CATHEPSIN C: A CHLORIDE-REQUIRING ENZYME

J. Ken McDonald, Thomas J. Reilly, Benjamin B. Zeitman
and Stanley Ellis
Environmental Biology Division, Ames Research Center, NASA
Moffett Field, California

Received August 8, 1966

As a result of our investigations of the Cl^- -requiring dipeptidyl arylamidases of the pituitary (McDonald et al., 1965, 1966) and liver[1], and because of the similarity of these enzymes to cathepsin C, we were prompted to look for, and consequently to find, a Cl^- requirement for cathepsin C. The Cl^- requirement was exhibited for the hydrolysis of Gly-Phe-NH_2, Gly-Phe-p-nitroanilide, and Gly-Phe-β-naphthylamide at pH 5.3. The Cl^- requirement appeared to be absolute, and to apply also to the transferase activity of cathepsin C at pH 6.8. A thiol activation of cathepsin C could not be demonstrated in the absence of Cl^-.

Since cathepsin C was first identified in swine kidney by Gutmann and Fruton (1948), this enzyme has been extensively purified (Tallan et al., 1952; de la Haba et al., 1955, 1959; Planta and Gruber, 1964; Metrione et al., 1966), its substrate specificities have been determined (Tallan et al., 1952; Wiggans et al., 1954; Izumiya and Fruton, 1956; Planta et al., 1964), its activation and kinetics have been described (Fruton and Mycek, 1956), and its physical properties have been investigated (de la Haba et al., 1959). It now appears as though cathepsin C is extremely limited as a tissue protease or cathepsin, and is actually relatively specific for the hydrolysis (Tallan et al., 1952; Planta et al., 1964) and transfer (Tallan et al., 1952; Planta et al., 1964; Jones et al., 1952; Fruton et al., 1953) of NH_2-terminal dipeptides having a penultimate, aromatic amino acid, and a small, unsubstituted terminal amino acid, typically, Gly-Phe-NH_2 (Tallan et al., 1952).

---

1. Studies conducted in this laboratory have provided evidence for a Cl^- dependent, dipeptide cleavage from His-Ser-β-naphthylamide by the "glucagon-degrading enzyme" of liver.

In view of the dipeptide transfer and polymerization reactions catalyzed by cathepsin C (Fruton et al., 1953; Wurz et al., 1962; Fruton and Knappenberger, 1962; Nilsson and Fruton, 1964), it was recently recommended (Metrione et al., 1966) that this enzyme be renamed "dipeptidyl transferase."

Cathepsin C has, to the best of our knowledge, been fortuitously studied in Cl^--containing reaction mixtures (Wiggans et al., 1954; Izumiya and Fruton, 1956; Jones et al., 1952; Planta and Gruber, 1963; Vanha-Perttula et al., 1965) as a consequence of using Cl^- containing buffers or enzyme solutions, or the hydrochloride salts of various amide substrates and sulfhydryl activators. For this reason, apparently, a Cl^- requirement has so far not been observed, or at least reported, for cathepsin C. The present communication demonstrates that cathepsin C has, in addition to a sulfhydryl requirement (Gutmann and Fruton, 1948; Tallan et al., 1952; Fruton and Mycek, 1956), an absolute requirement for Cl^-.

Methods. The results presented in this report were obtained with an acid extract of rat spleen, prepared according to Metrione et al., (1966), and a purified cathepsin C preparation which was a generous gift from Dr. M. Gruber of the Biochemisch Laboratorium, The University, Groningen. Dr. Gruber's preparation was prepared by the method of de la Haba, et al. (1959). Both cathepsin C preparations were dialyzed overnight against 0.05 M sucrose. The spleen extract was adjusted from pH 3.5 to pH 7.0 prior to dialysis. The concentration of cathepsin C stated for any reaction mixture actually represents the amount of protein contained in the added enzyme sample as determined spectrophotometrically according to the method of Kalckar (1947). The rates of hydrolysis of amide substrates were followed by the ammonia diffusion method of Seligson and Seligson (1951), p-nitroanilide substrates by the colorimetric method of Planta and Gruber (1963), and β-naphthylamide substrates by the fluorescence method of McDonald et al. (1966). Transferase activity was measured at pH 6.8 using the transamidation assay of de la Haba et al. (1959), as modified by Metrione et al. (1966).

Results. As found for the rat-spleen extract, and for Gruber's preparation of cathepsin C (Table I), a requirement for Cl^- was exhibited for the hydrolysis of Gly-Phe-NH_2, Gly-Phe-p-nitroanilide, and Gly-Phe-β-naphthylamide. The possibility of a stepwise (aminopeptidase) hydrolysis of these substrates was discounted since the corresponding phenylalanine derivatives were hydrolyzed at relatively low rates by the spleen extract, and at undetectable rates by Gruber's

cathepsin C. Mercaptoethylamine (hydrochloride), as well as cysteine (hydrochloride), are the recommended sulfhydryl activators (Fruton and Mycek, 1956), however, since the results shown in Table I indicate that the chloride ion is also essential, perhaps it was for this reason that these activators were found to be so effective.

TABLE I. EFFECT OF CL^- ON CATHEPSIN C ACTIVITY

mμmoles substrate hydrolyzed/min/mg cathepsin C

Substrate, Concentration	MEA-HAc	MEA-HCl
Gly-Phe-NH_2, 0.05 M	88	1150
Gly-Phe-p-nitroanilide, 2.0 mM	0	137
Gly-Phe-β-naphthylamide, 0.2 mM	5	488

Reaction mixtures contained 0.1 M sodium citrate, pH 5.3, and 5 mM 2-mercaptoethylamine (MEA) which was prepared from the free base by adjustment to pH 5.3 with either acetic acid (HAc) or hydrochloric acid (HCl). The concentration of (Gruber's) cathepsin C used in the various reaction mixtures was: 0.15 mg/ml in the amide assay, 20 μg/ml for the nitroanilide assay, and 0.5 μg/ml for the naphthylamide assay. These substrate concentrations give zero-order rates in the respective assay systems.

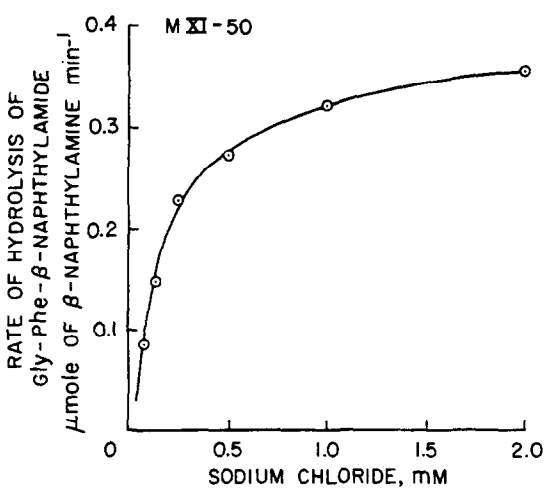

Fig. 1. Effect of Cl^- concentration on the rate of Gly-Phe-β-naphthylamide hydrolysis by (Gruber's) cathepsin C. Reaction mixtures contained 1.5 μg cathepsin C in 0.2 mM Gly-Phe-β-naphthylamide; 0.1 M sodium citrate, pH 5.3; volume, 4 ml. Thiol activation was achieved with 5 mM 2-mercaptoethylamine previously adjusted to pH 5.3 with acetic acid. The rates of hydrolysis are expressed for 1 mg of cathepsin C.

As illustrated in Fig. 1, the rate of hydrolysis of Gly-Phe-β-naphthylamide is proportional to the concentration of Cl^- in the reaction mixture, and the shape of the rate-response curve suggests that the Cl^- requirement is absolute.

Hydroxylamine, made Cl^--free according to the method of Eldredge and Robertson (1965), was found to be a poor "acceptor" for a dipeptide transfer from Gly-Phe-NH_2 by cathepsin C. However, the incorporation of 10 mM NaCl into the reaction mixture produced a transamidation rate, as measured in terms of Gly-Phe-NHOH formation (Metrione et al. 1966), that was equal to the transamidation rate obtained when hydroxylamine hydrochloride was used as the "acceptor."

Table II shows the degree of cathepsin C activation that was obtained with the various anions. Acetate and phosphate failed to substitute for chloride, and the halide requirement was still demonstrable when 3,3-dimethylglutarate buffer was used in place of citrate buffer. The relative halide effect, as well as the NO_3^- effect, were not too different from what has been reported for other halide-requiring peptidases such as yeast polypeptidase (Johnson, 1941), the "converting enzyme" of blood plasma (Skeggs et al. 1954), the "glucagon-degrading enzyme" (Kakiuchi and Tomizawa, 1964), and "dipeptidyl arylamidase I" (McDonald et al. 1966).

TABLE II. EFFICACY OF VARIOUS SALTS AS ACTIVATORS OF GLY-PHE-β-NAPHTHYLAMIDE HYDROLYSIS BY CATHEPSIN C

Salt	Activation	Salt	Activation
	%		%
NaCL	100	$NaNO_3$	33
NaBr	91	$NaNO_2$	12
NaI	30	Sodium acetate	0
NaF	0	NaH_2PO_4	0

Reaction mixtures contained Gly-Phe-β-naphthylamide, 0.2 mM; cathepsin C (Gruber's), 0.5 μg/ml; sodium citrate buffer, 0.1 M, pH 5.3; 2-mercaptoethylamine, 5 mM; and the appropriate anion, 5 mM.

*Cl^- activation taken as 100%.

These observations on cathepsin C warrant its inclusion in the small but growing class of peptidases having an absolute requirement for halide. In view of the Cl^- requirement exhibited by cathepsin C, it may be necessary in some instances to reevaluate negative activity

findings, and to assess past and future specific activity data with regard for the Cl^- as well as the -SH requirement of cathepsin C.

REFERENCES

1. Gutmann, H.R., and Fruton, J.S., J. Biol. Chem., 174, 851 (1948).
2. Tallan, H.H., Jones, M.E., and Fruton, J.S., J. Biol. Chem., 194, 793 (1952).
3. de la Haba, G., Cammarata, P.S., and Fruton, J.S., in S. P. Colowick and N.O. Kaplan (Editors), Methods in Enzymology, Vol. II, Academic Press, Inc., New York, 1955, p. 64.
4. de la Haba, G., Cammarata, P.S., and Timasheff, S.N., J. Biol. Chem., 234, 316 (1959).
5. Planta, R.J., and Gruber, M., Biochim. Biophys. Acta, 89, 503 (1964).
6. Metrione, R.M., Neves, A.G., and Fruton, J.S., Biochemistry 5, 1597 (1966).
7. Wiggans, D.S., Winitz, M., and Fruton, J.S., Yale J. Biol. Med., 27, 11 (1954).
8. Izumiya, N., and Fruton, J.S., J. Biol. Chem., 218, 59 (1956).
9. Planta, R.J., Gorter, J., and Gruber, M., Biochim. Biophys. Acta, 89, 511 (1964).
10. Fruton, J.S., and Mycek, M.J., Arch. Biochem. Biophys., 65, 11 (1956).
11. Jones, M.E., Hearn, W. R., Fried, M., and Fruton, J.S., J. Biol. Chem., 195, 645 (1952).
12. Fruton, J.S., Hearn, W.R., Ingram, V.M., Wiggans, D.S., and Winitz, M., J. Biol. Chem., 204, 891 (1953).
13. Wurz, H., Tanaka, A., and Fruton, J.S., Biochemistry, 1, 19 (1962).
14. Fruton, J.S., and Knappenberger, M.H.,Biochemistry, 1, 674 (1962).
15. Nilsson, K.K., and Fruton, J.S., Biochemistry, 3, 1220 (1964).
16. McDonald, J.K., Ellis, S., and Reilly, T.J., J. Biol. Chem., 241, 1494 (1966).
17. McDonald, J.K., Reilly, T.J., and Ellis, S., Life Sci., 4, 1665 (1965).
18. Planta, R.J., and Gruber, M., Anal. Biochem., 5, 360 (1963).
19. Vanha-Perttula, T., Hopsu, V.K., Sonninen, V., and Glenner, G.G., Histochemie, 5, 170 (1965).
20. Kalckar, H.M., J. Biol. Chem., 167, 461 (1947).
21. Seligson, D., and Seligson, H., J. Lab. Clin. Med., 38, 324 (1951).
22. Eldredge, N.T., and Robertson, W.V.B., Anal. Biochem., 13, 385 (1965).
23. Johnson, M.J., J. Biol. Chem., 137, 575 (1941).
24. Skeggs, L.T., Marsh, W.H., Kahn, J.R., and Shumway, N.P., J. Exptl. Med., 99, 275 (1954).
25. Kakiuchi, S., and Tomizawa, H.H., J. Biol. Chem., 239, 2160 (1964).