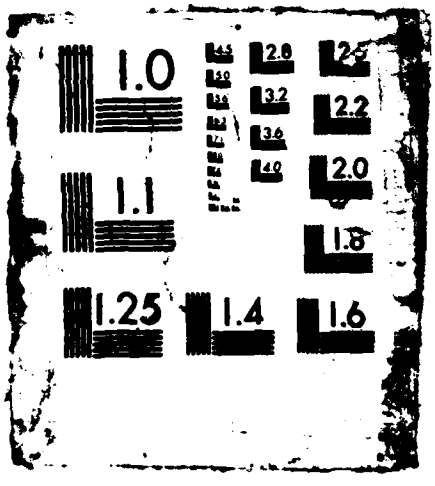
AD-A178 987 MINIATURE ORGANIC MODEL OF CHYMOTRYPSIN: SYNTHESIS AND EVALUATION(U) NORTHWESTERN UNIV EVANSTON IL DEPT OF CHEMISTRY M L BENDER 23 MAR 87 N00014-86-K-0241 1/1

UNCLASSIFIED F/G 6/1 NL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REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION (U)	1b. RESTRICTIVE MARKINGS NA
2a. SECURITY CLASSIFICATION AUTHORITY NA	3. DISTRIBUTION/AVAILABILITY OF REPORT distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Northwestern University/Dept. of Chemistry	5. MONITORING ORGANIZATION REPORT NUMBER(S) Office of Naval
6a. NAME OF PERFORMING ORGANIZATION Northwestern University 6b. OFFICE SYMBOL (If applicable) NA	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code) Dept. of Chemistry 2145 Sheridan Road Evanston, IL 60201	7b. ADDRESS (City, State, and ZIP Code) 536 S. Clark St. Chicago, IL 60635
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N0014-86-K-0241
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification)

Miniature Organic Model of Chymotrypsin: Synthesis and Evaluation

12. PERSONAL AUTHOR(S)

Myron L. Bender

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 3/24/86 TO 3/23/87		

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
			Enzyme models, chymotrypsin

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Three artificial enzymes (benzymes), based on α, β and γ-cyclodextrins (cycloamyloses) were synthesized by attaching the three catalytic groups of chymotrypsin; (1) hydroxyl group (2) imidazolyl group and (3) carboxylate ion to known binders, cyclodextrins. These molecules have both a catalytic portion and a binding portion in them and thus they can serve as complete, albeit artificial enzymes. β-Benzyme has approximately the same catalytic activity as the real enzyme although the molecular weight of the artificial enzyme (1365) is 1/18 the molecular weight of the real enzyme (24,800). Results indicate that both the artificial chymotrypsin and the real chymotrypsin have similar mechanisms. The artificial enzyme shows increased activity at high pH and temperature conditions whereas the real enzyme denatures at relatively milder pH and thermal conditions. The artificial enzymes show a specificity based on the size of the cyclodextrin cavity. Five publications, one patent and three major presentations have resulted from this study. Synthetic, mechanistic and specificity investigations of these enzymes are planned for the second year.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS		
22a. NAME OF RESPONSIBLE INDIVIDUAL Myron L. Bender	22b. TELEPHONE (Include Area Code) (312)491-7675	22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

87 4 0 2

Myron L. Bender;
ONR Contract No. N0014-86-K-0241

ANNUAL REPORT ON

MINIATURE ORGANIC MODEL OF CHYMOTRYPSIN: SYNTHESIS AND EVALUATION

ACCOMPLISHMENTS IN YEAR I

1. We have synthesized three artificial enzymes (benzymes), based on α-, β- and γ-cyclodextrins (cycloamyloses) by attaching the three catalytic groups of chymotrypsin; (1) hydroxyl group (2) imidazolyl group and (3) carboxylate ion to known binders, cyclodextrins. These molecules have both a catalytic portion and a binding portion in them and thus they can serve as complete, albeit artificial enzymes.

2. We have investigated the catalytic activity of the artificial enzyme. β-Benzyme has approximately the same catalytic activity as the real enzyme: $[(k_{cat}/K_m)_{real}/(k_{cat}/K_m)_{art.} = 1.3]$ in ester hydrolysis although the molecular weight of the artificial enzyme (1365) is 1/18 the molecular weight of the real enzyme (24,800).

3. We have investigated the mechanism of action of the artificial enzyme. Results indicate that both the artificial chymotrypsin and the real chymotrypsin have similar mechanisms, i.e they both proceed through an acyl-enzyme intermediate and that imidazole in both the enzymes acts as a general base catalyst $[(k_{H_2O}/k_{D_2O}) = 3]$ and not a nucleophile.

4. We have investigated the stability of the artificial enzyme. The most important difference between the real and artificial enzymes is their stability in adverse conditions. The artificial enzyme in fact shows increased activity at high pH and temperature conditions whereas the real enzyme denatures at relatively milder pH and thermal conditions.

5. We have investigated the specificities of these artificial enzymes. The artificial enzymes show a specificity based on the size of the cyclodextrin cavity i.e. whereas m-t-butylphenyl acetate is hydrolyzed fastest by β-benyzme; γ-benzyme is found to be the best for the hydrolysis of tryptophan ethyl ester.

6. Publications and presentations resulted from the study are:

   (a) Publications

   1. Synthesis and Evaluation of a Miniature Organic Model of Chymotrypsin; Valerian T. D'Souza, K. Hanabusa, T. O'Leary, R. C. Gadwood and M. L. Bender, Biochem. Biophys. Res. Commun., 129, 727 (1985).

   2. Miniature, Organic Models of Chymotrypsin Based on α-, β- and γ-cyclodextrins; M. L. Bender, V. T. D'Souza, X. L. Lu, Trends in Biotechnology, 4, 132, (1986).

   3. M. L. Bender, "Cyclodextrins (Cycloamyloses) as Enzyme Models" in Enzyme Mechanisms, M. I. Page and A. Williams, eds., Royal Society of Chemistry, in press, (1987).

4. Miniature Organic Models of Enzymes; V. T. D'Souza and M. L. Bender, Acc. Chem. Res., in press, (1987).

5. The Thermal and pH Stability of β-Benzyme; V. T. D'Souza, X. L. Lu, R. D. Ginger and M. L. Bender, Proc. Natl. Acad. Sci., in press, (1987).

(b) Patent

1. Chymotrypsin Model, U.S. 876,278, pending, (filed June 19, 1986).

(c) Major Presentations

1. Second International Symposium on Models of Enzyme Action, Plenary Lecture, University of Sussex, Brighton, England, September 15-17, 1986.

2. Boston University, Dept. of Chemistry, Colloquim, Boston, Mass. October 6, 1986.

3. Southern Methodist University, Department of Chemistry, Colloquim, Dallas, Texas, November 6, 1986.

RESEARCH GOALS FOR YEAR II

1. To synthesize a second set of artificial chymotrypsins, with the carboxylate ion attached to an aliphatic system, because theory does not allow us to differentiate the model containing (A) the carboxylate attached to an aromatic ring, from (B) the one containing the carboxylate attached to an aliphatic system.

2. To determine whether an artificial enzyme can be made from a cyclodextrin polymer (To be supplied by American Maize Products Company). This artificial enzyme will have the advantage of being a solid, through which a substrate solution can be passed and will resemble an immobilized enzyme.

3. To determine whether there are proton transfers, and how many there are in the artificial chymotrypsins made from cycloamyloses. We know that there are two general mechanisms for these reactions, both in real and artificial chymotrypsins. One mechanism involves multiple proton transfers. The other mechanism involves only the movement of pK's (in the acyl-chymotrypsin, where a dioxane water solvent was used). These two mechanisms can be differentiated by carrying out the reactions in D_2O where deuteron transfer should affect the rate, but a change in pK should not affect the rate. Determining the rate in different mixtures of H_2O and D_2O (proton inventory) should enable us to say how many proton transfers take place.

4. The identity of the groups in real chymotrypsin involves inhibition of the enzyme by various groups. We will determine if the same is true with artificial chymotrypsin: inhibition by p-t-butylphenylsulfonyl chloride (to ascertain whether there is a hydroxyl group); Inhibition by methyl-p-t-butylphenyl sulfonate, (to see if there is an imidazolyl group) and inhibition by a glycine ethyl ester and p-t-butylphenylcarbodiimide (to determine whether there is a carboxylate ion).

5. To determine the specificity of artificial chymotrypsin. There is much known about the specificity of real chymotrypsin, e.g., tryptophan derivatives> tyrosine derivatives > phenylalanine derivatives. The specificity of artificial chymotrypsin will depend on cycloamylose specificity and will be different from that of real chymotrypsin specificity. p-t-Butylphenyl esters will presumably be specific (fast) as we learned from previous results. In order to resolve this issue, an experimental approach involving natural and artificial substrates will have to be taken:

   (A) phenylalanine, trosine, tryptophan esters
   (B) phenylalanine, tyrosine, tryptophan amides
   (C) phenylalanine, tyrosine, tryptophan peptides
   (D) phenylalanine, tyrosine, tryptophan containing proteins

   It is predicted that tryptophan compounds will be too large to be included in the cyclodextrin cavity and thus will be the slowest. The amides will definitely be slower than the esters. We will determine whether peptides and proteins are cleaved, through experiments.

6. We will determine the proper solvent for these artificial enzyme reactions. We know that the best solvent for the artificial acyl-enzyme reactions is dioxane-water, as the dioxane simulates the apolar nature of the active site. We also know that the optimum solvent for cycloamylose reactions is pure water, as that leads to optimal apolar binding. So we must ascertain this important difference, which can only be determined by experiment.

7. To determine the exact structure of the artificial enzyme including the distances between and the orientation of the catalytic groups. This will be done by X-ray crystallographic analysis of the artificial enzyme.

8. To determine the rates of carboxylate ion production in ester hydrolysis by the artificial enzyme and compare it with the spectrophotometric rate of phenol production. This will indicate turn-over rate. This will be done by pH-stat experiments.

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