The N-Terminal Sequence of Bovine Carboxypeptidase A and Its Relation to Zymogen Activation*

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A pentadecapeptide derived from the N-terminal portion of bovine carboxypeptidase A_γ was isolated by the action of cyanogen bromide on the protein. Its sequence was established as Asn.Tyr.Ala.Thr.Tyr.His.Thr.Leu.Asp.Glu.Ile.Tyr.Asp.Phe.Met. An analogous peptide, derived from carboxypeptidase A_α, differs from carboxypeptidase A_γ by seven residues at the N terminus, rather than the five residues previously indicated by Sampath Kumar *et al.* (1963). The correct N-terminal sequence is proved to be: Ala.Arg.Ser.Thr.Asn.Thr.Phe.Asn.Tyr.Ala.Thr.Tyr.His.Thr.Leu.Asp.Glu.Ile.Tyr.Asp.Phe.Met. A revised mechanism for the origin of the various chemical species of bovine carboxypeptidase A from subunit I of procarboxypeptidase A is presented. Carboxypeptidase A_α could be converted to a new form of carboxypeptidase A, A_β, with N-terminal serine. Conversion to carboxypeptidase A_γ could be effected only to a limited extent.

The major chemical species in preparations of carboxypeptidase A, isolated from autolyzing tissues of the bovine pancreas (Anson, 1937) is carboxypeptidase A_γ. Its N-terminal residue has been identified as asparagine (Thompson, 1953; Bargetzi *et al.*, 1963, 1964) and its N-terminal sequence has been established by Coombs *et al.* (1964) as Asn.Tyr.Ala.... Carboxypeptidase A_α is the major chemical species in preparations of the enzyme isolated after activation of partially purified procarboxypeptidase A (Cox *et al.*, 1964) and it contains an N-terminal alanine residue (Bargetzi *et al.*, 1963, 1964). The chemical relationship between these two enzymes derived from the same source was clarified by Sampath Kumar *et al.* (1963) by subjecting chymotryptic digests of the two proteins, after performic acid oxidation, to the technique of "peptide mapping." An additional pentapeptide, having the sequence H.Ala.Arg.Ser.Thr.Asn.OH, was found in the digest of carboxypeptidase A_α. It was concluded that this sequence precedes the N-terminal sequence Asn.Tyr.Ala... of carboxypeptidase A_α and hence that different mechanisms of activation of the precursor give rise to the different chemical species of the enzyme.

In the course of investigations on the primary sequence of carboxypeptidase A_γ, it was found that cleavage by cyanogen bromide yields a pentadecapeptide which appeared to be derived from the N terminus of the molecule. A parallel study on carboxypeptidase A_α was expected to liberate an eicosapeptide containing the additional pentapeptide sequence at its amino terminus. This fragment was found to contain, in addition, the sequence Thr.Phe, which had not been identified in the previous investigation. The present communication describes the isolation and amino acid sequence of the 22-amino acid peptide derived from the N terminus of carboxypeptidase A_α and proposes a revised scheme for the mechanism of activation of procarboxypeptidase and for the conversion of carboxypeptidase A_α to other chemical species.

Materials and Methods

Carboxypeptidase A, obtained from Worthington Biochemical Corp., contained predominantly the chemical species A_γ, identified by its N-terminal asparagine (Bargetzi *et al.*, 1964). Carboxypeptidase A prepared by the method of Cox *et al.* (1964) from partially purified procarboxypeptidase A contained predominantly the chemical species A_α, as shown by its N-terminal alanine.

Trypsin, chymotrypsin, and pepsin were all twice-crystallized products obtained from Worthington Biochemical Corp. Succinyltrypsin was prepared according to Terminiello *et al.* (1958). Nagarse (a crystalline protease from *B. subtilis*) was purchased from the Biddle Sawyer Corp. Cyanogen bromide and trifluoroacetic acid were obtained from Eastman Organic Chemicals.

Dinitrophenylation and identification of terminal groups were carried out by conventional procedures (Fraenkel-Conrat *et al.*, 1955). Amino acid analyses of peptides were carried out on a Spinco amino acid analyzer. Occasionally, the amino acids were separated on paper (Richmond and Hartley, 1959) and analyzed by the method of Tigane *et al.* (1961).

For sequence determination of the peptides, conventional methods of digestion and separation of the peptide mixtures were employed. The peptides were separated by electrophoresis at 34 v/cm on Whatman No. 3 paper in volatile buffers (*p*H 6.5, 3.6, and 2.1) by adaptation of the techniques of Michl (1953) and Naughton *et al.* (1960), and by paper chromatography in butanol–acetic acid–water (3:1:1) as required. The peptide zones were detected by staining with ninhydrin. The purity of the peptides was judged by homogeneity during electrophoresis and chromatography, by end-group analysis, and by observations that the measured ratios of amino acid residues were integral numbers.

Edman degradations of the peptides were carried out by the subtractive procedure essentially according to the method of Konigsberg and Hill (1962). In some cases the terminal amino acids were identified as phenylthiohydantoins according to Sjöquist (1960).

C-terminal residues were identified by analyzing the amino acids liberated by the action of carboxypeptidase A_γ on the peptides.

Isolation of the N-Terminal Pentadecapeptide by the Action of Cyanogen Bromide on Carboxypeptidase A_γ.— The conditions for cleaving quantitatively at methionine residues of carboxypeptidase A_γ are essentially those described by Bargetzi *et al.* (1964). Four hundred mg of the enzyme dissolved in 32 ml of 75% trifluoroacetic acid was incubated with 800 mg of cyanogen bromide at room temperature for 24 hours. The

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solution was diluted with water to 5% acid concentration, stirred for 1 hour, and centrifuged. The insoluble fragments were dissolved in 10 ml of trifluoroacetic acid, diluted to 5% acid concentration, and centrifuged. The precipitate was washed once again with 5% trifluoroacetic acid. The three supernatants were combined, extracted three times with ether, concentrated to a small volume *in vacuo* at 35–40°, and lyophilized.

The lyophilized material was extracted with water repeatedly and most of it was solubilized. The soluble material was absorbed on a column of talc (15 × 2.2 cm) equilibrated with water. The column was washed with several volumes of water and the washings were discarded. The column was then treated with 0.1 N ammonia and the ammonia eluate containing the pure peptide was concentrated to dryness. This peptide derived from carboxypeptidase A_γ will be referred to as peptide N_γ. Alternatively, the peptide could be precipitated at *p*H 4.4 from the water extract of the lyophilized material, but invariably this procedure yielded incomplete recoveries of the peptide.

Isolation of a 22-Residue Peptide from Carboxypeptidase A_α.—The enzyme was treated with cyanogen bromide as in the previous case. The 5% trifluoroacetic acid–soluble fraction and the washings of the insoluble fragments were combined, extracted three times with ether, concentrated to a small volume *in vacuo* at 35–40°, and lyophilized. Attempts to precipitate the peptide or absorb it selectively on a column resulted in impure peptides and incomplete recoveries. The lyophilized material was therefore repeatedly extracted with water. The insoluble fraction, on analysis, proved to be the desired fragment. The water extract was again concentrated to a small volume *in vacuo* and lyophilized. Repeated extraction of this material with water left behind a second crop of the insoluble peptide. Very little peptide could be recovered from the second water extract. The peptide was stored frozen as a suspension in water. This peptide will be referred to as peptide N_α.

Results

Sequence of Peptide N_γ Isolated from Carboxypeptidase A_γ.—The composition of the peptide is given in Table I. The N-terminal amino acid residues were determined by subtractive Edman procedure which yielded the following compositions of the residual peptides after cleaving the N-terminal residue at each step:

1st degradation: *Asp 2.00*, Thr 1.9, Glu 1.4, Ala 0.9, Leu 1.0, Ile 0.8, Tyr 2.3, Phe 0.8, His 0.8, HomoSer 1.0

Table I
Composition of N-Terminal Peptide Fragments Derived from Carboxypeptidases A_α and A_γ

Amino Acid	Peptide N_α	Nearest Integer (residues per molecule)	Peptide N_γ	Nearest Integer (residues per molecule)
Aspartic acid	4.0	4	3.0	3
Threonine	3.8	4	1.9	2
Serine	1.2	1		
Glutamic acid	1.4	1	1.1	1
Alanine	1.8	2	1.0	1
Isoleucine	0.9	1	1.0	1
Leucine	1.2	1	1.0	1
Tyrosine	2.6	3	2.7	3
Phenylalanine	2.0	2	1.0	1
Histidine	1.0	1	1.0	1
Arginine	0.9	1		
Methionine[a]	1.1	1	1.0	1

[a] Sum of homoserine and homoserine lactone.

2nd degradation: Asp 1.9, Thr 1.8, Glu 1.4, Ala 1.0, Leu 0.9, Ile 0.8, *Tyr 1.6*, Phe 1.0, His 0.8, HomoSer 1.0

3rd degradation: Asp 2.0, Thr 1.7, Glu 1.1, *Ala 0.5*, Leu 1.0, Ile 0.8, Tyr 1.6, Phe 1.0, His 0.8, HomoSer 1.0

The results suggest an N-terminal sequence of Asp.-Tyr.Ala.., which is consistent with the structure of the isolated peptides (*vide infra*). Since it has been established that the N-terminal sequence of carboxypeptidase A_γ is Asn.Tyr.Ala.., the data strongly suggest that this fragment is derived from the N-terminal portion of the protein.

Digestion of the peptide (0.5 μmole) in 0.05 M ammonium acetate, *p*H 8.6, with 500 μg chymotrypsin for 5 hours at room temperature yielded a mixture of five peptides, which were separated as described. The composition of the peptides is given in Table II.

Table II
Compositions of Chymotryptic Peptides Derived from Peptide N_γ

Amino Acid	γ-C1	γ-C2	γ-C3	γ-C4	γ-C5
	(residues per molecule)				
Aspartic acid	1.1			1.1	1.0
Tyrosine	0.8	0.8	0.9		
Alanine		1.0			
Threonine		1.0	1.0		
Histidine			1.0		
Leucine			1.0		
Glutamic acid			1.1		
Isoleucine			1.0		
Phenylalanine				0.9	1.0
Methionine[a]					1.0

[a] Sum of homoserine and homoserine lactone.

Sequence of Peptide γ-C1.—The sequence of this peptide was established by Edman degradation. The residue after cleavage of the N-terminal acid yielded the following composition: Asp 0.1 and Tyr 1.0. From its neutral behavior during electrophoresis at *p*H 6.5, it was inferred that the peptide contains asparagine rather than aspartic acid. Its sequence is therefore Asn.Tyr.

Sequence of Peptide γ-C2.—This peptide, which is always contaminated with small amounts of γ-C4 and γ-C5, could not be purified by any of the conventional systems of electrophoresis or chromatography. The solvent system of tertiary amyl alcohol saturated with water was used for chromatography, and the peptide could be separated from its contaminants after 4 days of chromatography. Incubation of 0.1 μmole of the peptide with 100 μg of carboxypeptidase A at *p*H 8.6 in 0.1 M ammonium acetate for 16 hours released 75% of the initial tyrosine and no other amino acid. Since the only alanine residue in peptide N_γ is in the N-terminal sequence Asn.Tyr.Ala.., the sequence of the peptide γ-C2 is deduced as Ala.Thr.Tyr, and it extends the N-terminal sequence of Peptide N_γ to Asn.Tyr.Ala.Tyr.

Sequence of Peptide γ-C3.—The N-terminal sequence of the peptide was deduced by Edman degradation.

1st degradation: *His 0.1*, Thr 0.9, Leu 1.0, Asp 1.0, Glu 1.0, Ile 0.9, Tyr 0.9

2nd degradation: His 0.0, *Thr 0.3*, Leu 1.0, Asp 1.0, Glu 1.0, Ile 0.9, Tyr 0.9

3rd degradation: His 0.0, Thr 0.2, *Leu 0.4*, Asp 1.0, Glu 1.0, Ile 0.9, Tyr 0.7

4th degradation: His 0.0, Thr 0.3, Leu 0.4, *Asp 0.5*, Glu 1.0, Ile 0.8, Tyr 0.7

The peptide (1.0 μmole) was digested in 0.1 M ammonium acetate, *p*H 8.6, with 200 μg of Nagarse at room temperature for 2 hours. Separation of the prod-

TABLE III
SUMMARY OF EVIDENCE FOR THE SEQUENCE OF PEPTIDE N_γ

Composition	$(Asp_3,Thr_2,Glu_1,Ala_1,Ile_1,Leu_1,Tyr_3,Phe_1,His_1,Met_1)$[a]
Edman on N_γ	Asp.Tyr.Ala
Peptide γ-Cl	(Asn,Tyr)
Edman on γ-Cl	Asn.Tyr
Peptide γ-C2	(Ala,Thr,Tyr)
Carboxypeptidase A on γ-C2	(Ala,Thr)Tyr
Peptide γ-C3	(His,Thr,Leu,Asp,Glu,Ile,Tyr)
Edman on γ-C3	His.Thr.Leu.Asp(Glu,Ile,Tyr)
Carboxypeptidase A on γ-C3	(His,Thr,Leu,Asp,Glu)Ile.Tyr
Peptide γ-C4	(Asp,Phe)
Peptide γ-C5	(Asp,Phe,Met)
Edman on γ-C5	Asp(Phe,Met)
Sequence	Asn.Tyr.Ala.Thr.Tyr.His.Thr.Leu.Asp.Glu.Ile.Tyr.Asp.Phe.Met

[a] Methionine determined as sum of homoserine and homoserine lactone.

ucts by high-voltage electrophoresis at *p*H 6.5 yielded an acidic and a basic peptide which gave the following compositions for their constituent amino acids:

Acidic peptide: Asp 1.0, Glu 1.2, Ile 0.9 and Tyr 1.0

Basic peptide: His 1.0, Thr 1.1 and Leu 1.0

The compositions of the peptides and the Edman-degradation data are consistent with the sequence His.Thr.Leu.Asp(Glu,Ile,Tyr). When 0.2 μmole of the peptide was digested with 100 μg of carboxypeptidase A at *p*H 8.6, 56% of the tyrosine and 26% of the isoleucine were liberated in 2 minutes, whereas in 5 minutes 71% of the tyrosine and 31% of the isoleucine were released. The sequence of the peptide γ-C3 was therefore established as His.Thr.Leu.Asp.Glu.Ile.Tyr.

Sequence of Peptide γ-C4 and γ-C5.—The peptide γC4 was acidic at *p*H 6.5, suggesting that it contains aspartic acid rather than asparagine. The peptide γC5 was acidic at *p*H 6.5, suggesting that the methionine degradation product must be present as homoserine and not as its lactone. One cycle of Edman degradation resulted in complete loss of aspartic acid. Since homoserine could be only C-terminal, the sequence of the peptide was established as Asp.Phe.HomoSer.

The evidence for the sequence of the peptide N_γ is summarized in Table III. The sequence is H.AspN.-Tyr.Ala.Thr.Tyr.His.Thr.Leu.Asp.Glu.Ile.Tyr.Asp.-Phe.HomoSer.OH.

Sequence of Peptide N_α Derived from Carboxypeptidase A_α.—Since the sequence of the 15 residues in peptide N_γ was consistent with its derivation from the N-terminal portion of the molecule, it was expected that analogous treatment of carboxypeptidase A_α would give a fragment with an additional N-terminal pentapeptide having the structure Ala.Arg.Ser.Thr.Asn.. (Sampath Kumar *et al.*, 1963). However, the amino acid composition of the fragment from A_α (given in Table I) revealed not only the anticipated 20 residues but also one additional residue each of threonine and phenylalanine. The N-terminal portion of this peptide was examined by Edman degradation:

1st degradation: His 1.1, Arg 1.0, HomoSer 1.0, Asp 4.0, Thr 3.7, Ser 1.2, Glu 1.5, *Ala 1.2*, Ile 0.9, Leu 1.3, Tyr 2.5, Phe 1.9

2nd degradation: His 0.9, *Arg 0.3*, HomoSer 0.9, Asp 4.0, Thr 3.6, Ser 1.0, Glu 1.8, Ala 1.3, Ile 1.0, Leu 1.3, Tyr 2.5, Phe 2.0

The eicosapeptide which remained after two cycles of the Edman degradation was digested with 2 mg of chymotrypsin at room temperature for 8 hours at *p*H 8.6 in 0.04 M ammonium acetate buffer. A peptide map of this digest showed an identical pattern to an analogous digest of peptide N_γ except for *two additional peptides*. One, with the mobility of aspartic acid in the butanol–acetic acid–water system had the composition Ser 0.9, Thr 0.8, Asp 1.1. Since it was neutral at *p*H 6.5, the aspartic acid must occur at the amide. The other peptide, with a mobility faster than leucine, had the composition Thr 1.0, Phe 0.9.

A peptic digest of peptide N_α was carried out using 1.2 μmoles in 4% formic acid with 2 mg of pepsin at room temperature for 16 hours. Only one basic, Sakaguchi-positive peptide was observed during electrophoresis at *p*H 6.5. It had the composition Asp 2.0, Thr 2.0, Ser 1.0, Ala 1.0, Phe 0.8, Arg 0.9.. From the basic character of this peptide, both aspartic acid residues were inferred to be amides. Dinitrophenylation yielded DNP-alanine as the only N-terminal amino acid. Since the only arginine residue in peptide Nα occurs penultimate to the N-terminal alanine, this octapeptide must be at the N terminus of N_α. Digestion of 0.08 μmole of the octapeptide with 250 μg of carboxypeptidase A in 0.05 M ammonium acetate at *p*H 8.6, room temperature, for 25 minutes, released about 0.75 residue each of phenylalanine and asparagine.

Taken together, these results indicate the following N-terminal sequence for peptide N_α: Ala.Arg(Ser,Thr,-Asn,Thr)(Phe,Asn). Since the C-terminal 15 residues coincide both in composition and in the above peptide map to peptide N_γ, it is possible to deduce that the sequence of N_α is Ala.Arg(Ser,Thr,Asn,Thr)Phe.Asn.Tyr.-Ala.Thr.Tyr.His.Thr.Leu.Asp.Glu.Ile.Tyr.Asp.Phe.-HomoSer.OH. Combining this information with the N-terminal sequence previously reported (Sampath Kumar *et al.*, 1963), carboxypeptidase A_α must have the N-terminal sequence NH_2-Ala.Arg.Ser.Thr.Asn.Thr.-Phe.Asn.Tyr.Ala.Thr.Tyr.His.Thr.Leu,Asp.Glu.Ile.-Tyr.Asp.Phe.Met.OH.

DISCUSSION

It has been previously suggested that the term "bovine pancreatic carboxypeptidase A" includes a family of chemical species that originate from the same precursor, subunit I of procarboxypeptidase A (Brown *et al.*, 1963). Differences among the species in the N-terminal amino acid residues were ascribed to alternate pathways of activation, which cause different peptide bonds in the common precursor to be cleaved. This conclusion was supported by the finding that carboxypeptidase A_α differed from A_γ in an additional pentapeptide sequence at the N terminus, and a pentapeptide having the expected composition was, in fact, isolated and its structure was determined (Sampath Kumar *et al.*, 1963). In the present investigation, the difference between carboxypeptidases A_α and A_γ was more fully

TABLE IV
CONVERSION OF CARBOXYPEPTIDASE A_α TO OTHER SPECIES

Digesting Enzyme[a]	Ratio of Enzyme to A_α	Ratios of N-Terminal Groups Before Ala/Ser/Asp	After Ala/Ser/Asp
Trypsin	1:6	97:3:0	13:82:5
Succinyltrypsin	1:4	74:26:0	23:77:0
α-Chymotrypsin	1:5	97:3:0	78:0:22

[a] Incubations carried out for 18–24 hours at 25–30°.

elucidated by isolating by chemical means an N-terminal peptide fragment from both enzymes. The presence of a methionine residue in the amino-terminal region of carboxypeptidase A_α afforded a means of obtaining this fragment by cleavage with cyanogen bromide, and of comparing it with the analogous peptide derived from carboxypeptidase A_γ. These results unequivocally establish that the two enzymes differ, not by 5, but by 7 residues, and that the amino-terminal sequence of carboxypeptidase $A\alpha$ is Ala.Arg.Ser.Thr.-Asn.Thr.Phe.Asn.Tyr.Ala.Thr.Tyr.His.Thr.Leu.Asp.-Glu.Ile.Tyr.Asp.Phe.Met. Apparently, in the previous peptide maps of oxidized proteins, the additional Thr.Phe peptide was overlooked, perhaps because it had the same mobility as some other neutral peptide. As expected, the inclusion of this additional dipeptide in this corrected N-terminal sequence provides even closer agreement with the compositional differences described by Bargetzi *et al.* (1963) than our calculations based on the pentapeptide difference (Sampath Kumar *et al.*, 1963) had previously indicated.

A mechanism depicting the origin of the various forms of carboxypeptidase A from its precursor, viz., subunit I of bovine procarboxypeptidase A (Sampath Kumar *et al.*, 1963), has been postulated in earlier studies, based on the identification of only one pentapeptide difference between carboxypeptidases A_α and A_γ. The present investigation modifies the scheme to account for the extension of the difference to 7 residues (Fig. 1). The difference peptide possesses a trypsin-susceptible Arg-Ser bond, and a Phe-Asn bond, the cleavage of which can be effected by either chymotrypsin or chymotrypsinlike enzymes. While the cleavage of the latter bond results in the formation of carboxypeptidase A_γ, the splitting of the former bond results in a new form of carboxypeptidase A, A_β, with N-terminal serine. In this regard, it is significant that all preparations of carboxypeptidase A invariably contain small amounts of a protein with N-terminal serine (Bargetzi *et al.*, 1964). Carboxypeptidase A_α must be formed by a rupture of the bond at the amino end of alanine, presumably by the action of trypsin. In this process, the role of the chymotrypsinlike endopeptidase derived from subunit II of procarboxypeptidase A (Brown *et al.*, 1963) remains to be clarified. It is not immediately apparent why the bond between arginine and serine is not readily split by trypsin during activation of procarboxypeptidase A, or why the bond between phenylalanine and asparagine is split in preference to others in the preparations of the enzyme from autolyzing glands. Nor is it apparent whether yet unrecognized forms of carboxypeptidase A may be formed by cleavage of susceptible bonds beyond asparagine.

Attempts to convert the preparation of carboxypeptidase A containing predominantly the species A_α into other species are summarized in Table IV. In the presence of high concentrations of trypsin, this preparation could be made to yield a crystalline enzyme which has predominantly serine as the N-terminal group,

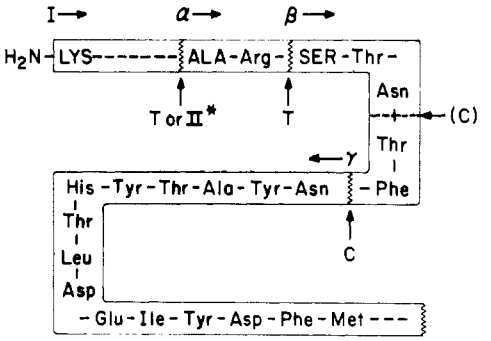

FIG. 1.—Scheme for the origin of the various species of bovine carboxypeptidase A from procarboxypeptidase A. I, subunit I of the precursor; II*, endopeptidase derived from subunit II of the precursor; T, trypsin; C, chymotrypsin; α, β, and γ, the chemical species of carboxypeptidase A, with N-terminal alanine, serine, and asparagine, respectively.

as shown by changes in the ratio of end groups before and after treatment. Succinyltrypsin also effects this conversion. Edman degradation of the preparation rich in carboxypeptidase A_β followed by dinitrophenylation gave mainly dinitrophenylthreonine, as predicted by the sequence at the N terminus of carboxypeptidase A_α. Attempts to convert the protein rich in carboxypeptidase A_α to A_γ by chymotrypsin have not been as successful, since only 20% of the enzyme could be converted to the species containing N-terminal asparagine. The reason for this poor yield is not clear, but it would appear that, once the zymogen is converted to A_α, the protein assumes a configuration which is resistant to further enzymatic cleavage. The protein rich in the β enzyme could not be converted to the species having N-terminal aspartyl residue.

The multiple forms of bovine carboxypeptidase A bear resemblance to the porcine system. Folk and coworkers (Folk, 1963; Folk and Schirmer, 1963) have shown that, as in bovine pancreas, the enzyme isolated by activation of porcine procarboxypeptidase A has an N-terminal alanine, and that this enzyme could be converted by trypsin to another chemical species having threonine as N-terminal residue. The new species was formed as a result of the release of a dipeptide, Ala-Arg. The nature of a third form of porcine carboxypeptidase A has not been clarified.

The present investigations illustrate yet another aspect of the structure and function of bovine carboxypeptidase A which is yet to be explored. The studies of Coombs *et al.* (1964) have shown that in carboxypeptidase A_γ the zinc atom which is essential for activity is bound by not only a –SH group but also by the α-amino group. The existence of multiple forms of the active enzyme differing in their N-terminal sequences suggests that neither the identity of the terminal residue nor the length of the peptide chain at the N terminus may have any qualitative effect on the binding of the metal in this region. In view of the necessarily different ligands, one might anticipate specific conformational changes at the N terminus or quantitative differences in the magnitude of the stability constants for the metals in the various species of carboxypeptidase. However, the absence of cysteine in the N-terminal 15 residues of carboxypeptidase A_γ indicates that the size of the chelate ring must be relatively enormous, and one might expect that alterations in the size of this ring by 7 residues would be minor enough to contribute virtually nothing to the magnitude of the stability constant, especially in view of the fact that the

stability of the metal-enzyme complex is predominantly due to the metal-sulfur bond.

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A Stochastic Approach to Enzyme-Substrate Reactions*

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This paper presents a stochastic approach to the study of enzyme-substrate reactions. Stochastic models are constructed for various enzyme-substrate systems including the Michaelis-Menten scheme. The stochastic models provide a framework from which the fluctuations in the number of reactant species during the early stages of the reaction may be obtained. Solutions of the stochastic equations for the second moment are obtained by assuming that the second moment of the concentration variable may be expressed in terms of the first moment. This technique reduces the calculation of the variance to the determination of the first moment. It is also shown that under certain conditions (e.g., $\langle s \rangle \gg \langle e \rangle$) the stochastic rate equations correspond to the deterministic equations. Rigorous solutions of these rate equations cannot be found; however, by invoking the stationary-state hypothesis, approximate expressions for the first moments are obtained.

It has been shown by several authors (Delbruck, 1940; Singer, 1953; Renyi, 1954; Bartholomay, 1958, 1959, 1962a,b; Ishida, 1960; McQuarrie, 1963, McQuarrie *et al.*, 1964) that the course of a chemical reaction can be treated as a stochastic process. The conventional approach to chemical kinetics may be called deterministic, since once the initial number of reactant molecules is known the value at subsequent times is predicted by mathematical expressions. Furthermore, the conventional approach predicts no fluctuations for the number of reactant molecules and any fluctuations are usually attributed to experimental error. On the other hand, the stochastic approach treats the number of reactant molecules as a time-varying discrete random variable and is concerned with determining the probability that the reaction system is in a certain state. The stochastic approach also provides a well-defined method of determining the extent of the fluctuations of the number of reactant species. Such fluctuations are inherent to some extent in all chemical reactions and in some reactions these fluctuations may be relatively large, e.g., systems containing a small number of interacting species. The stochastic model also provides a method of determining the extent to which the conventional rate expressions are applicable to small systems.

This paper discusses stochastic models for various enzyme-substrate mechanisms. Bartholomay (1962a, b) considered a stochastic model for enzyme-substrate reactions in terms of the Michaelis-Menten mechanism. Bartholomay showed that the stochastic model corresponds "in the mean" with the deterministic model, but no mathematical expression for the variance of the reactant species was found. The inability to determine the fluctuations is owing to the appearance of nonlinear stochastic equations. In a previous paper (McQuarrie *et al.*, 1964) we proposed an approximation that permits the determination of the second moment with relative ease. We shall illustrate the application of this approximate method to stochastic models of various enzyme-substrate mechanisms including the Michaelis-Menten scheme.

EXAMPLES

Michaelis-Menten Scheme.—

$$S + E \underset{2}{\overset{1}{\rightleftarrows}} C$$

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