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THE INFLUENCE OF pH ON THE DEGRADATION OF BOVINE MYELIN BASIC PROTEIN BY BOVINE BRAIN CATHEPSIN D *

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The degradation of bovine myelin basic protein by bovine brain cathepsin D (EC 3.4.23.5) was studied over a pH range of 2.75–6.0. Throughout this pH range pepstatin, an inhibitor of cathepsin D, prevented the degradation. The degradation at a pH away from the optimum of pH 3.5 was predictably slower, but also resulted in more restricted cleavage. Above pH 4.5 bovine basic protein peptide 1–42 was not degraded further to peptide 1–36 as occurs at pH 3.5. Additionally, at pH 5.5 another fragment of basic protein, peptide 1–91, persisted indicating that under certain conditions basic protein as well as basic protein peptide 43–169 may be cleaved in the molecular region of basic protein around the phenylalanyl-phenylalanine residues at position 88–89. The small amount of peptides 1–91 and 92–169 detected at pH 5.5 suggests that the bond between residues 91 and 92 in intact basic protein is a minor cleavage site. The options and variation in cleavage around residues 88–92 of basic protein presumably result from pH-dependent changes in conformation in this region but could also be due to changes in conformation of cathepsin D. These results indicate that local tissue changes such as pH may affect not only the velocity of the reaction but also the nature of the product formed by the degradation of basic protein by brain cathepsin D.

Introduction

Cathepsin D (EC 3.4.23.5) is the major acidic endopeptidase of brain [1]. By immunocytochemical methods cathepsin D has been demonstrated in neurons, choroid plexus epithelium, oligodendrocytes and ependymal cells but not astrocytes [2]. The presence of cathepsin D in oligodendrocytes [2] indicates that this enzyme may be involved in the in vivo metabolism of myelin proteins. Bovine brain cathepsin D degrades bovine myelin basic protein in a sequential, limited manner [3]. Bovine basic protein, a molecule of 169 residues, is cleaved in two major steps at pH 3.5. An initial cleavage at residues 42–43 results in the formation of peptides 1–42 and 43–169 which are subsequently degraded to peptides 1–36, 43–88, 89–169 and 92–169 and minor amounts of peptides 43–89 and 43–91. The conditions and factors regulating this degradation in vivo are unknown. Thus, the cellular compartments in which these reactions occur, the operative pH range and the presence of stimulatory or inhibitory influences remain to be defined. During investigation of the range of pH in which brain cathepsin D degrades basic protein, a pH-dependent difference in cleavage of basic protein was demonstrated. Results of the present investigation indicate further restrictions in the molecular site and extent of degradation at different pH values.

Materials and Methods

Materials. Trasylol was purchased from Mobay Chemical Corporation (New York, NY); phenyl-

methanesulphonyl fluoride, *N*-ethylmaleimide, 1,10-phenanthroline, soybean trypsin inhibitor and bacitracin from Sigma Chemical Company (St. Louis, MO); and antipain, chymostatin, leupeptin and pepstatin from Peptide Research Institute (Osaka, Japan).

Digestion of basic protein with brain cathepsin D. Basic protein microheterogeneous components and brain cathepsin D were purified as previously described [3]. For the pH-related studies, the incubation mixture consisted of 400 μg myelin basic protein and 4 μg enzyme in a total volume of 1 ml. The solutions for incubation were 0.005 M citric acid, pH 2.75, or 0.005 M citrate buffer with the pH adjusted with sodium citrate to 3.0, 3.5, 4.0, 4.5, 5.0, 5,5, 6.0, 7.0 or 8.0. The mixture of citric acid and sodium citrate was chosen to control fluctuations in ionic strength which also affect cathepsin D activity [4]. The 0.005 M concentration of citrate was selected because higher concentrations interfered with the clarity of bands on polyacrylamide gels. Inhibitors were added in a 100 μl volume solution containing 10-times the final concentration of inhibitor. Not all of the inhibitors could be completely dissolved under these conditions. The solutions of incompletely solubilized inhibitors were sonicated prior to being mixed with basic protein and enzyme. Other incubations of basic protein or basic protein peptides and cathepsin D contained different amounts of substrate and enzyme as described. The incubations were allowed to proceed at 37°C for varying periods of time after which the solutions were frozen and lyophilized and the residue processed for polyacrylamide disc-gel electrophoresis at pH 8.8 [3,5] or column chromatography [3].

Other methods. Protein concentrations were determined by the Folin-Phenol method [6]. Large scale basic protein digestion by bovine brain cathepsin D, carboxymethyl-cellulose chromatography at pH 7.5, gel filtration on Sephadex G-50 superfine, amino acid analysis and amino acid sequencing were performed as previously described [3].

Results

Bovine brain cathepsin D effectively degraded bovine myelin basic protein over a pH range of 2.75–5.5 and to a limited extent at pH 6. At a protein-enzyme ratio of 100 : 1 with incubation at 37°C for 8 h, there was no degradation of basic protein outside this pH range. Based on the assumption that the migration of basic protein peptides under the conditions of polyacrylamide gel electrophoresis is the same as that previously described [3], the major differences noted were the persistence of peptide 43–91 at pH 2.75, the reduced or lack of conversion of peptide 1–42 to peptide 1–36 at pH 4.5 and above, and the presence or persistence of a new peptide, ultimately identified as basic protein peptide 1–91, at pH 5.5 (Fig. 1). This newly observed peptide, which migrated more cathodally than intact basic protein but less than basic protein peptide 43–169, was distinctly present after digestion at pH 5.5 and in trace amounts at pH 6.0. There was no degradation of basic protein incubated alone under the same conditions outside the range of pH 2.75–6.0. On the polyacrylamide gel electrophoresis performed at pH 8.8, the band migrating just on the cathodal side of basic protein peptide 43–88 and present in preparations of intact basic protein is a basic protein dimer.

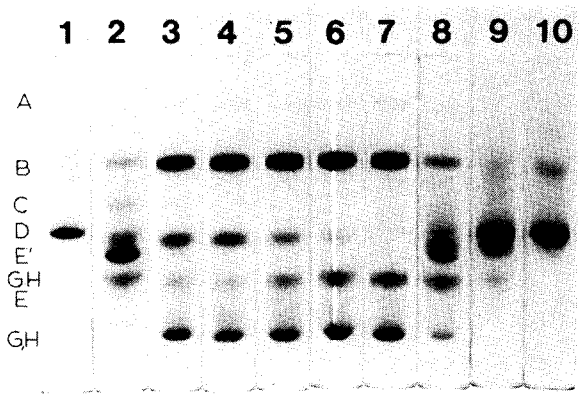


Fig. 1. Polyacrylamide disc-gel electrophoretic study at pH 8.8 of the digestion of basic protein by brain cathepsin D over a pH range of 2.75–7.0. Myelin basic protein microheterogeneous component one was incubated alone [1] or with brain cathepsin D at a protein-enzyme ratio of 100 : 1 at 37°C for 8 h at pH 2.75 (2), 3.0 (3), 3.5 (4), 4.0 (5), 4.5 (6), 5.0 (7), 5.5 (8), 6.0 (9) and 7.0 (10). Designations to the left based on previously identified peptides (Ref. 3): A (peptide 43–89); B (peptide 43–88); C (peptide 43–91); D (peptide 1–36 or intact BP); E' (peptide 1–91); GH (peptide 43–169; E (peptide 1–42); and G, H (peptide 89–169 or peptide 92–169). 40 μg protein applied per gel except gel 1 to which 20 μg were applied. Cathode at bottom.

Effects of proteolytic inhibitors

Pepstatin (1 μg/ml) totally inhibited the degradation of basic protein by cathepsin D throughout its pH range of activity. Although the purity of the brain cathepsin D used has been established [7], the influence of other proteinases, possible contaminating the preparation of cathepsin D on the degradation of basic protein, was examined through the use of proteolytic inhibitors. The inhibitors were included in a 5 h incubation period at 37°C along with basic protein and cathepsin D mixed at an enzyme-substrate ratio of 1 : 100. At both pH 3.5 and 5.0 the degradation of basic protein by the preparation of barin cathepsin D was partially inhibited by bacitracin (1.45 mg/ml), whereas chymostatin (50 μg/ml), leupeptin (50 μg/ml), antipain (50 μg/ml), EDTA (2 mM), 1,10-phenanthroline (1 mM) *N*-ethylmaleimide (125 μg/ml), phenylmethanesulphonyl fluoride (87 μg/ml), soybean trypsin inhibitor (1 μg/ml), ε-aminocaproic acid (650 μg/ml) and trasylol (500 units/ml) were without effect.

Degradation of basic protein by cathepsin D at pH 5

Because of the differences observed in the digestion related to pH, the degradation of basic protein

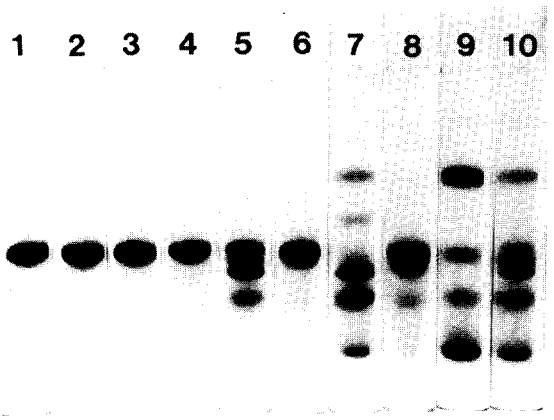


Fig. 2. Polyacrylamide disc-gel electrophoretic study at pH 8.8 of the digestion of basic protein by varying amounts of cathepsin D at pH 3.5 (odd numbered gels) or pH 5.5 (even numbered gels). Basic protein microheterogeneous component one was incubated alone [1,2] or with brain cathepsin D at a protein-enzyme ratio of 1 000 : 1 [3,4], 500 : 1 [5,6], 250 : 1 [7,8], or 100 : 1 [9,10] for 4 h at 37°C. 40 μg protein per gel. Cathode at bottom.

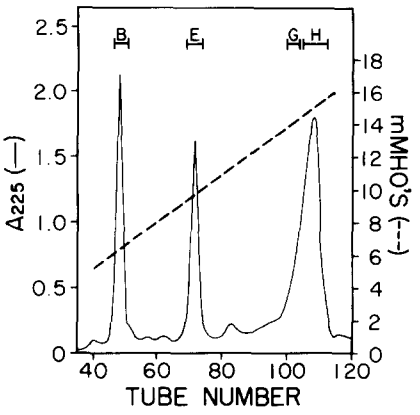


Fig. 3. CM-cellulose (2.6 × 18 cm) chromatography in 0.02 M NH_4HCO_3 of 60 mg bovine basic protein microheterogeneous component one digested with brain cathepsin D at a protein-enzyme ratio of 200 : 1 at 37°C and pH 5 for 120 min. The NaCl gradient was begun at tube 17 and was from 0 to 0.3 M NaCl. Horizontal bars indicate the tube contents (9.2 ml/tube) pooled together. Letter designations are those previously used (Ref. 1) (see Tables I and II).

by cathepsin D above its optimum pH of 3.5 was investigated further. Although the velocity of the reaction could not be quantitated because of the restrictions in separating products from each other and from substrate, the rates of disappearance or formation of bands was slower in the digestion under the higher pH conditions, particularly at pH 5.5 (Fig. 2), than at pH 3.5. In contrast to the incubation at pH 3.5, the digestion at pH 5 did not show the formation of fragments migrating as basic protein peptides 43–91 or 1–36 (Fig. 1) [3]. To identify with certainly the basic protein peptides formed at pH 5.0, basic protein component one was digested with brain cathepsin D at pH 5 and the resultant peptides purified by ion-exchange chromatography on CM-cellulose and gel filtration on Sephadex G-50 superfine [3]. From the pH 5 digest, three distinct peaks eluted from CM-cellulose with an ascending shoulder to the third peak (Fig. 3). Similar to the results demonstrated by electrophoresis (Fig. 1), the elution pattern of the pH 5 digest showed fewer peaks than that of the pH 3.5 digest [3]. The three peaks and the ascending shoulder to the third peak eluted from Sephadex G-50 superfine as single peaks (data not shown). The purity of the isolated basic protein peptides was demonstrated by polyacrylamide gel electrophoresis at pH 8.8 (Fig. 4). According to amino

TABLE I

AMINO ACID ANALYSIS OF PEPTIDES OF BOVINE MYELIN BASIC PROTEIN

Letters in the column headings have the same designation as that used for bands seen on gel electrophoresis and peaks present on column chromatography. Numbers in parentheses following the letters indicate the appropriate basic protein peptide. The first number was derived by amino acid analysis of the peptide. Numbers in parentheses represent the number of residues based on the known sequence of bovine basic protein [11,12]. N.D. not determined.

Amino acid	E (1–42)	B (43–88)	E' (1–91)	H (89–169)	G (92–169)
Lysine	2.1 (2)	3.1 (3)	6.3 (6)	8.1 (8)	8.0 (7)
Histidine	3.0 (3)	5.0 (5)	7.7 (8)	2.0 (2)	2.0 (2)
Arginine	6.1 (6)	4.1 (4)	9.8 (10)	7.0 (7)	6.7 (7)
Aspartic acid	3.0 (3)	4.0 (4)	8.1 (8)	4.0 (4)	3.6 (3)
Threonine	2.0 (2)	2.1 (2)	3.9 (4)	3.0 (3)	2.8 (3)
Serine	5.2 (5)	2.6 (3)	7.6 (8)	10.3 (10)	9.6 (10)
Glutamic acid	2.0 (2)	3.9 (4)	6.3 (6)	3.9 (4)	3.7 (4)
Proline	2.2 (2)	3.9 (4)	6.4 (6)	6.0 (6)	5.9 (6)
Glycine	3.3 (3)	6.8 (7)	10.3 (10)	14.8 (15)	14.7 (15)
Alanine	5.4 (5)	4.0 (4)	9.1 (9)	5.3 (5)	5.2 (5)
Valine	0 (0)	1.3 (2)	1.5 (2)	0.6 (1)	0.9 (1)
Methionine	0.6 (1)	0 (0)	1.0 (1)	0.9 (1)	0.9 (1)
Isoleucine	0.8 (1)	0 (0)	0.8 (1)	1.6 (2)	2.0 (2)
Leucine	4.1 (4)	1.0 (1)	5.2 (5)	5.2 (5)	5.0 (5)
Tyrosine	0.9 (1)	1.0 (1)	1.8 (2)	2.0 (2)	1.8 (2)
Phenylalanine	2.0 (2)	2.0 (2)	4.5 (5)	4.0 (4)	3.0 (3)
Tryptophan				N.D. (1)	N.D. (1)

acid composition (Table I) and partial sequence analysis (Table II), the three major peaks were shown to contain basic protein peptides 1–42, 43–88 and 89–169. The ascending shoulder of peak three, designated previously as G [3], showed only a small amount of peptide which by partial sequence analysis contained an admixture of peptides beginning at residues 89 and 92 (Table II). The amino acid composition for this material was the same as that for basic protein peptide 89–169 except for a reduction in phenylalanine content (Table I). The failure to show a reduction in lysine (residue 90) and aspartic acid (residue 91) in the amino acid content of this peptide is most likely due to the contamination of basic protein peptide 92–169 with basic protein peptide 89–169. Residue 91 is asparagine [11,12] but is deaminated during acid hydrolysis and detected as aspartic acid during amino acid analysis.

TABLE II

NH_2-TERMINAL SEQUENCE OF FRAGMENTS OF BASIC PROTEIN

B	43	44	45	46	47	48	49	50
	Phe	-Gly	-Ser	-Asp	-Arg	-Gly	-Ala	-Pro
E	Blocked NH_2-terminal							
E'	Blocked NH_2-terminal							
G	89	90	91	92	93	94	95	96
	Phe	-Lys	-Asn	-Ile	-Val	-Thr	-Pro	-Arg (1) [a]
	92	93	94	95	96	97	98	99
	Ile	-Val	-Thr	-Pro	-Arg	-Thr	-Pro	-Pro (2) [a]
H	89	90	91	92	93	94	95	
	Phe	-Lys	-Asn	-Ile	-Val	-Thr	-Pro	

[a] Numbers in parentheses indicate ratio, i.e., 1 : 2, of peptides in G.

Since the appearance of basic protein peptide 1–36 at pH 3.5 was the major difference from the outcome at pH 5.0, the susceptibility of basic protein peptide 1–42 to digestion by brain cathepsin D at pH 3.5 and 5.0 was examined. At pH 3.5 basic protein peptide 1–42 was readily degraded to basic protein peptide 1–36, whereas at pH 5.0 no degradation was evident (Fig. 5). It was previously demonstrated that at pH 3.5 bovine brain cathepsin D degrades basic protein microheterogeneous components two–five in a manner similar to that for

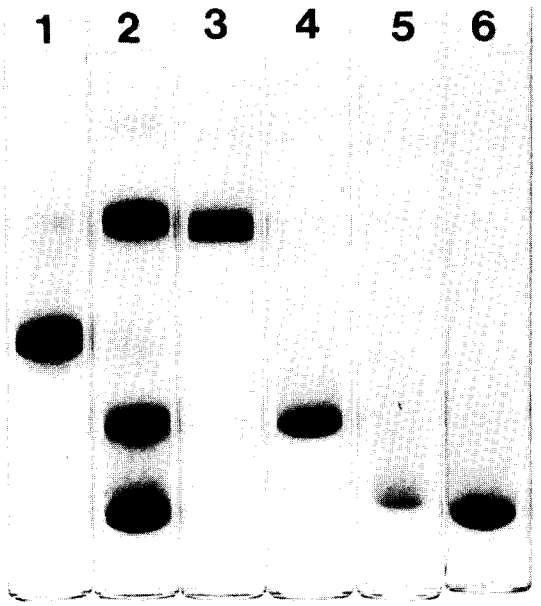


Fig. 4. Polyacrylamide disc-gel electrophoretic study at pH 8.8 of basic protein peptides prepared by the degradation of bovine basic protein microheterogeneous component one by brain cathepsin D at pH 5.0. Gels contain basic protein component one incubated alone (1) or with brain cathepsin D at a protein-enzyme ratio of 200 : 1 (2) at 37°C and pH 5 for 8 h and fragments B (peptide 43–88) (3), E (peptide 1–42) (4), G (mixture of peptides 89–169 and 92–169) (5) and peptide H (peptide 89–169) (6) obtained by CM-cellulose chromatography (Fig. 3) followed by gel filtration on Sephadex G-50 superfine. 40 μg protein applied to gels 1 and 2 and 20 μg to gels 3–6. Cathode at bottom. Tables I and II contain amino acid analysis data on these peptides.

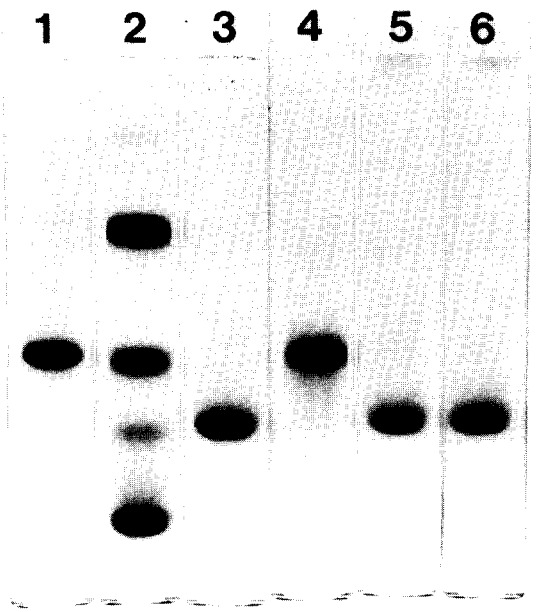


Fig. 5. Polyacrylamide disc-gel electrophoretic study at pH 8.8 of basic protein peptide 1–42 exposed to brain cathepsin D at pH 3.5 and 5.0. Gels contain basic protein component one incubated alone (1) or with brain cathepsin D at a protein-enzyme ratio of 200 : 1 (2) at 37°C and pH 3.5 for 5 h, basic protein peptide 1–42 incubated alone (3) or with brain cathepsin D at a protein-enzyme ratio of 100 : 1 (4) at 37°C and pH 3.5 for 8 h, and basic protein peptide 1–42 incubated alone (5) or with brain cathepsin D at a protein-enzyme ratio 100 : 1 (6) at 37°C and pH 5.0 for 8 h. 20 μg protein applied to gel one, 40 μg to gel two, and 30 μg each to gels 3–6. Cathode at bottom.

component one [3]. This effect was re-examined at pH 5. At pH 5 the patterns for all five components were identical for peptides 43–88 and 1–42 and the lack of formation of peptide 1–36 (data not shown). The differences in the more cathodally migrating peptides 89–169 and 92–169 were explicable on the basis of microheterogeneity [8]. Similar to the results at pH 3.5 [3], the presence of basic protein peptide 43–89 was more distinct in the gels of components two–five than component one.

Degradation of basic protein by cathepsin D at pH 5.5

The digestion of basic protein at pH 5.5 was less complete than at pH 5.0 and, in contrast to digestion at pH 5.0, revealed the appearance or persistence of a new peptide migrating on gels to a position between intact basic protein and basic protein peptide 43–169 (Fig. 1). Even with digestion at pH 5.5 for up to 4 h this band remained (Fig. 2). This new peptide as well as the others formed by digestion of basic protein with cathepsin D at pH 5.5 was isolated by CM-cellulose chromatography and gel filtration over Sephadex G-50 superfine. The profile on chromatography of the pH 5.5 digest was qualitatively similar to those of the early pH 3.5 [3] and 5.0 (Fig. 3) digests with the exception of a more prominent peak, designated E′, eluting after basic protein peptide 1–42. Electrophoresis of the isolated peptides at pH 8.8 showed the separation and inferential identifica-

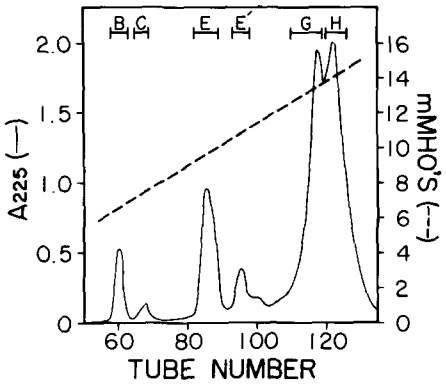
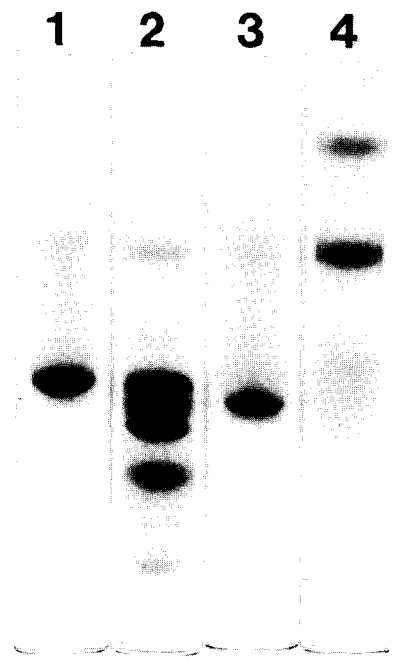


Fig. 6. CM-cellulose chromatography in 0.02 M NH_4HCO_3 of 51 mg bovine basic protein microheterogeneous component one digested with brain cathepsin D at a protein-enzyme ratio of 500 : 1 at 37°C and pH 5.5 for 45 min. The NaCl gradient was begun at tube 25 and was from 0 to 0.3 M NaCl. Horizontal bars indicate tube contents pooled together. Letter designations are those previously used (Ref. 3) (see Fig. 1, Table I, and Table II).

tion of the peptides (Fig. 7). Amino acid analysis (Table I) and partial sequencing (Table II) indicated that basic protein peptide E′ (Fig. 6; Fig. 7, lane 3) present in the pH 5.5 digest was basic protein peptide



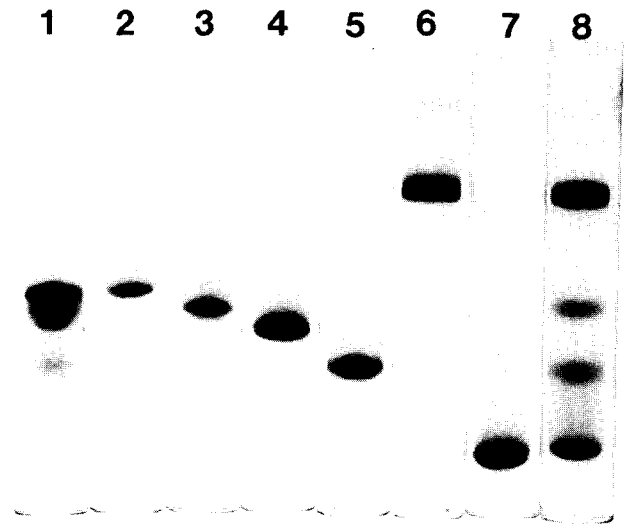


Fig. 7. Polyacrylamide disc-gel electrophoretic study at pH 8.8 of the digestion of bovine basic protein microheterogeneous component one by bovine brain cathepsin D at pH 5.5 (1) or pH 3.5 (8) at a protein-enzyme ratio of 100 : 1 for 4 h at 37°C and of the constituents (2–7) of the pH 5.5 digestion mixture separated by CM-cellulose chromatography (Fig. 6) and gel filtration over Sephadex G-50 superfine. Gels contain intact basic protein (from peak H) (2), peptide 1–91 (peak E′) (3), peptide 43–169 (from peak G) (4), peptide 1–42 (peak E) (5), peptide 43–88 (peak B) (6), and peptide 92–169 (from peak H) (7). (Letter designations same as used in Figs. 1 and 6, Table I, and Table II). 40 μg protein applied to gels 1 and 8, and 20 μg applied to gels 2–7. Cathode at bottom.

1–91. Its amino terminal was blocked, and its amino acid composition agreed well with the expected composition of this peptide. Further studies of peptide 1–91 were conducted by digesting it with brain cathepsin D at pH 3.5. These digests contained products migrating at positions of basic protein peptides 43–88, 43–89 and 1–42 (Fig. 8). The less

Fig. 8. Polyacrylamide disc-gel electrophoresis at pH 8.8 of the digestion of basic protein and basic protein peptide 1–91 by bovine brain cathepsin D. Gels contain basic protein component one before (1) and after (2) digestion by cathepsin D at a protein-enzyme ratio of 100 : 1 for 4 h at 37°C at pH 5.5, peptide 1–91 (Fig. 6, peak E′; Fig. 7, gel 3) purified from the digest (3), and basic protein peptide 1–92 exposed to cathepsin D at a peptide-enzyme ratio of 100 : 1 for 4 h at 37°C at pH 3 5 (4). 20 μg per gel for gels 1 and 3 and 40 μg per gel for gels 2 and 4. Cathode at bottom.

distinct band of peptide 1–42 is related to its lower dye-binding capacity for Coomassie brillant blue which is even lower in basic protein peptide 89–169 on a weight basis (data not shown). Basic protein peptide 43–91 is known to be present only transiently during the sequential degradation of basic protein [3], and it presumably has been further cleaved to form basic protein peptide 43–88 and 43–89.

Discussion

An acid pH optimum, the lack of requirement for cofactors, and inhibiton by pepstatin are well known features of cathepsin D [4], and activation by glycine ethyl ester [9] and ribonucleotides [10] has been reported. Other factors which influence the activities of cathepsin D are incompletely understood [4]. Results of the present study indicate that the pH environment of the degradative activity of cathepsin D on basic protein influences not only the rate but also the extent and sites of cleavage.

Cathepsin D degrades basic protein over a pH range of 2.75–6.0. Based on the inhibition of this degradation by pepstatin and the failure of many other inhibitors to prevent the digestion, cathepsin D is implicated as the active enzyme throughout this range. Conclusions about the previously demonstrated sequential, limited cleavage of basic protein by cathepsin D at its optimal pH of 3.5 must be modified to account for the observations made at higher pH values. First, above pH 4.5 basic protein peptide 1–42 is not cleaved to form basic protein peptide 1–36. The measured intralysosomal pH of 4.7–4.8 [11] indicates that peptide 1–36 would be an unlikely product of bovine basic protein digestion by cathepsin D. This block in the degradation of bovine basic protein resembles the outcome at the optimal pH of 3.5 for human and guinea pig basic protein [12]. The explanation for the difference among species presumably involves structural differences in basic protein. There is an isoleucine substitution for leucine at position 39 and a glycine substitution for serine at position 45 in human BP compared to bovine basic protein [13–15].

At pH 5.5 another intermediate basic protein fragment, peptide 1–91, was identified. This peptide was more clearly seen at this pH although it sometimes appeared transiently in digestions at pH 3.5 (Fig. 2, gel 5). The recognition of peptide 1–91 at pH 5.5 may be due to the reduced velocity of the reaction at the higher pH, but it helps in delineating the sequence of events occurring around the scission point of the phenylalanyl-phenylalanine bound at residues 88–89. The present results suggest that the asparagine-isoleucine bond at residues 91–92 can be cleaved in intact myelin basic protein with subsequent removal by cathepsin D of asparagine and lysine or these two residues plus phenylalanine from the carboxyl terminal to form basic protein peptide 43–89 and 43–88, respectively. The environmental influence, such as by pH, on the conformation in this part of the basic protein molecule around residues 88–92 [16,17] is likely to be a major factor in controlling the sites and extent of cleavage and determining the types and amounts of basic protein peptides formed. Alterations in the conformation and activity of cathepsin D by the same environmental influences should also be considered; however, the pattern of digestion of basic protein by cathepsin D appears to be determined more by the features of basic protein than the source of cathepsin D [12]. A more quantitative analysis of various environmental changes on the degradation of basic protein by cathepsin D awaits an assay that can readily separate product from substrate.

On the basis of the present and previous investigation [3] of bovine basic protein degradation by cathepsin D, the sequence of cleavage may be summarized as follows: the initial cleavage site is at the phenylalanyl-phenylalanine bond of residues 42–43 to form peptides 1–42 and 43–169. A minor cleavage site is at residues 91–92 to form peptides 1–91 and 92–169. As digestion proceeds, peptides 43–88 and 43–89 are formed from peptides 1–91 and 43–169, and peptide 43–169 gives rise to additional amounts of peptide 92–169 and 89–169. At lower pH values only peptide 1–42 is degraded to 1–36. This interpretation is made with the awareness of the difference in dye-binding capacity (peptide 43–88 > peptide 1–42 > peptide 89–169) of basic protein peptides for Coomassie brilliant blue. The only differences among the five microheterogeneous components of basic protein noted so far is that more peptide 43–89 is formed from components two–five than component one [3]. Because of the poor resolu-

tion of the more cathodal peptides of components two–five in the pH 8.8-gel electrophoresis system, other differences among components could have ascaped detection.

The shift in enzyme activities by pH, ionic strength, or other factors is not restricted to cathepsin D as indicated by the findings with murine macrophage lysozyme [18] and chick liver B-glucuronidase [19]. Cathepsin D activity is increased in the tissue lesions of multiple sclerosis [20] and may be involved in the formation of basic protein peptides appearing in cerebrospinal fluid of patients with acute damage to central nervous system myelin [21]. The present observations indicate that the size and amounts of fragments of basic protein formed or released may be altered by a number of conditions of the reaction in addition to the major cleavage sites recognized by the enzyme. The basic protein peptides formed by selective endopeptidases during normal catabolism or as a result of myelin damage may influence the extent of further digestion by exopeptidases. The pathological processes leading to myelin injury and basic protein degradation [22] may be affected by local environmental conditions so that the basic protein-like material released into body fluids may be comprised of different basic protein peptides having different catabolic pathways and biological effects.

References

1 Marks, N. and Lajtha, A. (1971) in Handbook of Neurochemistry (Lajtha, A., ed.), Vol. V, Part A, pp. 49–139, Plenum, New York

2 Whitaker, J.N., Terry, L.C. and Whetsell, W.O. (1981) Brain Res. 216, 109–124

3 Whitaker, J.N. and Seyer, J.M. (1979) J. Biol. Chem. 254, 6 956–6 963

4 Barrett, A.J. (1977) in Proteinases in Mammalian Cells and Tissues (Barrett, A.J., ed.), pp. 209–248, North Holland, Amsterdam

5 Benuck, M., Marks, N. and Hashim, G. (1975) Eur. J. Biochem. 52, 615–621

6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275

7 Whitaker, J.N. and Seyer, J.M. (1979) J. Neurochem. 32, 325–333

8 Chou, F.C.-H., Chou, C.-H.J., Shapira, R. and Kibler, R.F. (1976) J. Biol. Chem. 251, 2 671–2 679

9 Dionyssiou-Asteriou, A. and Rakitzis, E.T. (1979) Biochem. J. 177, 355–356

10 Watabe, S., Terada, A., Ikeda, T., Kouyama, H., Taguchi, A. and Yago, N. (1979) Biochem. Biophys. Res. Commun. 89, 1 161–1 167

11 Ohkuma, S. and Poole, B. (1978) Proc. Natl. Acad. Sci. 75, 3 327–3 331

12 Whitaker, J.N. (1981) Comp. Biochem. Physiol. 68B, 215–220

13 Eylar, E.H., Brostoff, S., Hashim, G., Caccam, J. and Burnett, P. (1971) J. Biol. Chem. 246, 5 770–5 784

14 Brostoff, S.W., Reuter, W., Hichens, M. and Eylar, E.H. (1974) J. Biol. Chem. 249, 559–567

15 Dunkley, P.R. and Carnegie, P.R. (1974) Biochem. J. 141, 243–255

16 Chapman, B.E. and Moore, W.J. (1976) Biochem. Biophys. Res. Commun. 73, 758–766

17 Whitaker, J.N., Chou, C.-H.J., Chou, F.C.-H. and Kibler, R.F. (1977) J. Exp. Med. 146, 317–331

18 Artman, M. and Seeley, R.J. (1979) Biochem. Biophys. Res. Commun. 89, 1 217–1 223

19 Glaser, J.H. and Conrad, H.E. (1980) J. Biol. Chem. 255, 1 879–1 884

20 Einstein, E.R., Csejtey, J. Dalal, K.B., Adams, C.W.M., Bayliss, O.B. and Hallpike, J.F. (1972) J. Neurochem. 19, 653–662

21 Whitaker, J.N. (1977) Neurology 27, 911–920

22 Bashir, R.M. and Whitaker, J.N. (1980) Ann. Neurol. 7, 50–57