toribara, T. Y. and Warner, H. (1956) Anal. Chem. 28, 1756-1758.
12. Findlay, J., Levvy, G. A. and Marsch, C. A. (1958) Biochem. J. 69, 467-476.
13. Feigelson, P. and Greengard, O. (1961) J. Biol. Chem. 236, 153-157.
14. Knox, W. E. and Mehler, A. H. (1950) J. Biol. Chem. 187, 419-430.
15. de Duve, C., de Barsy, T., Poole, B., Trouet, A., Tulkens, P. and Van Hoof, F. (1974) Biochem. Pharmacol. 23, 2495-2531.
16. Wibo, M. and Poole, B. (1974) J. Cell Biol. 63, 430-440.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randell, R. J. (1951) J. Biol. Chem. 193, 263-275.
18. Schutz, G. and Feigelson, P. (1972) J. Biol. Chem. 247, 5327-5332.
19. Leskowitz, S. and Waksman, B. (1960) J. Immunol. 84, 58-72.
20. Lazarow, P. B. and de Duve, C. (1973) J. Cell Biol. 59, 491-506.
21. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
22. Gerard, K. W., Jacobs, J. W. and Schneider, D. L. (1977) Arch. Biochem. Biophys. 181, 94-102.