THE CARBOXYPEPTIDASE B-CATALYZED HYDROLYSIS OF poly-ε-N-METHYL-L-LYSINE and poly-ε-N,ε-N-DIMETHYL-L-LYSINE*

John H. Seely and N. Leo Benoiton**

Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada.

Received September 26, 1969

Summary

Poly-ε-N-methyl-L-lysine was hydrolyzed by porcine carboxypeptidase B at a rate comparable with that for the hydrolysis of poly-L-lysine. Poly-ε-N,ε-N-dimethyl-L-lysine was hydrolyzed, but at a slower rate. All three curves describing the release of free amino acid are characterized by an initial rate lasting one hour followed by a slower rate which remained constant for up to four hours.

Carboxypeptidase B*** catalyzes the hydrolysis of the peptide bond liberating the C-terminal amino acid of a peptide chain when this amino acid is arginine or lysine (1). Little is known about the effect of substitution on the side-chain amino or guanidino group of the amino acid forming the susceptible bond. In view of the finding of Lys(Me) (2-6), Lys(Me_2) (3-5), and Lys(Me_3) (4) in histones and other sources (7,8), it was of interest to establish whether such modified lysine residues in a protein would still be liberated by CPB. To this end, the action of CPB on poly-L-lysine, poly-Lys(Me) and poly-Lys(Me_2) was investigated by measuring the release of amino acid from the polymers

---

* Supported by a grant from the Medical Research Council of Canada

** Associate of the Medical Research Council of Canada

*** Abbreviations: carboxypeptidase B, CPB: ε-N-methyl-L-lysine, Lys(Me); ε-N,ε-N-dimethyl-L-lysine, Lys(Me_2); tris (hydroxymethyl)aminomethane sulfonic acid, TES.

with an amino acid analyzer. The hydrolysis of poly-L-lysine by CPB has been reported previously (9).

Materials

Poly-L-lysine·HBr (approx. m. wt. 3000; IR max: 1470(s), 1550(s), 1650(s)) was obtained from Sigma Chemical Co., St. Louis, Missouri. Poly-Lys(Me)·HBr was prepared as described by Katchalski for the synthesis of poly-L-lysine (10) except that the required α-N-carboxy anhydride was prepared by the method of Fasman et al. (11). Upon treatment with phosgene, ε-N-carbobenzoxy, ε-N-methyl-L-lysine (12) gave the ε-N-carbobenzoxy-ε-N-methyl-α-N-carboxy-L-lysine anhydride (after 5 recrystallizations from ethyl acetate-petroleum ether, yield 85%, m.p. 83°, IR max; 1420(w), 1460(w), 1500(w), 1665(s), 1780(s), 1840(m)). This was polymerized as usual to the poly-ε-N-carbobenzoxy, ε-N-methyl-L-lysine (amorphous powder, yield 69%), which was treated with 30% HBr in acetic acid to give poly-Lys(Me)·HBr as the hydrate from water-acetone (yield 86%; approx. m. wt. 2240; IR max: 1470(s), 1550(s), 1650(s)). Anal. Calcd. for $(C_7H_{15}BrN_2O\cdot H_2O)_n$: C, 34.85; H, 7.1; N, 11.6; Br, 33.2. Found: C, 34.3; H, 7.2; N, 11.6; Br, 32.95.

Poly-Lys(Me_2)·HBr was obtained by the methylation of poly-L-lysine·HBr with formic acid and formaldehyde after an unsuccessful attempt at methylation by hydrogenation in the presence of formaldehyde (Table I) as was done for the preparation of Lys(Me_2) (13). The reaction mixture was repeatedly evaporated to dryness with the addition of ethanol, and finally after the addition of HBr in acetic acid. The product was precipitated with ether, filtered, and washed with ethanol

TABLE I

Chemical methylation of poly-L-lysine

Reaction conditions	Amino acid content (%)*		
HCHO H_2 Pd/C 23°**	Lysine	Lys(Me)	Lys(Me_2)
3 days	81.8	17.5	0.6
7 days	56.1	37.5	6.4
HCHO/HCOOH 100°***	Lysine	Lys(Me)	Lys(Me_2)
6 hours	4.7	13.5	81.6
12 hours	0.5	1.1	98.4

* Determined with an amino acid analyzer, after hydrolysis in a sealed tube, using a 0.9 x 15 cm Aminex A-5 (Bio•Rad Labs) resin column eluted with 0.35 N sodium citrate, pH 6.48 (1 hour) (15).

** An aqueous solution of poly-L-lysine•HBr (0.86 g) brought to pH 7 with NH_4OH was hydrogenated over 10% palladium on charcoal in the presence of 37% formaldehyde (2.4 ml). After 3 days, catalyst and reagents were replaced, and hydrogenation continued.

*** A solution of poly-L-lysine•HBr (0.21 g) and 37% formaldehyde (0.18 ml) in 90% formic acid (1 ml) was refluxed 6 hours. The solution was evaporated to dryness, the reagents replaced, and solution again heated.

---

and ether (hygroscopic).

The poly-L-lysine and poly-Lys(Me) were shown to be free of amino acids or peptides containing less than six residues by thin layer chromatography using a series of lysine oligopeptides obtained commercially as reference compounds. They also gave the calculated amounts of amino acid after acid hydrolysis.

The enzyme was the diisopropyl fluorophosphate-treated porcine CPB (code COBDFP) from Worthington Biochem. Corp., Freehold, New Jersey. The concentration of the Worthington solution (3.11 mg protein/ml; 9.05×10^{-5} M) was determined by measuring its absorbance at 278 $m\mu$ using a molar absorbancy

of 7.4×10^4 (14) and assuming a molecular weight of 34,300 (14).

Results

The results appear in Fig. 1. It is seen that the three polymers tested were all substrates for CPB. Moreover, in all cases the release of amino acid was unusual in that it occurred initially at a fast rate, which lasted for about 1 hour, followed by a slower rate which remained constant for up to 4 hours, at which time about 8% of the poly-L-lysine had been hydrolyzed. When the amount of enzyme was doubled, the amount

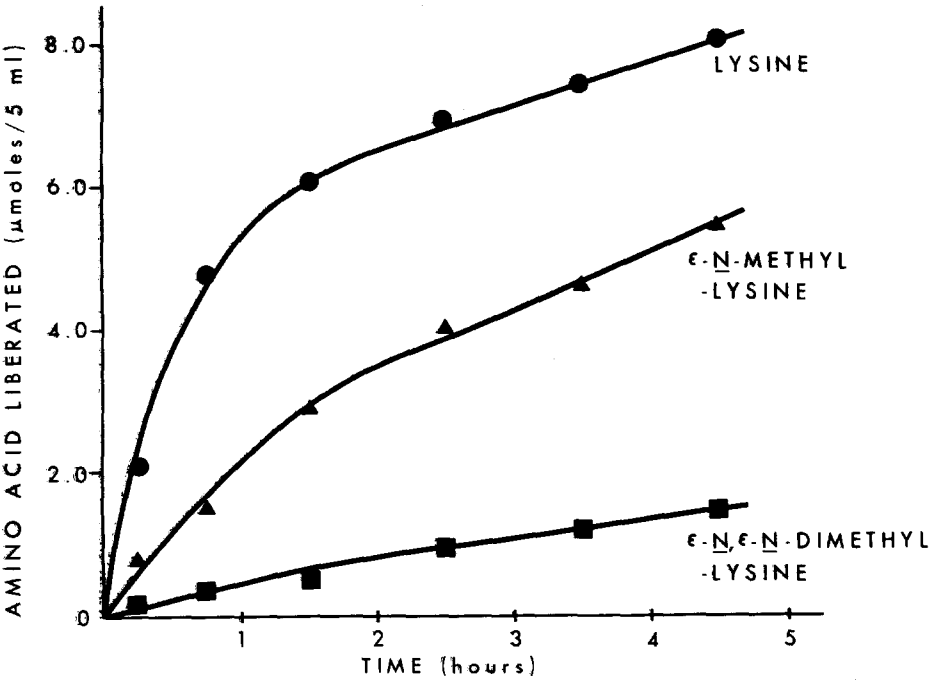

Fig. 1. Liberation of amino acids from poly-Lys(Me_x) by porcine CPB at pH 7.50 and 25°C. Polymer (25 mg) was dissolved in 5.0 ml 0.1 M TES buffer containing 0.2 M NaCl in a small test-tube. Enzyme solution (50 or 165 μl) was added at time 0. Aliquots (0.1 ml) were removed and added to 0.2 ml of 10% sulfosalicylic acid. 0.2 ml of the mixture was analyzed on the short column of a Beckman model 120B amino acid analyzer (see (15) for data). The mixture was stirred magnetically during the digestion. ●--● x = 0, 0.156 mg of protein; ▲--▲ x = 1, 0.156 mg of protein; ■--■ x = 2, 0.518 mg of protein.

of lysine liberated from poly-L-lysine was also doubled. In the presence of 8% (w/w) L-lysine, the hydrolysis of poly-L-lysine was unaffected.

The calculated rates for both sections of each curve in Fig. 1 are recorded in Table II. The initial rate of release of lysine from poly-L-lysine was three times the rate for the release of Lys(Me) from poly-Lys(Me), however, the constant rate eventually attained for the hydrolysis of poly-L-lysine was actually 16% lower than for the hydrolysis of poly-Lys(Me). Poly-Lys(Me_2) was hydrolyzed initially at a rate 2% that of poly-L-lysine but eventually at a rate 11% of that of the unsubstituted polymer.

The reason for the drop in rates after first hour of incubation is not apparent. The hydrolysis of hippuryl-L-lysine by CPB is known to be inhibited by L-lysine (16), however L-lysine was shown to have no effect on the hydrolysis of poly-L-lysine. This would indicate that the latter is bound much more tightly to the enzyme than is the monomer.

The results presented here extend our present knowledge on the susceptibility to hydrolysis of peptide bonds formed

TABLE II

Rates of release of amino acid from corresponding polymer

	μmoles/hr/mg protein*		
	Lysine	Lys(Me)	Lys(Me_2)
Initial rate	55.8	19.0	1.0
Final rate**	4.26	5.06	0.47

* Five-ml reaction mixture
** Best straight line obtained by the least squares method using an Olivetti Programme 101.

from the carboxyl groups of ε-N-methylated lysine residues by the basic amino acid-directed peptidases used in protein sequence studies. Our demonstration that the N,N-dimethyl-lysine polymer was hydrolyzed by CPB constrasts with the demonstrated resistance to hydrolysis of Lys(Me_2) derivatives by trypsin (17,18). However, as has been shown here for CPB, the latter also hydrolyzes Lys(Me) derivatives (12,18).

References

1. R.P. Ambler, in Methods in Enzymology (C.H.W. Hirs, Ed.), Academic Press, New York, 1967. Vol. XI, p. 437
2. K. Murray, Biochemistry, 3, 10 (1964).
3. W.K. Paik and S. Kim, Biochem. Biophys. Res. Commun., 27, 479 (1967).
4. K. Hempel, H.W. Lange and L. Birkofer, Naturwissenschaften, 55, 36 (1968); Z. Physiol. Chem., 349, 603 (1968).
5. W.C. Starbuck, C.M. Mauritzen, C.W. Taylor, I.S. Saroja and H. Busch, J. Biol. Chem., 243, 2038 (1968).
6. R.J. DeLange, D.M. Fambrough, E.L. Smith and J. Bonner, J. Biol. Chem., 244, 319 (1969).
7. T.L. Perry, S. Diamond and S. Hansen, Nature, 222, 668 (1969).
8. R.J. DeLange, A.N. Glazer and E.L. Smith, J. Biol. Chem., 244, 1385 (1969).
9. J.A. Gladner and J.E. Folk, J. Biol. Chem., 231, 393 (1958).
10. E. Katchalski, in Methods in Enzymology (S.P. Colowick and N.O. Kaplan, Eds.), Academic Press, New York, 1957. Vol. III, p. 540.
11. G. Fasman, M. Idelson and E. Blout, J. Am. Chem. Soc., 83, 709 (1961).
12. L. Benoiton and J. Deneault, Biochim. Biophys. Acta, 113, 613 (1966).
13. L. Benoiton, Can. J. Chem., 42, 2043 (1964).
14. J.E. Folk, K.A. Piez, W.R. Carroll and J.A. Gladner, J. Biol. Chem., 235, 2272 (1960).
15. J.H. Seely, R. Edattel and N.L. Benoiton, J. Chromatog., accepted for publication.
16. E.C. Wolff, E.W. Schirmer and J.E. Folk, J. Biol. Chem., 237, 3094 (1962).
17. M. Gorecki and Y. Shalitin, Biochem. Biophys. Res. Commun., 29, 189 (1967).
18. J.H. Seely and N.L. Benoiton, Can. J. Biochem. to be published.