The sequence of subtilisin has been reported recently.[22] Its histidine and active serine sequences are completely different from those of the pancreatic enzymes. The present evidence indicates therefore that bacterial serine proteases have evolved along at least two independent pathways.

Acknowledgments. We thank Miss C. Nayler and Mr. E. Paradowski for expert technical assistance. This work has been supported by the Medical Research Council of Canada, The Life Insurance Medical Research Fund, and the National Institutes of Health, U. S. Public Health Service (Grant AM-06287).

(22) E. L. Smith, F. S. Markland, C. B. Kasper, R. J. De Lange, M. Landon, and W. H. Evans, *J. Biol. Chem.*, 241, 5974 (1966).

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Received March 4, 1967

Kinetic Properties of the α-Lytic Protease of *Sorangium sp.*

Sir:

An accompanying communication[1] gives evidence that the amino acid sequence around the only histidine residue of a bacterial protease is homologous with the sequence around histidine-57 of chymotrypsin. This evidence, coupled with previous evidence[2] of homology

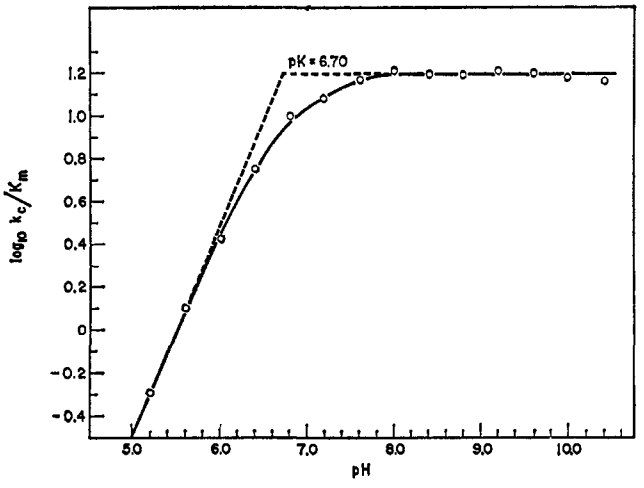

Figure 1. Hydrolysis of N-acetyl-L-valine methyl ester in 0.10 *M* KCl at 25.0°.

around the active serine residue, raises the question as to whether the reaction mechanism of the *Sorangium* enzyme differs in any way from that of chymotrypsin for, if it does not, reaction mechanisms which require chymotrypsin to have two catalytically functional histidines are clearly in need of reappraisal.

The kinetic data reported in this communication are based on measurements of esterase activity in a pH-Stat. The enzyme was prepared by a simplified version[3] of the original procedure. Enzyme concentrations were determined from amino acid analyses and the known amino acid composition per mole of enzyme.[4]

Table I gives a comparison of esterase activities toward various N-benzoyl- and N-acetylamino acid esters under reaction conditions which gave initial rates in direct proportion to both the enzyme and the substrate concentration. As the over-all kinetics are Michaelis–Menten kinetics, the second-order velocity constants are designated as k_c/K_m ratios where k_c is the catalytic rate constant (k_{cat}).[5] It is evident from the range of substrate concentration which gave second-order kinetics that all values of K_m are greater than 10 m*M*. An earlier comparison[6] of the enzyme's action pattern on the A and B chains of oxidized insulin had indicated a pancreatic elastase-like specificity for linkages involving the carbonyl groups of neutral, aliphatic amino acids; the specificity shown by Table I is in accordance with these findings.

Table I. Esterase Activities of the α-Protease at pH 8.0 in 0.10 *M* KCl at 25.0°[a]

Substrate	$[S]_0$, m*M*	k_c/K_m, M^{-1} sec^{-1}
Bz-Arg-OEt	11	0.00
Ac-Tyr-OEt	6.3	0.00
Ac-Trp-OMe	2.3	0.00
Bz-Gly-OMe	2.6–10.5	8.07
Ac-Val-OMe	0.60–6.0	16.3
Ac-Ala-OMe	1.0–3.0	26.3
Bz-Ala-OMe	1.02–10.2	723
Bz-D-Ala-OMe	11.0	0.00

[a] The enzyme concentration was 8.0×10^{-8} *M* for Bz-Ala-OMe and 5.0×10^{-6} *M* for the other esters.

Figure 1 shows the pH dependence of the hydrolysis of N-acetyl-L-valine methyl ester. The values of k_c and K_m for this substrate (Table II) were estimated from Eadie plots at higher substrate concentrations. When

Table II. K_m and k_c for Hydrolysis of N-Acetyl-L-valine Methyl Ester in 0.10 *M* KCl at 25.0°

pH	K_m, m*M*	k_c, sec^{-1}
6.30	61 ± 8	0.28 ± 0.01
7.00	72 ± 6	0.94 ± 0.04
8.00	65 ± 4	1.1 ± 0.1

water is replaced by D_2O, the p*K* shifts from 6.7 (Figure 1) to 7.35, and k_c/K_m at the plateau is reduced 2.03-fold. The pH dependence of the hydrolysis of N-benzoyl-L-alanine methyl ester is essentially the same as that shown in Figure 1 (p*K* = 6.55) although, as indicated in Table I, the value of k_c/K_m at the plateau is much higher. Activity toward this substrate over the same pH range is unaffected by acetylation of the enzyme with acetic anhydride at pH 6.8 and 0°. The N-terminal alanine residue and the two ε-amino groups of the enzyme no longer react with 1-fluoro-2,4-dinitrobenzene or with cyanate[7] after this treatment; the electrophoretic mobility of the enzyme at pH 5.0 is reduced by about one-third.

(1) L. B. Smillie and D. R. Whitaker, *J. Am. Chem. Soc.*, 89, 3350 (1967).
(2) D. R. Whitaker, L. Jurášek, and C. Roy, *Biochem. Biophys. Res. Commun.*, 24, 173 (1966); D. R. Whitaker and C. Roy, *Can. J. Biochem.*, 45, 911 (1967).
(3) D. R. Whitaker, *ibid.*, in press.
(4) L. Jurášek and D. R. Whitaker, *ibid.*, 45, 991 (1967).
(5) B. Zerner and M. L. Bender, *J. Am. Chem. Soc.*, 86, 3669 (1964).
(6) D. R. Whitaker, C. Roy, C. S. Tsai, and L. Jurášek, *Can. J. Biochem.*, 43, 1961 (1965).
(7) G. R. Stark and D. G. Smyth, *J. Biol. Chem.*, 238, 214 (1963).

The pH dependence of k_c/K_m below pH 8 stems from the dependence of k_c, not of K_m, on pH (Table II). This pH dependence of k_c, the pK value of 6.7, its shift to 7.35 by D_2O, and the one-unit slope of the log k_c/K_m function all match the data for chymotrypsin and are consistent with a requirement for a single, unprotonated imidazole group.[8] The isotope effect of 2.03 with D_2O is of the same magnitude as for chymotrypsin and is consistent with general basic catalysis by an imidazole group.[9] The constancy of k_c/K_m from pH 8 to 10.5 and the activity of the acetylated enzyme indicate that the binding of neutral substrates is completely independent of pH and is not influenced by ionizations of α- or ϵ-amino groups of the enzyme. This represents the only contrast with chymotrypsin, as the latter undergoes a change in conformation at alkaline pH values and loses its ability to bind substrates.[10]

The above comparison thus supports the indications of the direct kinetic evidence for chymotrypsin, *i.e.*, that its reaction mechanism involves only one histidine group.

(8) M. L. Bender, G. E. Clement, F. J. Kezdy, and H. d'A. Heck, *J. Am. Chem. Soc.*, **86**, 3680 (1964); F. J. Kézdy, G. E. Clement, and M. L. Bender, *ibid.*, **86**, 3690 (1964).

(9) M. L. Bender, E. J. Pollock, and M. C. Neveu, *ibid.*, **84**, 595 (1962).

(10) H. L. Oppenheimer, B. Labouesse, and G. P. Hess. *J. Biol. Chem.*, 241, 2720 (1966); M. L. Bender, M. J. Gibian, and D. J. Whelan, *Proc. Natl. Acad. Sci. U. S.*, **56**, 833 (1966).

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Octahedral *vs.* Trigonal-Prismatic Coordination. The Structure of $(Me_4N)_2[V(mnt)_3]$[1]

Sir:

It has recently been shown that a large number of uncharged and monoanionic tris complexes of bidentate sulfur donor ligands possess trigonal-prismatic coordination and thus constitute the first examples of nonoctahedral six-coordinate complexes.[2-6] However, prior to this work there has been no unequivocal evidence relating to the structures of the more highly reduced species having charges 2− and 3−. It has in fact been suggested[3] that some of these complexes could be closer to the classical octahedral configuration. In this communication, we report the molecular structure of $V(mnt)_3^{2-}$, which exhibits the first nontrigonal-prismatic coordination geometry for this class of compounds.

Black monoclinic crystals of $(Me_4N)_2[V(mnt)_3]$[7-9] were grown from acetone–2-propanol solutions. Precession photography revealed systematic extinctions indicating the space group C2/c or C_c, with a cell of dimensions a = 20.53 A, b = 10.19 A, c = 16.98 A, β = 124° 15′ and vol. = 2935 A^3. The measured density of 1.37 ± 0.05 g cm^{-3} is consistent with four anions and eight cations in the unit cell of calculated density 1.40 g cm^{-3}. Assuming the space group C2/c, the four vanadium atoms are required to occupy the 4e special positions of the space group and thus the anion is required to have a twofold symmetry axis. This assumption is verified by the satisfactory agreement ultimately obtained.

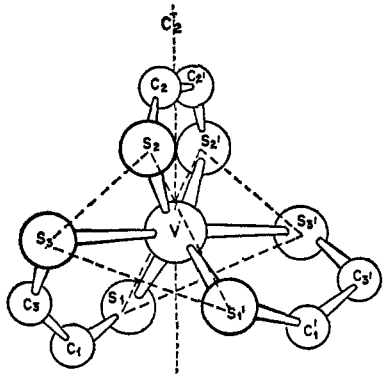

Figure 1. Perspective drawing of the molecular structure of the $V(mnt)_3^{2-}$ anion. The cyano groups are omitted.

The intensity data were collected by the multiple film equiinclination Weissenberg technique using Cu Kα radiation. Intensities were estimated visually and reduced to values of F_o by standard methods. The structure was solved by standard Patterson, least-squares, and Fourier methods. The R factor for 88 positional and thermal parameters (allowing the vanadium and three independent sulfurs to have anisotropic temperature factors) is currently 0.125 for 1187 independent nonzero reflections.

A perspective drawing of the coordination geometry is shown in Figure 1; some of the important bond distances are given in Table I. The vanadium lies on a twofold rotation axis which bisects one of the three ligands. The six sulfur donor atoms are located around the vanadium at an average distance of 2.36 ± 0.01 A. The polyhedron described by these sulfur atoms is by no means a regular one, but for some purposes it is usefully described as a very distorted octahedron (*vide supra*). The intraligand S–S dis-

Table I. Important Bond Lengths in the $V(mnt)_3^{2-}$ Anion[a]

Bond	Bond length, A	Bond	Bond length, A
V–S_1	2.36 ± 0.01	C_1–C_3	1.37 ± 0.02
V–S_2	2.35 ± 0.01	C_2–$C_{2'}$	1.29 ± 0.03
V–S_3	2.36 ± 0.01	S_2–$S_{2'}$	3.17 ± 0.01
S_1–C_1	1.72 ± 0.02	S_1–S_3	3.11 ± 0.01
S_2–C_2	1.74 ± 0.02	$S_{1'}$–S_2	3.43 ± 0.01
S_3–C_3	1.69 ± 0.02	S_2–S_3	2.98 ± 0.01
		$S_{1'}$–S_3	3.18 ± 0.01

[a] The prime denotes an atom related by the twofold axis.

(1) Acknowledgment is made to the National Science Foundation for support of this research. We thank Professor R. Eisenberg of Brown University for several helpful discussions.

(2) E. I. Stiefel, R. Eisenberg, R. C. Rosenberg, and H. B. Gray, *J. Am. Chem. Soc.*, **88**, 2956 (1966).

(3) R. Eisenberg, E. I. Stiefel, R. C. Rosenberg, and H. B. Gray, *ibid.*, **88**, 2874 (1966).

(4) R. Eisenberg and J. A. Ibers, *ibid.*, **87**, 3776 (1965); *Inorg. Chem.*, **5**, 411 (1966).

(5) A. E. Smith, G. N. Schrauzer, V. P. Mayweg, and W. Heinrich, *J. Am. Chem. Soc.*, **87**, 5798 (1965).

(6) R. Eisenberg and H. B. Gray, to be published.

(7) The anion was first reported as the Ph_4As^+ salt by Davison and co-workers[8] and later isolated using a somewhat different procedure as the $Ph_3P(Me)^+$ salt.[9] The Me_4N^+, Et_4N^+, and Bu_4N^+ salts described in this study were prepared by a procedure only slightly modified from that given in ref 8.

(8) A. Davison, N. Edelstein, R. H. Holm, and A. H. Maki, *J. Am. Chem. Soc.*, **86**, 2799 (1964).

(9) N. M. Atherton, J. Locke, and J. A. McCleverty, *Chem. Ind.* (London), **29**, 1300 (1965).