Vol. 99, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1981 Pages 830-836

LYSOSOMAL POOL OF FREE-AMINO ACIDS

Erik Harms[1,3], Nathan Gochman[2], and Jerry A. Schneider[1]

Departments of [1]Pediatrics and [2]Pathology, University of California, San Diego, La Jolla, CA 92093, and [2]Laboratory Services, Veterans Administration Hospital, San Diego, CA 92161.

Received February 24,1981

SUMMARY: Free (non-protein) amino acids were measured in whole rat liver and in unmodified lysosomes which were prepared from rat liver by the technique of free-flow electrophoresis. Significant intralysosomal pools of threonine, serine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine were found. No efflux occurred from rat liver lysosomes in isotonic buffered sucrose at 0^{0}C, but all amino acids showed various degrees of efflux at 20^{0} and 37^{0}.

Lysosomes are cell organelles which degrade macromolecules. The processes by which macromolecules enter the lysosomal apparatus have been intensely studied. However, relatively little is known about the fate of digestion products. Liver perfusion experiments have indicated that one class of these digestion products, amino acids, is present in a distinct lysosomal pool (1). A more recent study, however, has contradicted this by finding that ^{14}C-valine was uniformly distributed in rat hepatocytes following the hydrolysis of previously labeled intracellular protein (2). Direct measurement of the free-amino acid content of isolated lysosomes has not been reported previously and should provide a more direct answer to the question of whether or not a pool of free-amino acids exists in lysosomes. Verification of such a pool would substantiate a number of studies of amino acid incorporation into protein which suggested that amino acids are not uniformly distributed within the cell (3-14). Such measurements might also provide additional information about lysosomal function in general, and may help answer recent divergent

[3]Present address: Universitäts-Kinderklinik, 6900 Heidelberg, West Germany.

0006-291X/81/070830-07$01.00/0

findings concerning the permeability of the lysosomal membrane for free-amino acids and small peptides (15).

MATERIALS AND METHODS: Unmodified rat liver lysosomes were prepared from male Sprague-Dawley rats (about 200 g body weight) by the method of free-flow electrophoresis according to Stahn et.al. (16) as modified by Henning and Heidrich (17). A VaP 5 model (Bender & Hobein, Munich, West Germany) was used for the final electrophoresis step. Details of this procedure in cultured fibroblasts and lymphoblasts, and references to enzyme assays for indentification of intracellular organelles have been recently published (18,19). The fractions of highest lysosomal enzyme activity were pooled and collected by 20 min centrifugation at 20,000 x g. The relative specific activity (activity/mg protein in lysosomes to activity/mg protein in crude homogenate) in the final lysosomal fraction was 79 ± 22 (mean ± S.D.). Electron micrographs of this fraction showed almost exclusively lysosomes.

Lysosomal efflux of free-amino acids was studied by incubating a granular fraction from rat liver (preparation without final separation by free-flow electrophoresis) resuspended to a protein concentration of about 2 mg/ml in isolation buffer (0.3 M sucrose, 10 mM triethanolamine-acetic acid, 1 mM EDTA pH 7.40). At different time intervals the organelles were collected by 5 min centrifugation at 20,000 x g and the pellet prepared for amino acid analysis (see below). The integrity of the lysosomal membrane was determined at all time points by measurement of the structure-linked latency of β-N-acetylglucosaminidase according to Sellinger et.al. (20) as modified by Baccino et.al. (21). Depending on the incubation time and temperature the structure-linked latency varied from 95% to 70%. All amino acid values of the efflux experiments were corrected accordingly.

Amino acid analysis was performed in both whole liver and isolated lysosomes using a Durrum D-500 analyzer (22,23). Rats were fasted overnight, killed by decapitation, and liver slices were briefly blotted on filter paper before preparation for amino acid analysis. Acid soluble supernatants of tissue and organelles were prepared by sonication in 10 mM potassium phosphate buffer pH 7.3, 5 mM N-ethylmaleimide with subsequent acidification to 3% sulfosalicylic acid. After centrifugation, protein was dissolved in base and then determined colorimetrically (24).

RESULTS AND DISCUSSION: The results of determinations of free-amino acids in rat liver are given in Table I. As a rule, all essential amino acids were found to be concentrated to various degrees within lysosomes as compared to whole cells. Of the non-essential amino acids only serine, cystine (disulfide) and arginine had higher concentrations within lysosomes. The preference for the lysosomal localization of each amino acid can be estimated from the ratio of lysosomal concentration to overall concentration. No correlation was found between lysosomal concentration and properties of individual amino acids such as polarity or isoelectric point.

TABLE I

FREE AMINO ACIDS IN RAT LIVER LYSOSOMES[a]

	ASP	THR	SER	GLU GLN	PRO	GLY	ALA	VAL	CYS[b]
LYSOSOMES (N = 4)	5.13 ±0.91	7.11 ±0.47	7.91 ±0.59	14.99 ±3.14	0.37[c]	2.39 ±0.38	2.92 ±0.78	6.92 ±0.73	0.91 ±0.27
UNFRACTIONATED LIVER (N = 4)	26.85 ±7.40	1.66 ±0.20	2.88 ±0.22	43.28 ±11.09	0.86 ±0.27	10.28 ±3.18	9.11 ±2.24	1.47 ±0.53	0.077 ±0.045
RATIO $\frac{\text{LYSOSOMES}}{\text{UNFRACTIONATED LIVER}}$	0.2	4.3	2.7	0.3	0.4	0.2	0.3	4.7	11.8

	MET	ILE	LEU	TYR	PHE	ORN	LYS	HIS	ARG
LYSOSOMES (N = 4)	2.32 ±0.32	4.35 ±0.83	8.39 ±0.98	4.93 ±0.26	3.01 ±0.40	0.80 ±0.30	10.98 ±2.00	3.37 ±0.21	2.24 ±0.54
UNFRACTIONATED LIVER (N = 4)	0.39 ±0.25	0.91 ±0.31	1.86 ±0.80	0.97 ±0.52	0.52 ±0.18	1.40 ±0.32	2.64 ±0.92	2.99 ±0.86	0.066[c]
RATIO $\frac{\text{LYSOSOMES}}{\text{UNFRACTIONATED LIVER}}$	5.9	4.8	4.5	5.1	5.8	0.6	4.2	1.1	33.9

[a] nmol/mg protein (mean ± S.D.)

[b] disulfide

[c] mean of two determinations; two additional determinations were below the level of detection

The pattern of the lysosomal amino acid pool is in good agreement with the recent study of Ward and Mortimore (1). Two major differences are the large pool contents found here for lysine and arginine. A high lysosomal concentration was expected by these authors because of the net positive charges of these amino acids at neutrality. However, they failed to observe any accumulation. The marked intralysosomal distribution of arginine and cystine may result from the rapid metabolism of these amino acids outside of the lysosome (25,26).

When studying incorporation of extracellular amino acids into cell proteins, many observations have suggested that amino acids can bypass a large intracellular pool of free amino acids. It is interesting that most of these observations were made using valine (9,12-14), leucine (4,6, 8-11) and lysine (7,8,10) as precursors, amino acids shown in this study to have considerable lysosomal pools. Many of the bypass-phenomena

reported could be interpreted as a result of the distinct lysosomal pool which fails to equilibrate rapidly with amino acids taken up the cells.

When granular fractions were separated by free-flow electrophoresis, almost all of the remaining free-amino acids were found within the lysosomal fraction whereas the small amount distributed with other organelles could easily be explained by a minor number of lysosomes not completely separated (data not shown). Therefore, efflux of free-amino acids from lysosomes could be studied using a lysosome-rich granular fraction without complete purification of lysosomes. A possible restriction of this system might be reuptake and metabolism of effluxed amino acids by non-lysosomal organelles present in the granular fraction. Although no evidence was found for this possibility, it could only be ruled out by studying completely isolated lysosomes which, because of the small amount of purified material available, is impractical at present.

The efflux data are summarized on Table II. When suspended in isolation buffer at $0^{o}C$, the lysosomal content of all amino acids was constant during the 100 min incubation period. At 20^{o} and $37^{o}C$, however, all amino acids efflux to various degrees. No clear correlation can be drawn between pool size and efflux rate. The impermeability of the lysosomal membrane at $0^{o}C$ makes it understandable that the lysosomal amino acid pool could be demonstrated in this study even after a time consuming isolation procedure of 8 hours which results in extensive dilution of the organelles. The lysosomal membrane was impermeable at $0^{o}C$ for all amino acids, including those not concentrated within lysosomes. Our findings are in good agreement with those of Ward and Mortimore (1) who have shown free-valine to stay within lysosomes when a granular fraction was incubated at $0^{o}C$ in a sucrose buffer similar to that used in this study.

The permeability properties of the lysosomal membrane have been reviewed by Reijngoud and Tager (15). No firm conclusions could be

TABLE II

TEMPERATURE DEPENDENCE OF FREE-AMINO ACID EFFLUX FROM RAT LIVER LYSOSOMES*

time (min)	0°	20°	37°	time (min)	0°	20°	37°
	Asparagine				Methionine		
0	7.45 ±.36	7.88 ±.26	7.38 ±.36	0	.52 ±.03	.94 ±.06	.64 ±.13
30	7.03 ±.35	6.36 ±.47	3.30 ±.30	30	.52 ±.08	.83 ±.18	.34 ±.09
60	6.96 ±.17	5.88 ±.29	.57 ±.00	60	.48 ±.03	.73 ±.03	.30 ±.01
100	6.97 ±.20	4.97 ±1.04	.57 ±.07	100	.47 ±.10	.53 ±.06	.25 ±.01
	Threonine				Isoleucine		
0	1.38 ±.07	1.83 ±.11	1.60 ±.11	0	1.04 ±.06	1.37 ±.07	1.14 ±.11
30	1.38 ±.10	1.83 ±.17	1.47 ±.17	30	1.04 ±.07	1.08 ±.15	.34 ±.27
60	1.33 ±.10	1.91 ±.06	1.22 ±.05	60	1.02 ±.04	.93 ±.06	.29 ±.15
100	1.41 ±.15	1.71 ±.05	.88 ±.95	100	1.04 ±.05	.65 ±.10	.20 ±.02
	Serine				Leucine		
0	1.53 ±.08	2.23 ±.11	1.83 ±.13	0	2.37 ±.16	4.17 ±.21	2.69 ±.25
30	1.52 ±.13	2.12 ±.30	1.23 ±.18	30	2.46 ±.23	3.68 ±.75	1.39 ±.55
60	1.45 ±.04	1.99 ±.03	.95 ±.04	60	2.33 ±.09	3.36 ±.14	1.22 ±.24
100	1.51 ±.17	1.66 ±.04	.72 ±.09	100	2.36 ±.16	2.41 ±.30	.82 ±.08
	Glutamic acid + Glutamine				Tyrosine		
0	6.00 ±.29	5.34 ±.93	6.36 ±.67	0	1.03 ±.06	1.60 ±.06	1.15 ±.07
30	6.43 ±.79	3.97 ±.83	3.66 ±.23	30	1.00 ±.06	1.50 ±.28	1.11 ±.16
60	6.62 ±.37	3.52 ±.86	2.84 ±.23	60	1.00 ±.03	1.45 ±.07	1.02 ±.05
100	6.73 ±.59	2.60 ±.45	1.64 ±.21	100	1.00 ±.06	1.17 ±.09	.77 ±.05
	Glycine				Phenylalanine		
0	1.12 ±.06	1.36 ±.08	1.24 ±.07	0	.81 ±.05	1.34 ±.10	.83 ±.08
30	1.07 ±.10	1.05 ±.14	.87 ±.12	30	.93 ±.10	.98 ±.30	.41 ±.12
60	1.01 ±.05	.99 ±.04	.85 ±.04	60	.78 ±.04	.86 ±.09	.37 ±.01
100	1.06 ±.19	.85 ±.01	.93 ±.05	100	.76 ±.07	.54 ±.10	.31 ±.01
	Alanine				Lysine		
0	.62 ±.06	1.56 ±.06	.61 ±.11	0	2.40 ±.11	3.27 ±.12	2.32 ±.11
30	.66 ±.09	1.42 ±.54	.51 ±.18	30	2.27 ±.14	2.72 ±.61	.96 ±.16
60	.60 ±.05	1.18 ±.15	.43 ±.08	60	2.26 ±.07	2.16 ±.16	1.08 ±.04
100	.57 ±.07	.77 ±.19	.31 ±.08	100	2.33 ±.09	1.46 ±.13	1.03 ±.05
	Valine				Histidine		
0	1.42 ±.16	1.89 ±.09	1.44 ±.16	0	.90 ±.21	1.33 ±.16	.84 ±.05
30	1.32 ±.02	1.74 ±.24	.70 ±.15	30	.81 ±.19	1.24 ±.37	.47 ±.14
60	1.30 ±.04	1.68 ±.06	.60 ±.04	60	.71 ±.25	1.15 ±.17	.57 ±.03
100	1.42 ±.10	1.33 ±.10	.48 ±.05	100	.68 ±.04	.93 ±.33	.44 ±.05
	Cystine (disulfide)				Arginine		
0	.20 ±.02	.29 ±.10	.14 ±.01	0	.29 ±.02	1.09 ±.18	.33 ±.04
30	.19 ±.01	.23 ±.01	.04 ±.01	30	.31 ±.03	1.03 ±.35	.28 ±.09
60	.20 ±.01	.21 ±.01	.04 ±.02	60	.29 ±.02	.77 ±.20	.25 ±.03
100	.20 ±.01	.19 ±.01	.02 ±.01	100	.31 ±.02	.48 ±.17	.26 ±.01

*A separate preparation was used for each temperature, and each time point represents four separate samples. Values are expressed as nmol/mg protein (mean ± S.D.).

drawn with respect to amino acids. The data and methodology presented in this study may aid further investigations of the permeability properties of the lysosomal membrane. The method by which efflux was measured in this study is not equivalent to the efflux of free-amino acids from lysosomes in an intact cell. In the present study, efflux was measured under approximate zero-trans conditions where the amino acid concentration is much greater inside compared to outside the lysosome (27). However, in the living, intact cell the efflux of lysosomal free-amino acids might take place in a more complex way. Detailed studies of the flux of individual amino acids using isolated lysosomes are necessary before a definite answer can be given about the mode of efflux of free-amino acids from the lysosomal pool.

ACKNOWLEDGEMENTS: We are greatful to Miss H. Kern, Mrs. O. Pellett and Mr. S. Smith for their excellent technical assistance, and to Dr. Stanley Mendoza for his help in preparation of the manuscript. This work was supported by grants AM 18434 and GMS 17702 from the NIH, NF1-373 from the March of Dimes Birth Defects Foundation, Ha 756/6 from the Deutsche Forschungsgemeinschaft, and the VA Research Services.

REFERENCES:

1. Ward, W.F., and Mortimore, G.E. (1978) J.Biol.Chem. 253, 3581-3587.
2. Solheim, A.E., and Seglen, P.O. (1980) Eur.J.Biochem. 107, 587-596.
3. Cowie, D.B., and McCluer, F.T. (1959) Biochim.Biophys.Acta 31, 236-245.
4. Kipnis, D.M., Reiss, E., and Helmreich, E. (1961) Biochim.Biophys. Acta 51, 519-524.
5. Garfinkel, D., and Lajtha, A. (1963) J.Biol.Chem. 238, 2429-2434.
6. Hider, R.C., Fern, E.B., and London, D.R. (1969) Biochem.J. 114, 171-178.
7. Portugal, F.H., Elwyn, D.H., and Jeffay, H. (1970) Biochim.Biophys. Acta 215, 339-347.
8. Righetti, P., Little, E.P., and Wolf, G. (1970) J.Biol.Chem. 246, 5724-5732.
9. Mortimore, G.E., Woodside, K.H., and Henry, J.E. (1972) J.Biol.Chem. 247, 2776-2784.
10. Venrooij, W.J., Poort, C., Kramer, M.F., and Jansen, M.T. (1972) Eur.J.Biochem. 30, 427-433.
11. Venrooij, W.J., Kuijper-Lenstra, A.H., and Kramer, M.F. (1973) Biochim.Biophys.Acta 312, 392-398.
12. Airhart, J., Vidrich, A., and Khairallah, E.A. (1974) Biochem.J. 140, 539-548.
13. Hod, Y., and Hershko, A. (1976) J.Biol.Chem. 251, 4458-4467.
14. Khairallah, E.A., and Mortimore, G.E. (1976) J.Biol.Chem. 251, 1375-1384.
15. Reijngoud, D.-J, and Tager, J.M. (1977) Biochim.Biophys.Acta 472, 419-449.
16. Stahn, R., Maier, K.P., and Hannig, K. (1970) J.Cell.Biol. 46,576-591.
17. Henning, R., and Hedrich, H.-G. (1974) Biochim.Biophys.Acta 345,326-335.
18. Harms, E., Kern, H., and Schneider, J.A. (1980) Proc.Nat.Acad.Sci. USA 77, 6139-6143.

19. Harms, E., Kartenbeck, J., Darai, G., and Schneider, J.A. (1981) Exp.Cell.Res. 131, 253-268.
20. Sellinger, O.Z., Beaufay, H., Jacques, P., Doyen, A., and deDuve, C. (1960) Biochem.J. 74, 450-456.
21. Baccino, R.M., and Zuretti, M.F. (1975) Biochem.J. 146, 97-108.
22. Lee, P.L.Y. (1974) Biochem.Med. 10, 107-121.
23. Bowie, L., Crawhall, J.C., Gochman, N., Johnson, K., and Schneider, J.A. (1976) Clin.Chim.Acta 68, 349-353.
24. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.
25. Rosenthal, O., Gottlieb, B., Gorry, J.D., and Vars, H.M. (1956) J.Biol.Chem. 223, 469-478.
26. Tietze, R. (1970) Arch.Biochem.Biophys. 138, 177-188.
27. Eilam, Y., and Stein, W.D. (1974) Methods.Membr.Biol. 2, 283-354.