two forms, C_{ss} and T_{ss} in Table . The ab initio results[4d] agree well with the predictions of rule 6. There is one disagreement for atom pair 2-3.

Acknowledgment. The author is grateful to the Center for Theoretical Physics and Chemistry for the support of this work. He wishes to thank also to Mr Geun Bae Ryu for calculating some of the results used in this work.

References

(1) M. J. S. Dewar, "The MO Theory of Organic Chemistry", McGraw-Hill, N.Y., 1969.

(2) T. K. Brunck and F. Weinhold, *J. Am. Chem. Soc.*, **101**, 1700 (1979).

(3) (a) M. J. S. Dewar and R. C. Dougherty, "The PMO Theory of Organic Chemitstry", Plenum Press, New York, 1975; (b) M-H. Whangbo, H. B. Schlegel and S. Wolfe, *J. Am. Chem. Soc.*, **99**, 1296 (1977); (c) R. Hoffmann, *ibid.*, **90**, 1475 (1968).

(4) (a) N. D. Epiotis, *J. Am. Chem. Soc.*, **95**, 3087 (1973); (b) N. D. Epiotis *et al.*, *ibid.*, **95**, 7588 (1973); (c) N. D. Epiotis *et al.*, *ibid.*, **96**, 4075 (1974); (d) N. D. Epiotis *et al.*, *ibid.*, **97**, 5961 (1975); (e) F. Bernardi *et al.*, *ibid.*, **98**, 2385 (1976).

(5) (a) R. S. Mulliken, *J. Chem. Phys.*, **23**, 1841 (1955); (b) I. G. Csizmadia, "Theory and Practice of MO Theory in Organic Chemistry", Elsevier, Amsterdam, 1977.

(6) D. Cremer, J. S. Binkley, J. A. Pople and W. J. Hehre, *J. Am. Chem. Soc.*, **96**, 6900 (1974).

(7) R. S. Mulliken, C. A. Rieke, D. Orloff and H. Orloft, *J. Chem. Phys.*, **17**, 1248 (1949).

(8) (a) K. Fukui, T. Yonezawa and H. Shingu, *J. Chem. Phys.*, **20**, 722 (1952); (b) K. Fukui *et al.*, *ibid.*, **22**, 1433 (1954); (c) K. Fukui *et al.*, *Bull. Chem. Soc. Japan*, **27**, 423 (1954); (d) K. Fukui *et al*, *J. Chem. Phys.*, **27**, 1247 (1957).

(9) R. Hoffmann and R. A. Olofson, *J. Am. Chem. Soc.*, **88**, 943 (1966).

(10) J. P. Lowe, *ibid.*, **92**, 3799 (1970).

(11) L. E. Sutton *Ed.*, "Tables of Interatomic Distances and Configuration in Molecules and Ions", The Chemical Soc., London, 1958.

(12) I. Lee, Unpublished results.

(13) I. Lee and D. W. Park, *J. Korean Chem. Soc.* Submitted for publication.

(14) (a) I. Fleming, "Frontier Orbitals and Organic Chemical Reactions", John Wiley and Sons, London, 1976; (b) W. L. Jorgensen and L. Salem, "The Organic Chemists Book of Orbitals", Academic Press, N. Y., 1973.

(15) H. Fujimoto and R. Hoffmann, *J. Phys. Chem.*, **78**, 1167 (1974).

Properties of Penicillin Amidohydrolase Immobilized on Nylon Fiber

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Penicillin amidohydrolase was partially purified from the fermented broth of *Bacillus megaterium*, and was immobilized on nylon fiber. The surface area of nylon fiber was increased by roughening it with fine sand and activated by acid treatment. The free amino groups on the nylon fiber exposed by such treatment were then utilized to immobilize the penicillin amidase. Enzymatic properties of penicillin amidohydrolase immobilized on the nylon fiber by covalent bonding and cross linking with glutaraldehyde were studied and compared with those of soluble enzyme. The optimal pH and temperature profile of immobilized enzyme showed only slightly broader peaks, and the values of kinetic constants, K_m, K_{ia}, and K_{ip}, of the immobilized enzyme are only slightly greater than those of the soluble enzyme. These rusults suggest that the mass transfer effect on the reaction rate for the penicillin amidase immobilized on nylon fiber is not so significant as the enzyme immobilized on some other support material like bentonite. The experimental results of batch reaction agreed well with the results of computer simulation for both the immobilized and soluble enzyme systems, confirming the validity of the rate equation derived which was based on the combined double inhibition by two reaction products.

Introduction

Penicillin amidohydrolase (or penicillin amidase) is an enzyme that hydrolyzes benzylpenicillin to yield 6-aminopenicillanic acid (6-APA) and phenylacetic acid (PAA). This enzyme is of considerable importance, since many semisynthetic penicillins and cephalosporins are prepared from 6-APA.

Penicillin amidase have been immobilized using various methods including adsorption on bentonite[1], covalent bonding on diethylaminoethyl cellulose[2], and entrapment in fibrous polymer matrices.[3] Nylon polymers have also been

used as a support matrix for the preparation of immobilized enzymes. The majority of enzyme immobilization on the nylon make use of chemical reactions to introduce reactive groups for the covalents binding of the enzyme protein[4~7].

These chemical reactions are usually accompanied by the chain scissions of the nylon polymer. This procedure has also been used for the immobilization of enzymes onto the inside surface of nylon tubing.

In the majority of cases, the nylon tube was treated with the methanolic solution of $CaCl_2$, to etch out the surface of inner wall by dissolving out the amorphous region and increasing available surface area. As an alternative, the mechanical treatment with fine sand particles proved to be good in obtaining the porous nylon fiber.

The penicillin amidase was immobilized on this nylon fiber and the enzymatic properties were studied. The purpose of this study, in part, is to show the possibility of developing the nylon fiber as enzyme carrier by devising a physical method of obtaining porous nylon fiber. The performance of the immobilized penicillin amidase batch reactor system was then compared with the results of computer simulation which was based on the kinetic model.

Materials and Methods

Materials. Nylon fibers were specially spun and supplied by Kolon Nylon Co. The potassium salt of benzylpenicillin (1544 units/mg) was obtained from Pfizer, and 6-APA from Wyeth Laboratories. Other chemicals were of the extra pure reagent grade from Wako Chemical.

Production and Purification of Enzyme. The cultur of mutant strain of *Bacillus megaterium* (ATCC 14945) was grown in 500 m*l* flasks according to the method described by Kim and Ryu[8]. The medium consisted of glucose, 1%, bactocasitone, 2.5%, and yeast extract, 0.5%. 0.1% of phenylacetic acid was added as an inducer of the enzyme. The pH was adjusted to 7.0 before sterilization. After 48 to 72 hr of incubation at 30°C, the broth was harvested, and the centrifuged supernatant was used as crude enzyme solution.

Purification of enzyme was ensued according to the method of Chiang and Bennett[9].

A batch of 6-liter supernatant was acidified to pH 6.4, and was mixed with 60 g of acid-washed celite. After 1 hr, the celite was collected by filtration. The filter cake was slurried in 24% ammonium sulfate solution in 0.1 *M* trisbuffer (pH 8.4), transferred to a glass column of suitable size, and was eluted with ammonium sulfate solution. The eluate was dialyzed and lyophilized.

Determination of Enzyme Activity. The enzyme activity was determined by *P*-dimethylaminobenzaldehyde method that was based on the measurement of the product of enzymatic hydrolysis, 6-APA[10] The hydrolysis of penicillin in 0.1 *M* borate buffer, pH 8.4, was carried out in water bath maintained at 40°C.

One unit of enzyme activity was defined as the activity of enzyme that is equivalent to one μ mole of 6-APA formed in an hour.

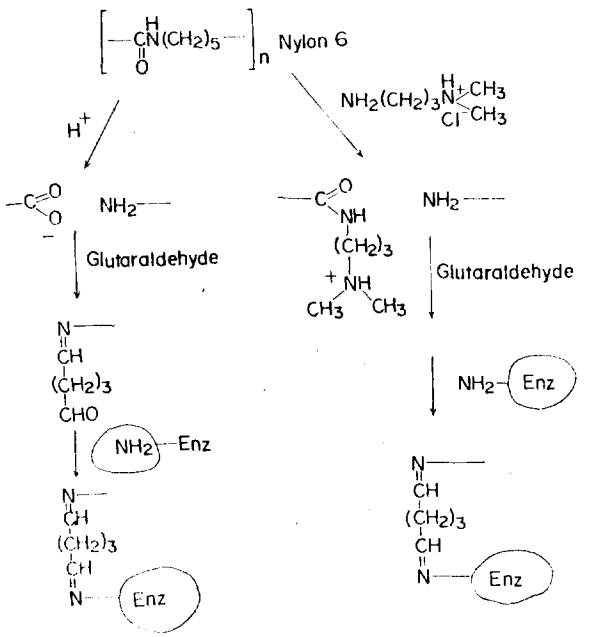

Figure 1. Reaction scheme for immobilization of enzyme on nylon fiber by partial hydrolysis with HCL and aminolysis with N,N-dimethylaminopropylamine.

Pretreatment of Nylon Fiber. Nylon fiber used in this experiment was the nylon filament of the diameter about 30μm. The filaments were cut into short slivers (2~5 mm), followed by scouring with carbon tetrachloride and then with methanol using Soxhlet apparatus to clear the nylon surface of any surface finishing chemicals[11].

In order to increase the surface area of nylon fiber, we first used the modified method of Hornby *et al.*[12] which requires incubation with $CaCl_2$ in methanolic water solution.

For mechanical treatment, the nylon fiber was slightly ground with fine sand particles in a mortar, followed by extensive water wash to remove the sand particles and the amorphous nylon fragments.

Enzyme Immobilization. For partial hydrolysis with HCl, about 600 mg of pretreated nylon fiber was added to the 100 ml Erlenmeyer flask containing 40 m*l* of 8.75 *N* HCl and preheated to 45~46°C on a magnetic stirrer.

After 1 to 2 hr stirring, distilled water was added to the vessel to make to 100 m*l* and stopped the hydrolysis, followed by filtration.

After activation with 5% (w/w) glutaraldehyde solution in 0.2 *M* borate buffer (pH 8.5) for 20 minutes, it was washed with water and immediately filled with enzyme solution. After standing overnight at 4°C, an extensive washing was followed according to the procedure of Axen *et al*[13].

For nonhydrolytic cleavage of amide bonds of nylon fiber, it was first immersed in methanol, filled with N,N-dimethylaminopropylamine, and incubated for 12 hr at 70°C.

The immobilization reactions are shown in Figure 1.

Operation and Simulation of Enzyme Reactor. For the batch reaction of immobilized enzyme, 50 m*l* Erlenmeyer flask equipped with a magnetic stirrer was used as a reactor vessel.

Using the kinetic constants determined from the experiments, a rate equation was derived to predict the reactor

performance[1] and to use it for computer simulation. Computer simulation was carried out at KAIS using NOVA computer system.

Materials and Methods

Purification of Enzyme. The results obtained in a typical enzyme purification experiment is shown in Table 1. Folin–Lowry method was employed for the determination of protein using bovine serum albumin as standard.

The amount of protein in final enzyme preparation was about 14 % (w/w) indicating that it contained substantial amount of other substances and the specific activity was 84 unit/mg.

Immobilization of Enzyme. In etching out the surface of nylon fiber, the chemical treatment with $CaCl_2$ solution was found to be unsuitable. The degree of etching could not be controlled well because of the sensitivity of nylon fiber even to a small variation in $CaCl_2$ concentration and temperature.

The nylon fiber ground with sea sand in a mortar showed very porous surface structure and also retained some mechanical strength. The fine structure of nylon fiber surface was photographed under microscopy, and it shows roughened and swollen surface that is quite different from the original fiber (Figure 2).

The effect of partial hydrolysis time on the amount of protein immobilized on nylon fiber is shown in Figure 3. The optimal hydrolysis time found was in the range of 5 to 10 minutes at 45°C when 3.75 *N* HCl was used. Aminolysis with N,N-dimethylaminopropylamine resulted in a retention of low enzyme activity. The comparison of the immobilization efficiencies resulted from aminolysis and hydrolysis is shown in Table 2.

TABLE 1: Purification of Enzyme by Celite Adsorption and Dialysis

	Volume (l)	Total Activity (unit)	Total Protein (mg)	Purification (fold)	Yield (%)
Broth	6	78720	4920	1	100
Eluate	0.52	60000	150	33	77
Dialyzate	0.51	45600	94	53	60
Lyophilization*	—	42000	70	71	54

*Specific activity of final preparation: 84 unit/mg

The retention of enzyme activity varied with different amount of enzyme loading during immobilization (Figure 4).

A constant immobilized enzyme activity was anticipated beyond a certain amount of enzyme loading which would saturate all the reactive groups on nylon fibers. Contrary to our anticipation, the activity gradually decreased above a certain amount of enzyme loading. The partially purified enzyme preparation contained certain amount of protein impurities the size of which are smaller than that of the enzyme molecule. The protein impurities may diffuse into the small pore matrix and become immobilized non-selectively. This results in a decrease in the number of the reactive groups for immobilization of enzyme molecules.

This result strongly suggests that the effect of enzyme loading should be considered seriously for enzyme immobilization, especially in the case of using partially purified enzyme.

Characterization of Immobilized Enzyme. Comparison of the effect of pH on the reaction rate of soluble and immobilized enzymes is shown in Figure 5. The optimal pH was almost the same for both, and overall activity profile became slightly broader for the immobilized enzyme. This broadening effect appears to be due to the increased mass transfer resistance for the immobilized enzyme.

The temperature effect on the activity of immobilized enzyme is just the same as that of soluble enzyme below 40°C, and shows only slightly increased stability toward higher temperature as shown in Figure 6.

Lineweaver–Burk plots were prepared to determine the Michaelis–Menten constant, K_m, the inhibition constant of phenylacetic acid, K_{ia}, and the inhibition constant of 6-APA, K_{ip}, of soluble and immobilized enzymes. The kinetic

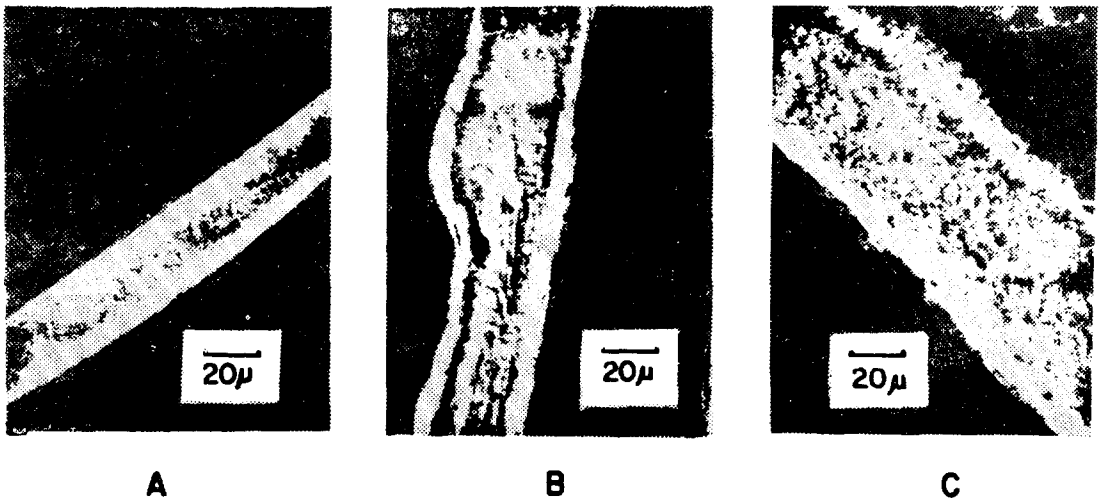

Figure 2. Microphotograph of treated nylon fiber. A. Intact nylon fiber scoured with carbon tetrachloride and methanol. B. Treated with the solution (one part water and four parts methanol) containing 18.6% (w/w) $CaCl_2$ for 20 min at 70°C. C. Slightly ground with fine sand particles.

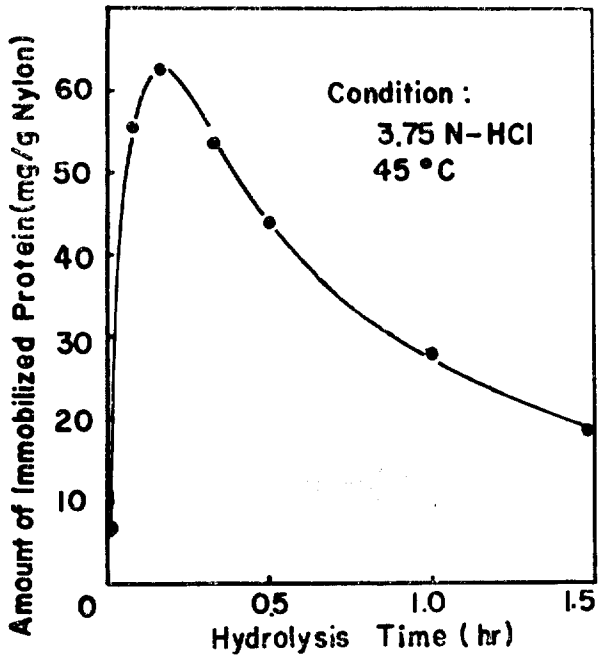

Figure 3. The effect of hydrolysis time on the amount of protein immobilized on nylon fiber.

TABLE 2: Comparison of the Efficiency of Enzyme Immobilization

Matrix	Immobilization method	Amount of protein retained (mg/g matrix)	Enzyme loading (units)	Activity retention (%)
Nylon fiber	Hydrolysis	63	3000	47
			5500	30
	Aminolysis	50	3000	11

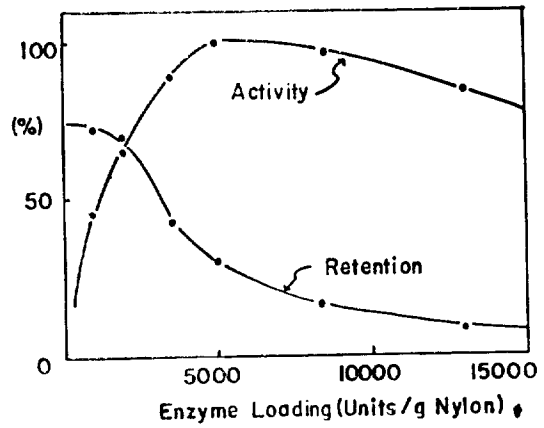

Figure 4. The effect of enzyme loading on the immobilized enzyme activity and the amount of enzyme reatined during immobilization.

constants of immobilized enzyme were determined from the reciprocal plots as shown in Figures 7, 8, 9, and 10. The values of kinetic constants determined are summarized in Table 3.

Storage stability of immobilized enzyme was also investigated. The time course of thermal deactivation obeyed approximately the first order decay as shown in Figure 11. The half-life of immobilized enzyme, $t_{1/2}$, determined was 187 hr (7.8 day), and the deactivation constant, $k_{1/2}$, 0.0037 hr^{-1}.

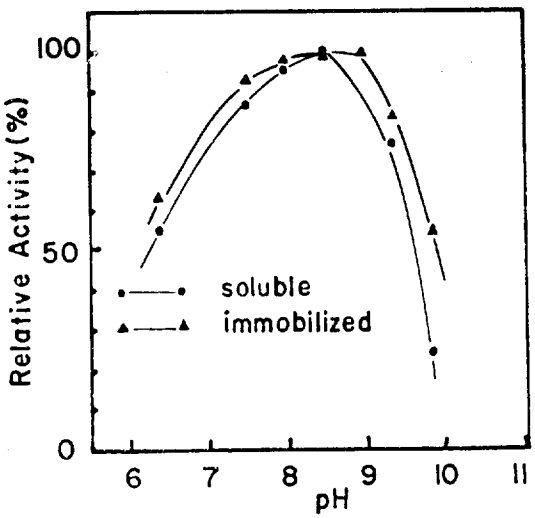

Figure 5. The relationship between pH and activity of soluble and immobilized enzymes. The buffer solutions used were: 0.1 *M* Phosphate buffer for pH 6.4, 0.1 *M* Tris buffer for pH 7.4, 7.9 and 8.5, 0.1 *M* Carbonate buffer for pH 9.0, 9.4 and 9.9.

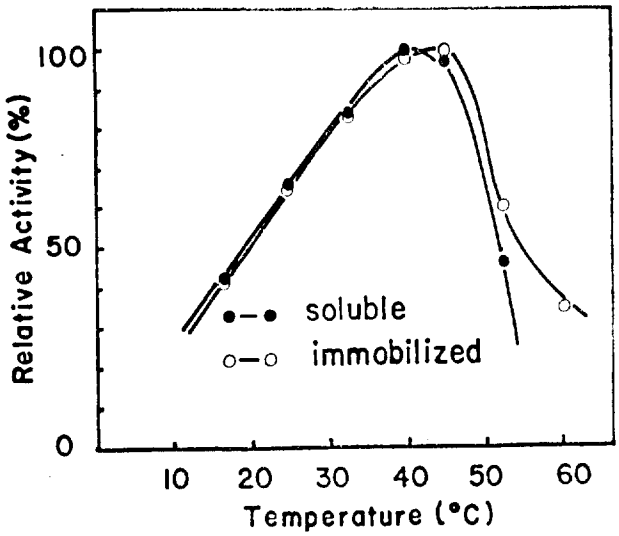

Figure 6. The effect of temperature on slouble and immobilized enzyme activity. The enzyme solutions were preincubated for 10 min at 17, 25, 33, 40°C, and for 5 min at 45, 54, 60°C, respectively.

The Batch Reaction of Soluble and Immobilized Enzyme. To predict the performance of the enzyme reactor system, simulation study was carried out with the sid of a computer.

Since two products of hydrolysis, 6-APA and PAA, are noncompetitive and competitive inhibitors of the enzyme respectively, the kinetic model that takes the combined inhibitory effects into consideration[1] is

$$V=\frac{K_0E_0}{1+\frac{K_m}{S}\left(1+\frac{A}{K_{ia}}\right)+\frac{P}{K_{ip}}\left(1+\frac{K_m}{S}\right)} \quad (1)$$

$S=S_0(1-X)$, $A=S_0X$, $P=S_0X$ then,

$$V=\frac{K_0E_oS_0(1-X)K_{ia}K_{ip}}{S_0(1-X)K_{ia}K_{ip}+K_mK_{ia}K_{ip}+K_mS_0K_{ip}X+S_0K_mK_{ia}X}$$
$$=f(s)$$

In a batch reactor system,

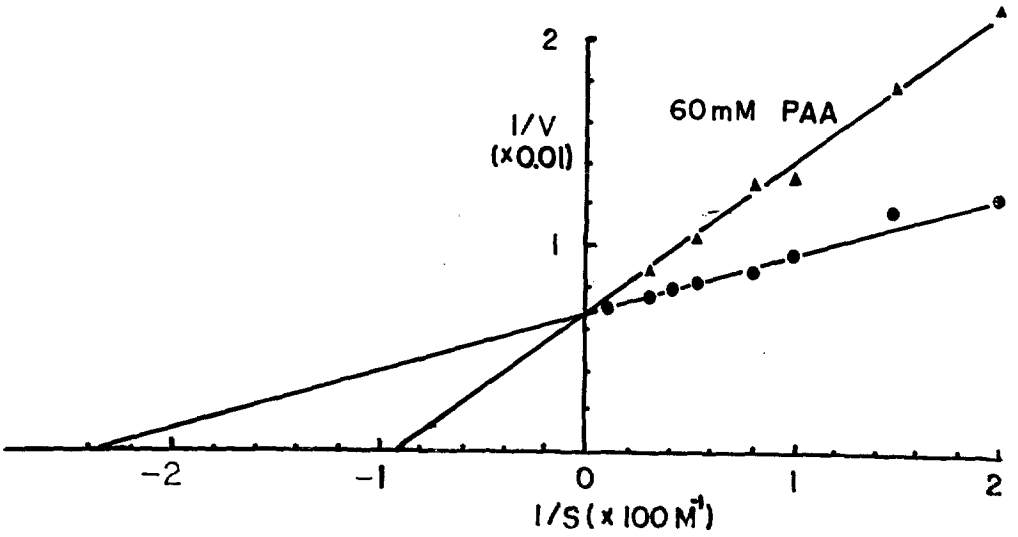

Figure 7. Determination of K_{ia} value of soluble enzyme.

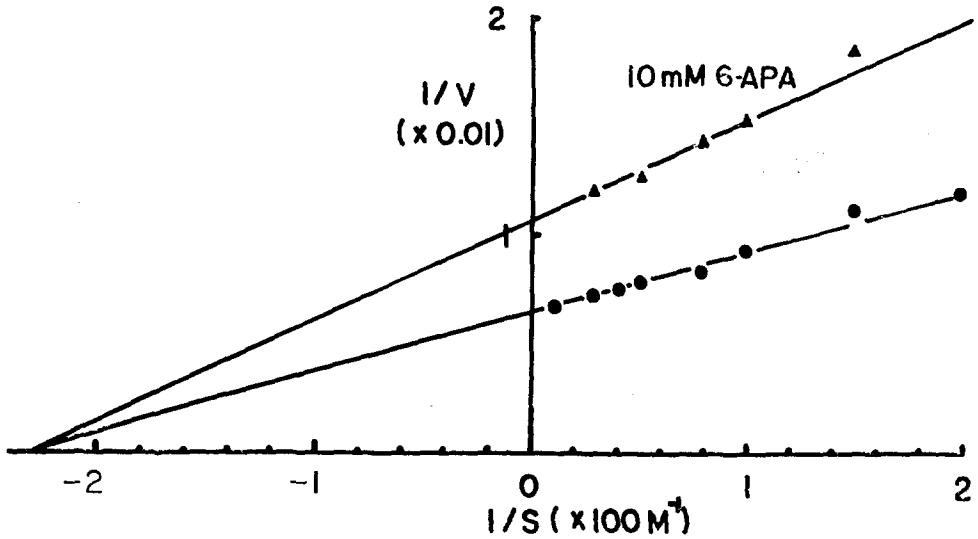

Figure 8. Determination of K_{ip} value of soluble enzyme.

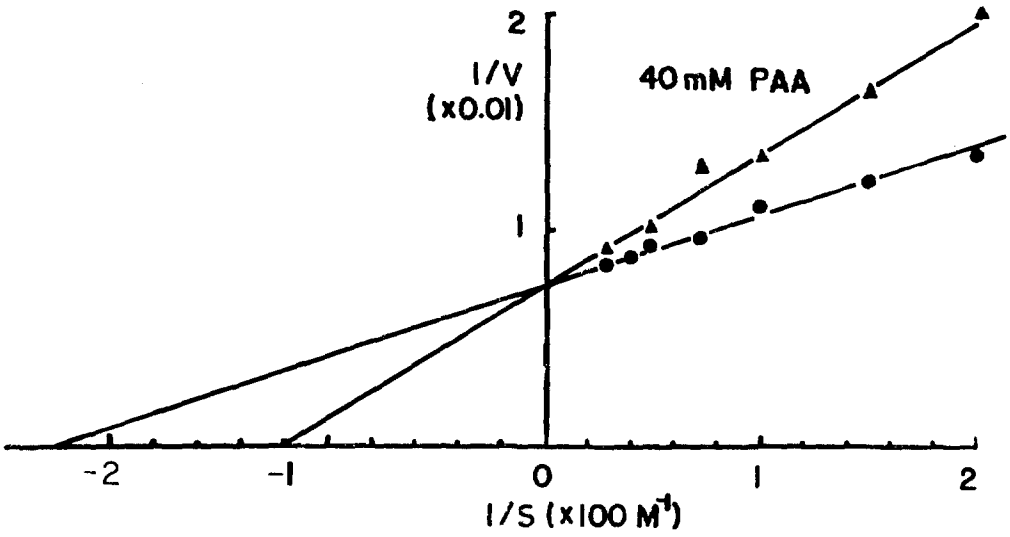

Figure 9. Determination of K_{ia} value of immobilized enzyme.

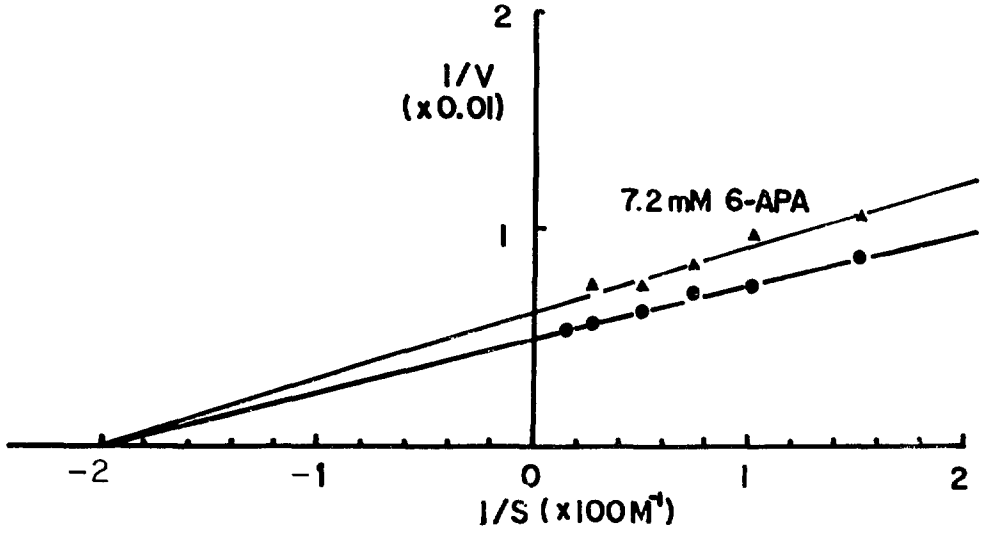

Figure 10. Determination of K_{ip} value of immobilized enzyme.

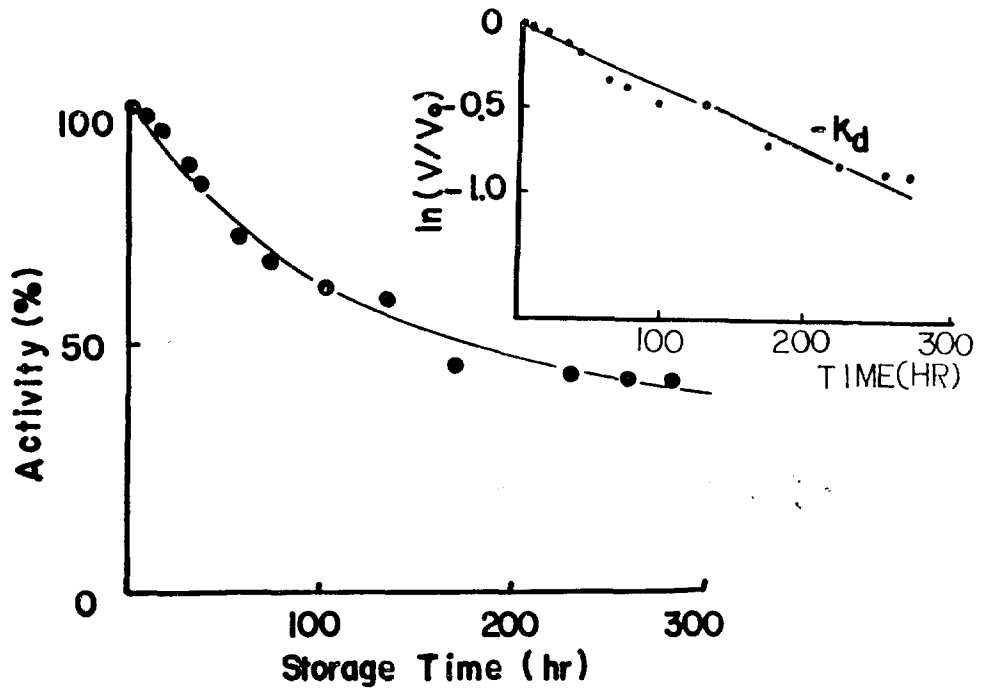

Figure 11. Stroage stability of immobilized enzyme and determination of deactivation constant (K_d). The first order deactivation of enzyme activity (the Figure 11-a). A stock of immobilized enzyme was stored at 40°C in 0.1 *M* borate buffer (pH 8.4).

$$V=-\frac{ds}{dt}=\frac{d}{dt}S_0(1-X)=\frac{dx}{dt}S_0=f(s) \qquad (2)$$

$$dx=\frac{1}{s_0}f(s)dt$$

$$X=\int dx=\frac{1}{S_0}\int f(s)dt$$

The batch reaction was simulated by equation (2) using MIMIC program. The hydrolysis of 10 m*M* and 20 m*M* of benzyl penicillin by soluble enzyme is shown in Figure 12, and the experimental results showed good agreement with the simulation results. The amount of enzyme used was 3.3×10^4 unit/*l*. About 90% conversion of 10 m*M* substrate was obtained in 1.5 hr, while about 84% conversion was attained for 20 m*M* solution.

The progress of batch hydrolysis by immobilized enzyme of varying enzyme loadings (9, 6, and 4×10^3 units/m*l*) in 13.5 m*M* of initial substrate solution was evaluated. The degree of conversion was determined at certain time intervals during 2.5 hr operation. The predicted values by simulation agreed well with the experimental results as shown in Figure 13. The variation of conversion efficiency as a function of the reactor operation time was also calculated for the experimental results as shown in Figure 14. It showed that

TABLE 3: Comparison of Kinetic Constants between Soluble and Immobilized Enzyme

	Soluble enzyme	Immobilized enzyme
K	4.3×10^{-3} *M*	4.8×10^{-3} *M*
K_{ia}	4.0×10^{-2} *M*	4.5×10^{-3} *M*
K_{ip}	2.2×10^{-2} *M*	5.0×10^{-2} *M*

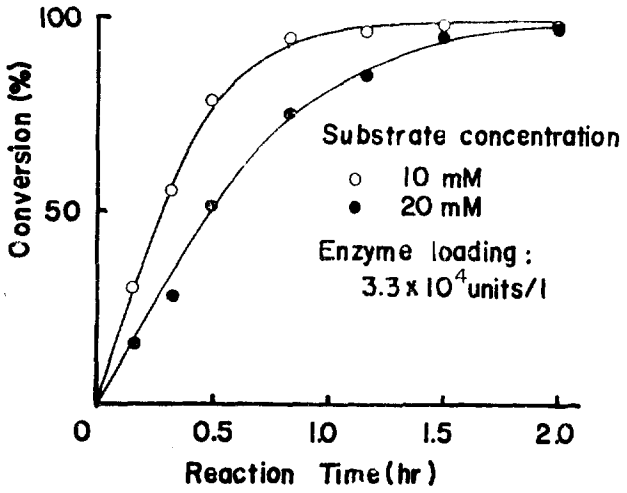

Figure 12. The batch reaction for the hydrolysis of benzyl penicillin by the soluble enzyme. o: Experimental result, —: simulation result.

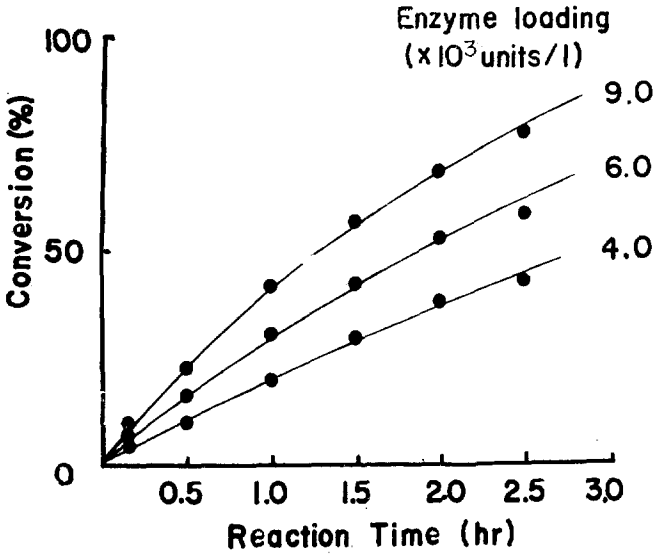

Figure 13. The batch reaction for the hydrolysis of benzyl penicillin by the immobilized enzyme. 13.5 m*M* of substrate solution was used. o: Experimental result, —: simulation result.

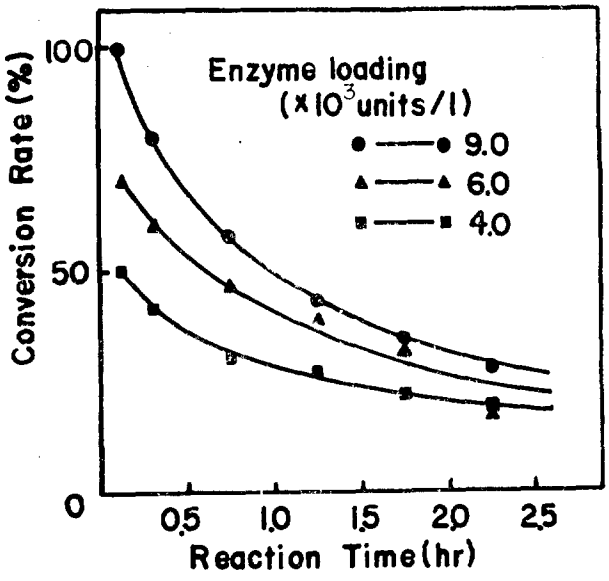

Figure 14. The variation of conversion efficiency as a function of the reactor operation time and enzyme loading.

the conversion efficiency of greater enzyme loading decreased more sharply than that of smaller enzyme loading.

The experimental results from batch reaction with immobilized and soluble enzymes corroborate the goodness of the kinetic model derived based upon the double inhibition by the both reaction products, 6-APA and PAA.

We have concluded that the penicillin amidohydrolase immobilized on nylon fibers has potential for commercial application to production of 6-aminopenicillanic acid because of good stability of nylon fiber as a support material, relative ease of covalent binding of the enzyme, and good handling properties of the nylon fiber in reactor operation.

Nomenclature

6-APA : 6-Aminopenicillaric acid
PAA : Phenylacetic acid
K_m : Michaelis-Menten constant
K_{ia} : Inhibition constant of PAA
K_{ip} : Inhibition constant of 6-APA
S_0 : Initial substrate concentration
S : Substrate concentration at time t
X : Fractional conversion
E : Enzyme loading
k : Reaction rate constant
K_d : Activity decay constant
$t_{1/2}$: Half life of enzyme activity
V : Rate of enzyme reaction

Results and Discussion

(1) D. Y. Ryu, C. F. Bruno, B. K. Lee and K. Venkatasubramanian, *Proc. IV IFS: Ferment Technol. Today*, 307–314 (1972).
(2) D. A. Self, G. Kay and M. D. Lilly, *Biotechnol. Bioeng.*, **11**, 337∼348 (1969).
(3) W. Marconi, F. Cecere, F. Morisi, G. Della Penna and B. Rappuoli, *J. Antibiotics*, **26**, 228–232 (1973).
(4) W. E. Hornby, J. Campbell, D. L. Inman and D. L. Morris, "Enzyme Engineering" 2, 401, Plenum, New York(1974).
(5) L. Goldstein, A. Freeman and M. Sokolovesky, *Biochem. J.*, **143**, 497 (1974).
(6) W. E. Hornby and H. Filippusson, *Biochem. Biophys. Acta*, **220**, 343 (1970).
(7) P. V. Sundaram and W. E. Hornby, *FEBS Lett.*, **10**, 325 (1970).
(8) K. H. Kim and D. Y. Ryu, *M. S. Thesis*, KAIS (1977).
(9) C. Chiang, and R. E. Bennett, *J. Bacteriol.*, **93**, 302–308 (1967).
(10) F. R. Batchelor, E. B. Chain, T. L. Hardy, K. R. L. Mansford and G. N. Rolinson, *Proc. Roy. Soc. Ser. B.*, **154**, 498 (1961)
(11) I. Cho and J. B. Kim, *Polymer (Korea)*, **1**, No. 2, (1977).
(12) D. J. Inman and W. E. Hornby, *Biochem. J.*, **129**, 255–262 (1972).
(13) Ralf Axen and Sverker Ernback, *Eur. J. Biochem.*, **18**, 351∼360 (1971).
(14) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265∼275 (1951).

(15) J. M. T, Hamilton-Miller, *Bacteriol. Rev.*, **30**, 760 771 (1966).
(16) Ralf A. Messing, "Immobilized Enzymes for Industrial Reactors", Academic Press, N.Y., 1975.
(17) K. Balashingham, D. Warburton, P. Warburton, P. Dunnil and M. D. Lilly, *Biochem. Biophys, Acta*, **276**, 250–256 (1972).
(18) D. Warburton, P. Dunnil and M. D. Lilly, *Biotechnol. Bioeng*, **15**, 13–25, (1972).
(19) B. L. Seong, *M. S. Thesis*, KAIS (1979).
(20) S. Murao, K. Sakaguchi and K. Knon, *U. S. Pat.* 3,144,395 (1964).

The Importance of One-Electron Effects in Conformationand Protonation of Acetamids*

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The CNDO/2 method was used to compute relative stabilities of various configurations and conformations of acetamide, N-methylacetamide and diacetamide and their protonated forms. It was found that: (a) nonbonded interactions play important roles in determining structural preferences of the compouds, (b) n-σ^* "throughbond" interactions always favor *cis*-protonation (relative to C-N).

In recent years, theoretical[1] as well as experimental[2] investigations have been conducted to determine conformational preferences of acetamides. Although some molecular orbital studies have been reported, the factors contributing to the conformational preferences have never been adequately clarified.

In the Hartree–Fock self-consistent field(SCF) framework, the total energy changed, ΔE_T, associated with various conformational variations can be decomposed into separate contributions, $\Delta\epsilon_i$, ΔV_{ee} and ΔV_{nn} in the form

$$\Delta E_T = \Delta(2\sum\epsilon_i) - \Delta V_{ee} + \Delta V_{nn}$$

where an orbital energy ϵ_i is an eigenvalue of the effective one electron operator, ΔV_{ee} is the sum of electron-electron repulsion energies and ΔV_{nn} is the sum of internuclear repulsion energies. Two important factors which control stereochemical preferences in molecules can thus be distinguished; (a) a one-electron factor, $\Delta(2\sum\epsilon_i)$, which is responsible for "through-bond" and "through-space" interactions[4]; (b) steric factors, ΔV_{ee} and ΔV_{ee}, which are responsible for sterically repulsive interactions.

One of us recently introduced[5] simple rules for predicting "through-space" nonbonded effects qualitatively based on the frontier orbital (FMO) concept[6]: (a) nonbonded interaction is significant only in a conjugated (or *iso*-conjugate) chain with two end atoms in close proximity: (b) the mode and extent of nonbonded interactions are dependent upon number of electrons in the conjugative chain and on the distance between the two end atoms. It was found convenient to adopt a notation of $(n\pi/m)$ representing $n\pi$ electrons delocalized over m atoms (or centers) of a chain forming a crowded structure, only systems with $n \geq 4$ and $m \geq 3$ being worth considering. It was shown that: (a) nonbonded interaction is attractive in decreasing order of 4N+2, 4N+1, and 4N-1 electron systems, while it is repulsive in a 4N electron system: (b) the closer the two ends approach, the stronger the nonbonded interaction becomes.

We report here results of MO studies on the rotational isomerism in acetamide, N-methylacetamide and diacetamide employing the CNDO/2 method. We have also carried out computations to investigate their protonation behaviors. We have stressed the importance of one-electron effects[4] in determining the conformational preferences applying our rules wherever possible.

Calculations

All calculations were carried out using the CNDO/2 method. For acetamide and N-methylacetamide experimental values of bond lengths and angles were used, while for diacetamide the CNDO/2 optimized geometry of Capparelli[1b] was used.

O-protonation only was considered[8], with a proton at 0.99A from O with 120° angle in the plane of NCO frame. Nitrogen was assumed to take planar form of sp^2 hybrids.

Result and Discussion

(I) Conformation

Acetamide. Two forms are possible depending on the arrangement of methyl group relative to C-N bond; the *E* form has a system, ($H_{ab}C_1C_2O$), π-isoconjugate to *cis* butadiene, a $(4\pi/4)$ system, which is both sterically and end-to-end repulsive,[11] while the *S* form contains a $(5\pi/4)$ system, ($H_{ab}C_1C_2N$), which should have "through-space"

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