N-FORMYL-METHIONINE DEFORMYLASE OF ANIMAL TISSUES

S. Grisolia, A. Reglero and J. Rivas

Department of Biochemistry
University of Kansas Medical Center
Kansas City, Kansas 66103

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SUMMARY. N-formyl-L-methionine is rapidly hydrolyzed by homogenates and cytosol fractions of rat tissues and of chicken liver, beef brain and pig kidney. Other N-formyl aminoacids are not, or are hydrolyzed at much slower rates. The cytosol fractions may be dialyzed, and their activity is not affected by Mn^{++}, Mg^{++} and Co^{++}. The optimum pH is about 7.4. The K_M varies from 3 to 12 mM with different rat tissues, i.e. of the same order as that of the deformylase from Euglena gracilis (3.8 mM). The N-formyl-methionine deformylase of animal tissues may represent a hitherto unrecognized activity of the previously described aminoacylases. This and possible physiological role(s) are discussed.

The important role of N-formyl-methionine in protein synthesis has been known for many years. Although several N-formyl-methionine deformylases have been described in many species including Euglena gracilis (1), no N-formyl-methionine deformylase for animal tissues has been reported.

While studying factors and conditions which may affect N-acetyl aminoacid levels, we checked the specificity of enzymes able to deacylate N-acyl aminoacids. We found N-formyl-methionine deformylase activity in rat brain, a tissue which has very high levels of acetyl aspartate and also N-acetyl-aspartate aminoacylase activity (2).* On further examination, we found marked N-formyl-methionine deformylase activity in all animal tissues tested. In fact, the activity in kidney and liver is so high that it approximates that of some of their glycolytic enzymes. This paper presents some properties of the N-formyl-

*It should be noted that the nomenclature is confusing; Greenstein (3) who discovered this enzyme called it acylase II. Other people refer to the enzyme and to the related acylase I of Greenstein as deacylases, others as aminoacylases and still others as N-acyl aminoacid amido hydrolases. To avoid confusion, it should be remembered that acylase I is [E.C. 3.5.1.14], and acylase II is [E.C. 3.5.1.15]; for simplification, we refer to them throughout this paper as aminoacylases.

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methionine deformylase activity in a number of animal tissues. From the data thus far obtained, it appears that the bulk of activity may be due to amino-acylase I of animal tissues (3).

MATERIALS AND METHODS. Male Wistar rats, weighing from 250-300 g, were from BioLab. Chicken livers were obtained from Pel-Freez Biologicals, Inc., Rogers, Arkansas. Beef brain was from a local slaughter house. N-formyl-L-aminoacids, N-acetyl-L-methionine, and pig kidney aminoacylase I (2640 U/mg) were from Sigma Chemical Co., St. Louis, Mo. Ninhydrin and cellosolve were obtained from Pierce, Rockford, Ill. All other chemicals were analytical reagent grade.

Rats were killed by decapitation. All tissues were homogenized 1:5 (1 vol tissue plus 4 vol 0.15 M KCl) with an Ultra-turrax for 15 s in an ice bath. After cooling for 2 min, the homogenization was repeated. Homogenates may be kept frozen for several months.

Mitochondria, microsomes and cytosol were prepared according to the method of Ragab, et al. (4). Mitochondria were washed twice with 0.25 M sucrose and once with 0.15 M KCl. Both mitochondria and microsomes were suspended in water, broken with an Ultra-turrax (20 s), protein adjusted to 100 mg/ml, and frozen and thawed once using a dry ice-acetone bath and water at 20°C, respectively. They were kept frozen until used. Cytosol was dialyzed against running tap water overnight.

Lactate dehydrogenase was assayed according to Kornberg (5) and phosphoglyceromutase according to Grisolia (6).

Incubations were carried out at 37°C in tightly covered tubes (Brinkmann disposable micro test tubes or regular test tubes covered with parafilm). Reactions were stopped with trichloroacetic acid to give a final concentration of 5%. After 10 min on ice, the mixtures were, depending on their volume, centrifuged at 12,000 x g for 4 min in a Brinkmann 3200 or for 10 min in a Sorvall RC-2B. Supernatants were carefully removed with Pasteur pipettes, and ninhydrin-positive material determined therein by the method of Spies (7). Leucine was used as a standard.

RESULTS. When dialyzed brain cytosol preparations were incubated with N-formyl-methionine, there was an increase in ninhydrin-positive material with the time of incubation as illustrated in Fig. 1. In the absence of N-formyl-methionine, there was little increase in ninhydrin-positive material indicating negligible proteolysis. As shown in the figure with the longest incubation, all the N-formyl-methionine was hydrolyzed.

Table I illustrates with brain, kidney and liver homogenates high activity with N-formyl-methionine. With any other N-formyl aminoacid tested there was no activity or the activity was much lower.

With dialyzed preparations, the activity was maximal at pH 7.4, and it decreased 7 and 19% at pH 6.5 and 8.5, respectively. The activity was not

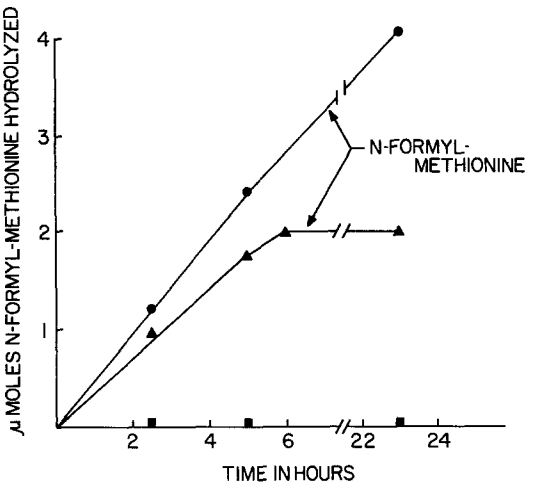

Fig. 1. The effect of time of incubation on hydrolysis of N-formyl-methionine with brain preparations. Incubation mixtures contained in 1.0 ml, 50 μmoles potassium phosphate buffer, pH 7.4, and 1 mg of a dialyzed (18 h) cytosol fraction of brain. When indicated, no ■, 2 mM ▲, or 4 mM ● N-formyl-methionine was added.

affected by 5 mM Mg^{++}, Mn^{++} or Co^{++}. There was 27% more activity with phosphate than with Tris-Cl^{-} buffer.

Table II presents a distribution of activity in rat tissues. As shown, activity is present in all tissues tested, including muscle. The K_M varied as indicated from ∿ 3 to 12 mM with the tissues tested. Interestingly, the K_M of Euglena gracilis N-formyl deformylase is 3.8 mM (1). Also, the activity is present in tissues of other species; for example, under the conditions of the table, we found 33 μmoles and 2.5 μmoles of N-formyl-methionine hydrolyzed per min per g of chicken liver and beef brain, respectively.

In all cases, the bulk of activity of the tissues listed in the table (over 95%) remained in the cytosol after centrifugation, with the exception of brain. With this tissue, there was considerable activity in the microsome and mitochondrial fractions. To clarify whether the activity in the particles was due to contamination with the cytosol proteins, we followed the distribution of phosphoglyceromutase and lactate dehydrogenase, typical cytosol enzymes. As

TABLE I. Specificity for N-formyl-aminoacids in some rat tissues.

SUBSTRATE	TISSUE		
	BRAIN	LIVER	KIDNEY
N-formyl-methionine	2.8	24.0	80.8
" " -alanine	0.2	0.8	2.0
" " -leucine	0.3	2.8	0.7
" " -glycine	0	0	0
" " -aspartate	0	0	0
" " -valine	0	0	0

Incubation mixtures contained in 1.0 ml, 50 μmoles potassium phosphate buffer, pH 7.4, 20 μmoles of the N-formyl aminoacid indicated, and appropriate amounts of tissue homogenates. Activities are given in μmoles/g/min.

TABLE II. Deformylase activity in rat tissues.

TISSUE	ACTIVITY	K_M
	μmols/g/min	(mM)
Kidney	80.8	6.5
Liver	24.0	12.5
Brain	2.8	5.0
Spleen	5.8	3.3
Muscle	2.5	5.0
Heart	3.5	5.0

Incubation mixtures contained in 1.0 ml, 50 μmoles potassium phosphate buffer, pH 7.4, 20 μmoles N-formyl-methionine, and appropriate amounts of tissue homogenates.

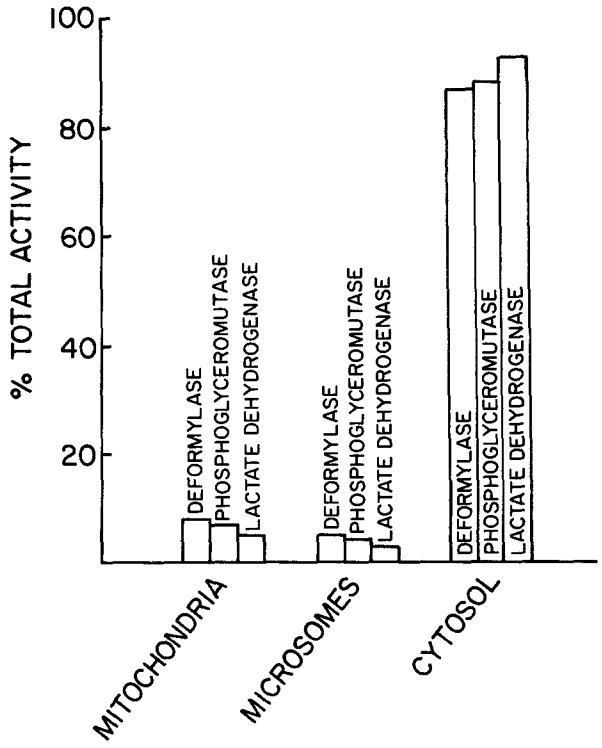

Fig. 2. The distribution of N-formyl deformylase, phosphoglyceromutase and lactate dehydrogenase in the mitochondrial, microsomal and cytosol fractions of brain homogenate. The activity for N-formyl-methionine deformylase was measured at pH 7.4 in 50 mM potassium phosohate buffer and 20 mM N-formyl-methionine The activities of phosphoglyceromutase and lactate dehydrogenase were measured by standard methods (5,6).

illustrated in Fig. 2, the distribution of these activities paralleled the deformylation activity.

Since N-acetyl-methionine is an excellent substrate (3) for aminoacylase I, we compared the ratios of activities with N-formyl-methionine and N-acetyl-methionine with kidney aminoacylase I before and after heating the enzyme at different temperatures. As illustrated in Fig. 3, the ratios of activity with N-formyl-methionine to N-acetyl-methionine remained constant with enzyme inactivation, indicating that the deformylase and deacylase activities are probably catalyzed by aminoacylase I.

The presence of aminoacylase II in rat brain, highly specific for N-acetyl-aspartate, has been described (2). Possibly the proportion and/or activities

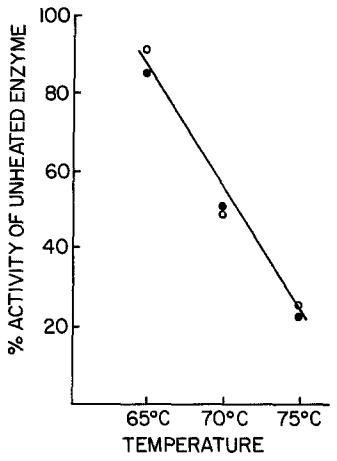

Fig. 3. Effect of heating pig kidney aminoacylase on the ratio of activity of N-formyl-methionine over N-acetyl-methionine. Enzyme solutions (1 mg/ml) were heated for 5 min in a waterbath at 65°, 70° and 75°. After cooling, the enzyme was assayed. ●-●, activity with 20 mM N-formyl-methionine; O-O, activity with 20 mM N-acetyl-methionine.

of aminoacylases and of N-formyl-methionine deformylase activity may vary with the tissue. Therefore, we compared the ratios of activity with N-formyl-methionine and N-acetyl-methionine (at 20 mM of either) with several tissues. The ratios of activity with N-formyl-methionine versus N-acetyl-methionine were as follows: kidney, 5.9; liver, 4.8; brain, 5.1; spleen, 2.0; muscle, 0.7; and heart, 2.4. The ratios of chicken liver and beef brain were 4.8 and 1.6, respectively, and that of pig kidney was 0.9. From these data, it appears that there may be different deformylases and/or aminoacylases (perhaps isoenzymes) which react somewhat differently with N-formyl- and N-acetyl-methionine.

DISCUSSION. N-acetyl-aminoacids have been known to be metabolized by animals for nearly 100 years (8). Also, the key role of N-acetyl-glutamate in citrulline synthesis by animals (9) and for ornithine synthesis by microorganisms has been known for many years (10). Because of the high concentration of N-acetyl-aspartate in brain, the aminoacylase II from brain has been studied, e.g. (2). Otherwise, little work has been carried out on aminoacylases since the pioneer work of Greenstein, perhaps because his interest was mainly to use the aminoacylases for preparative purposes (2).

It seems that the very high activity of the aminoacylases and/or deformylase must have some physiological significance beyond the control of N-acetyl-aspartate and N-acetyl-glutamate levels (11). Since many proteins possess N-acetyl residues, the aminoacylases may control the function of these proteins and/or serve as an agent permitting the further utilization of free aminoacids. Large quantities of fMet-tRNA^{fMet} exist in some animal tissues, e.g. Hela Cell mitochondria (12), which may play a role as protein change initiator and therefore may be controlled by deformylases. As pointed out by Aronson and Lugay (1), Euglena gracilis deformylase may play a role in protein biosynthesis by preventing the accumulation of a cellular pool of N-formyl-methionine formed via aberrant formylation of methionine or by cleavage of N-formyl-methionine from newly synthesized N-formyl-peptides.

Interestingly, it seems that neither Greenstein nor other workers tested for formyl aminoacids as substrates. Whether or not the deformylase activity of animal tissues is totally or partially due to the aminoacylases I and II of Greenstein or different entity(s) remains to be determined. It appears to us that whether or not there are several enzymes, the clarification of the significance of the deformylase activity of animal tissues may have considerable interest.

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REFERENCES.

1. Aronson, J.N. and Lugay, J.C. (1969) Biochem. Biophys. Res. Commun., 34, 311-314.
2. D'Adamo, A.F., Smith, J.C., and Woiler, C. (1973) J. of Neurochem., 20, 1275-1278.
3. Greenstein, J.P. and Winitz, M. (1961) Chemistry of the Amino acids, 2, pp. 1753-1778, John Wiley and Sons, New York.
4. Ragab, H., Beck, C., Dillard, C., and Tappel, A. (1967) Biochim. Biophys. Acta, 148, 501-505.
5. Kornberg, A. (1955) in Methods in Enzymology, I (Colowick, S.P. and Kaplan, N.O., eds) pp. 441-443, Academic Press, New York.
6. Grisolia, S. (1962) in Methods in Enzymology, V (Colowick, S.P. and Kaplan, N.O., eds) pp. 236-242, Academic Press, New York.
7. Spies, J.R. (1957) in Methods in Enzymology, III (Colowick, S.P. and Kaplan, N.O., eds) pp. 468-471, Academic Press, New York.

8. Schmiedeberg, O. (1881) Arch. exotl. Pathol. Pharmakol., 14, 379-392.
9. Grisolia, S. and Marshall, R.O. (1955) in Amino Acid Metabolism, pp. 258-276, Johns Hopkins, Baltimore, Md.
10. Vogel, H.J. (1955) in Amino Acid Metabolism, pp. 335-346, Johns Hopkins, Baltimore, Md.
11. Tatibana, M. and Shigesada, K. (1976) in The Urea Cycle (Grisolia, S., Baguena, R., and Mayor, F., eds) pp. 301-313, John Wiley and Sons, New York.
12. Galper, J.B. and Darnell, J.E. (1969) Biochem. Biophys. Res. Commun., 34, 205-214.