and previously sited literature.[5,11-14,19]

Discussion

A general model was developed in this paper to account for the proton inventories of associative reactions of serine proteases. The proton inventories determined in this work were of k_E for the reactions of αPI with PPE, MeOSuc-Ala-Ala-Pro-Val-pNA with HLE, and Suc-Ala-Ala-Ala-pNA with PPE and can be described by similar mechanisms. For reactions of peptide *p*-nitroanilides with proteases, it appears that as the reactants approach each other in solution, extensive solvent reorganization must occur to allow initial contacts to be established. At least for the reactions reported herein, this process generates inverse solvent isotope effects, suggesting an overall strengthening of hydrogen bonds and ordering of solvent structure. This view is supported by the large negative entropy of activation of −36 eu observed for the association of MeOSuc-Ala-Ala-Pro-Val-pNA with HLE.[20]

These initial reaction steps ultimately lead to the formation of an encounter complex of enzyme and substrate. Reaction from this intermediate proceeds to the acyl-enzyme through a virtual transition state composed of transition states for the chemical steps of acylation and the transition state for a physical step preceding the acyl-enzyme.[18] Although the identity of the physical step and its transition state are unknown, likely candidates include substrate binding, conformation changes of enzyme-bound intermediates, and release of the first product. The acylation transition state corresponds to either formation or decomposition of the tetrahedral addition adduct.

For the association of αPI with PPE, the transition state for the physical step not only makes a major contribution to determining the structure of the virtual transition state but also is accompanied by a large solvent isotope effect. That this step might correspond to a conformation change is supported by a recent X-ray crystallographic study[21] of αPI in which a major structural rearrangement was observed upon bond cleavage of inhibitor at its reactive center. The large inverse isotope effect on this step would then correspond to tightening of many hydrogen bonds within the structure of the enzyme–inhibitor complex as the transition state for the conformational change is reached.

The other major contribution to the virtual transition state for association comes from acylation. This suggests that the stable complex, E:I, following the virtual transition state of k_1 is either a tetrahedral intermediate or the acyl-enzyme itself.

More generally, the results of this paper alert us to the importance of solvent reorganization in associative processes and suggest that dome-shaped proton inventories of k_E will frequently have their origins in the solvent reorganization that accompanies binding processes. Finally, these results provide an alternative view to the complex interpretations of previously reported proton inventories.[22-25]

Acknowledgment. I gratefully acknowledge Prof. Michael S. Matta (Southern Illinois University) for having first suggested to me the importance of solvent reorganization in interpretation of solvent isotope effects and proton inventories. I also thank Barbara R. Viscarello (Yale University) for preparing α-1-protease inhibitor.

Registry No. αPI, 9041-92-3; PPE, 9004-06-2; Suc-$(Ala)_3$-pNA, 52299-14-6; MeOSuc-Ala-Ala-Pro-Val-pNA, 70967-90-7; deuterium, 7782-39-0.

(20) This is an unpublished result of the author and represents the average of two entropies of activation, −27 and −45 eu, obtained from a biphasic Arrhenius plot. These values are consistent with an associative process involving extensive reordering of solvent structure.

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Binding of Calcium to Amino Acids: The Crystal Structure of Pentaaquobis(hydroxy-L-prolinato)calcium, $Ca(C_5H_8O_3N)_2 \cdot 5H_2O$

Eunice E. Kim, Andrew Sicignano, and Klaas Eriks*

Contribution from the Department of Chemistry, Boston University, Boston, Massachusetts 02215. *Received May 24, 1984*

Abstract: Crystals of pentaaquobis(hydroxy-L-prolinato)calcium were obtained from aqueous solution at pH 11.0. They are monoclinic, space group $P2_1$, with a = 6.213 (1) Å, b = 14.905 (4) Å, c = 9.483 (3) Å, β = 96.41 (2)°, Z = 2, D(calcd) = 1.486 g cm^{-3}, and D(measd) = 1.49 g cm^{-3}. The structure was determined by using least-squares and difference Fourier calculations. The final values of R and R_w were 0.035 and 0.042, respectively, for 1222 reflections with $I_o > 3\sigma(I_o)$. Positional and thermal parameters were refined for the non-hydrogen atoms. Hydrogen atoms were included but not refined. The calcium atom is seven-coordinated by a carboxyl oxygen atom and a nitrogen atom from each of the two hydroxy-L-proline molecules and three water molecules. The hydroxy-L-proline molecules act as bidentate ligands. The coordination geometry around calcium is that of a distorted pentagonal bipyramid. Ca–O distances range from 2.330 to 2.452 Å; Ca–N distances are 2.595 and 2.613 Å. The conformation of the two crystallographically independent hydroxy-L-proline molecules is almost identical with that in the free amino acid. The structure consists of single, monomeric units of composition $Ca(Hyp)_2 \cdot 3H_2O$ that are tied together by hydrogen bonding involving the two additional water molecules. The extensive hydrogen-bonding network is discussed in detail, and structural comparisons are made with 11 other calcium amino acid complexes. It appears that binding of calcium to nitrogen will only occur in crystals grown from solutions of pH >10.

The structure determination described below was motivated by our interest in the manner in which calcium is capable of binding to amino acids and organic bases. Knowledge of the structure of small calcium amino acid complexes has a direct bearing on the understanding of the calcium-binding ability of proteins such as calmodulin and possibly of the mechanism of calcification in living tissue. The hydroxy-L-proline complex of calcium was found to be comparatively easy to obtain in single-

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Table I. Summary of Crystal Data

crystal size, mm^3	0.33 × 0.24 × 0.11
a, Å	6.213 (1)
b, Å	14.905 (4)
c, Å	9.483 (3)
β, deg	96.41 (2)
V, $Å^3$	872.7 (4)
Z, formula units/cell	2
F_w, amu	390.40
D(calcd), g/cm^3	1.486
D(measd), g/cm^3 (at 23 ± 1 °C)	1.49
absorpt coeff, cm^{-1}	35.5
T of data collection, °C	20 ± 1
wavelength of Cu radiat, Å	K_α = 1.541 78
scan range, deg	2.0–2.1
scan rate, deg min^{-1}	2.02–29.3
tot bkdg/scan time	0.5
max 2θ, deg	114.7
no. of reflect measd, excl. checks	2625
no. of unique reflect	1238
no. of unique reflect with $I > 3\sigma(I)$	1222
check reflect, % decay	$(0\bar{8}\bar{1})$, $(30\bar{2})$; none
diffractometer used	Nicolet $P2_1$

crystal form. It is of particular interest because collagen, the substrate for calcification in tissues, has a high content of hydroxy-L-proline. Urry[1] suggested that the initial step for the deposition of calcium phosphate mineral on collagen is the binding of a calcium ion.

Experimental Section

(a) Preparation. Initial experiments involved adding excess $Ca(OH)_2$ to a 1 M solution of 4-hydroxy-L-proline, stirring for 3 days, filtering, and placing the filtrate in a closed container over a 50:50 by volume mixture of ethanol and ether. Clear, colorless, prismatic crystals formed after a few days of vapor diffusion, but these crystals were air sensitive and very unstable when exposed to X-rays.

A stable modification was obtained accidentally in experiments that were intended to prepare a mixed amino acid complex containing both hydroxy-L-proline and L-serine. A solution that was 1 M in both amino acids was treated with excess $Ca(OH)_2$ as above and yielded well-formed clear crystals in the vapor diffusion process. The pH of the solution was 11.0. Atomic absorption showed them to contain calcium, and thin-layer chromatography (solvent system of 8:2:2 by volume *n*-butanol, acetic acid, and water,[2] silica gel G plate) displayed only a spot for hydroxyproline (orange) after staining with ninhydrin. The crystal density, measured by flotation in mixtures of benzene and carbon tetrachloride, was 1.49 g cm^{-3}.

(b) Data Collection. A fragment (0.33 × 0.24 × 0.11 mm^3) of a larger $Ca(C_5H_8NO_3)_2 \cdot 5H_2O$ crystal was mounted at the end of glass fiber with epoxy cement. The crystal was centered on a Nicolet $P2_1$ diffractometer equipped with a copper tube (λ = 1.54178 Å). Unit cell parameters, calculated from 15 centered reflections with 2θ values between 35° and 60°, are given in Table I, together with information on data collection. Systematic extinctions occurred from $0k0$ with $k = 2n + 1$ which uniquely determines the space group as $P2_1$ (space group no. 4 in Vol. I of International Tables for X-ray Crystallography).[3]

A total of 2739 reflections was measured including check reflections. The reflections were collected in two sets: the first set for $6° < 2\theta < 100°$, the second for $100° < 2\theta < 115°$. All reflections were corrected for Lorentz and polarization factors. No absorption corrections were applied because of the irregular shape of the crystal fragment.

(c) Structure Determination and Refinement. The positions of calcium and its surrounding oxygen atoms were determined from a Patterson map. From least-squares and difference Fourier calculations, all other non-hydrogen atoms could be located. In the initial calculations, all atoms were treated isotropically. Subsequent calculations with anisotropic thermal parameters led to $R = 0.050$, where $R = \sum||F_o| - |F_c||/\sum|F_o|$. At this point, all hydrogen atoms could be located. In the final cycles of refinement, hydrogen atoms were included in fixed positions obtained from a difference Fourier and with fixed $U = 0.065$. Convergence was reached at values of $R = 0.033$ and $R_w = 0.042$, where $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$. A total of 1222 reflections with $|I_o| \geq 3\sigma(I_o)$ contributed to the calculations and 216 parameters were refined. Program X-ray 76[4] was used for all calculations. Scattering factors for all non-hydrogen atoms were taken from Cromer and Mann[5] and for hydrogen from Stewart et al.[6] Final positional parameters for non-hydrogen atoms are listed in Table II. Other information is available as

Table II. Positional Parameters of Non-Hydrogen Atoms

atom	X	Y	Z
Ca	0.0434 (1)	0.250 00	0.2427 (1)
O1A	0.3653 (5)	0.181 2 (2)	0.1846 (3)
O2A	0.5410 (5)	0.056 0 (2)	0.1451 (3)
O3A	−0.1340 (5)	0.038 9 (2)	−0.2340 (3)
NA	−0.0088 (5)	0.132 1 (2)	0.0378 (3)
C1A	0.3730 (6)	0.104 9 (2)	0.1298 (4)
C2A	0.1743 (7)	0.069 7 (3)	0.0383 (4)
C3A	0.2277 (8)	0.061 2 (4)	−0.1190 (5)
C4A	0.0342 (8)	0.104 6 (3)	−0.2042 (5)
C5A	−0.0330 (9)	0.175 3 (3)	−0.1035 (4)
O1B	0.2298 (5)	0.233 9 (2)	0.4684 (3)
O2B	0.3698 (5)	0.276 8 (2)	0.6795 (3)
O3B	−0.1821 (5)	0.528 3 (2)	0.5790 (4)
NB	−0.0234 (5)	0.380 4 (2)	0.4139 (3)
C1B	0.2497 (7)	0.289 4 (3)	0.5656 (4)
C2B	0.1220 (7)	0.376 7 (3)	0.5483 (4)
C3B	−0.0203 (9)	0.392 7 (4)	0.6679 (5)
C4B	−0.2266 (7)	0.435 0 (3)	0.5941 (4)
C5B	−0.2497 (7)	0.387 5 (3)	0.4526 (5)
OW1	0.1667 (6)	0.372 2 (2)	0.1122 (3)
OW2	−0.1688 (5)	0.134 2 (2)	0.3483 (3)
OW3	−0.3031 (5)	0.292 5 (2)	0.1411 (4)
OW4	0.4346 (5)	0.611 5 (2)	0.4279 (3)
OW5	0.4624 (5)	0.377 1 (2)	−0.0835 (3)

Table III. Interatomic Distances (Å) and Angles (deg)

Ca Coordination			
Ca–O1A	2.367 (3)	OW1–Ca–OW2	166.2 (1)
Ca–NA	2.613 (3)	OW1–Ca–O1A	83.1 (1)
Ca–O1B	2.330 (3)	OW1–Ca–O1B	113.7 (1)
Ca–NB	2.595 (3)	OW1–Ca–OW3	85.1 (1)
Ca–OW1	2.376 (3)	OW2–Ca–O1A	107.3 (1)
Ca–OW2	2.452 (3)	OW2–Ca–O1B	77.7 (1)
Ca–OW3	2.345 (3)	OW2–Ca–OW3	81.2 (1)

bonds or ang	this study		free hydroxy-L-proline	
	molec A	molec B	X-ray[8]	neutron[9]
Hydroxy-L-proline				
C_1–O_1	1.254 (5)	1.233 (5)	1.25	1.244
C_1–O_2	1.268 (5)	1.256 (5)	1.27	1.242
C_1–C_2	1.520 (5)	1.524 (6)	1.52	1.530
C_2–C_3	1.570 (6)	1.532 (7)	1.53	1.526
C_3–C_4	1.516 (6)	1.526 (7)	1.50	1.520
C_4–C_5	1.512 (6)	1.509 (6)	1.52	1.523
C_4–O_3	1.437 (5)	1.428 (5)	1.46	1.403
C_5–N	1.480 (5)	1.496 (6)	1.48	1.487
N–C_2	1.469 (5)	1.479 (5)	1.50	1.498
O_1–C_1–O_2	122.9 (3)	122.9 (4)	126	125.6
O_1–C_1–C_2	118.9 (3)	119.0 (3)	119	116.6
O_2–C_1–C_2	118.2 (3)	118.1 (3)	115	117.8
C_1–C_2–C_3	109.0 (3)	113.0 (3)	113	111.5
C_1–C_2–N	111.3 (3)	112.7 (3)	111	110.8
C_3–C_2–N	107.1 (3)	106.5 (3)	105	105.0
C_2–C_3–C_4	103.5 (4)	104.4 (3)	108	106.1
C_3–C_4–C_5	102.7 (4)	101.9 (4)	104	103.0
C_3–C_4–O_3	109.3 (3)	106.6 (4)	106	109.5
C_5–C_4–O_3	110.7 (4)	111.6 (4)	109	111.7
C_4–C_5–N	105.1 (3)	104.6 (3)	105	
C_5–N–C_2	106.2 (3)	106.9 (3)	109	109.1

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Table IV. Hydrogen Bonding Distances (Å) and Angles (deg)

X-H···Y	X-H	X-H···Y	H···Y	ang X-H···Y	symm operat for Y
O3A-HO3A···OW4	1.03	2.697	1.73	154	$-x, y-{}^1/_2, -z$
O3B-HO3B···OW4[b]	1.03	2.917	2.60	97	$x-1, y, z$
NA-HNA···O2A[a]	1.00	3.283	2.32	163	$x-1, y, z$
NB-HNB···O3A	0.96	3.134	2.18	172	$-x, {}^1/_2+y, -z$
OW1-HW11···O3A	1.00	2.758	1.78	165	$-x, {}^1/_2+y, -z$
OW1-HW12···OW5	1.04	2.754	1.74	164	x, y, z
OW2-HW22···O2A	1.00	2.748	1.78	163	$x-1, y, z$
OW2-HW21···O3B	0.99	2.718	1.73	173	$-x, y-{}^1/_2, 1-z$
OW3-HW31···O1A	0.92	2.712	1.86	153	$x-1, y, z$
OW3-HW32···OW5	0.94	2.749	1.93	145	$x-1, y, z$
OW4-HW41···OW2	1.12	2.851	1.79	157	$-x, {}^1/_2+y, 1-z$
OW4-HW42···O1B[b]	0.92	2.861	2.64	95	$1-x, {}^1/_2+y, 1-z$
OW4-HW42···O2B	0.92	2.977	2.16	147	$1-x, {}^1/_2+y, 1-z$
OW5-HW51···O2B	1.06	2.706	1.66	170	$x, y, z-1$
OW5-HW52···O2A	1.01	2.730	1.73	172	$1-x, {}^1/_2+y, -z$

[a] Too long to be considered a hydrogen bond. [b] Poor geometry for hydrogen bond.

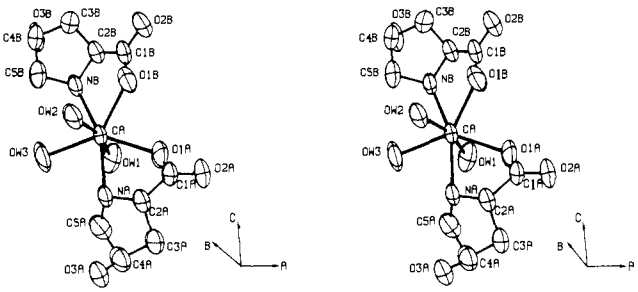

Figure 1. Coordination polyhedron of the calcium ion, showing single unit of $Ca(Hyp)_2 \cdot 3H_2O$.

supplementary material.[7] Pertinent interatomic distances and angles are listed in Table III, along with those for free hydroxy-L-proline.[8,9] Table IV contains data about hydrogen bonding in the structure.

Description. (1) Calcium Coordination. The calcium ion is seven-coordinated, by five oxygen and two nitrogen atoms (Figure 1). Each of the two crystallographically independent hydroxyproline molecules acts as a bidentate ligand, contributing one carboxyl oxygen atom and the nitrogen atom of the pyrrolidine ring to the coordination. The other three oxygen atoms are from water molecules 1, 2, and 3. Thus, the structure contains discrete units of composition $Ca(hydroxyproline)_2(H_2O)_3$. The average Ca-N distance is 2.604 Å. The geometry of the calcium coordination can best be described as a distorted pentagonal bipyramid, with OW1 and OW2 as the apexes. The deviations from the least-squares plane through calcium and the five equatorial atoms range from 0.05 Å (for OW3) to 0.69 Å (for O1B), and the calcium atom lies 0.06 Å from this plane. The angle OW1-Ca-OW2 is 166.2°. Alternatively, NA and NB could be taken as the apexes of a pentagonal bipyramid. In that case, the NA-Ca-NB angle is 162.5°, and the calcium ion lies at 0.02 Å from the least-squares plane through calcium and the equatorial atoms, but O1A and O1B deviate considerably from that plane, by −1.22 and +1.25 Å, respectively.

(2) Hydroxyproline Molecules. The agreement of distances and angles between the two independent hydroxyproline molecules A and B in the present study is remarkably good, as is that between the calcium-coordinated molecules and free hydroxyproline (Table III). The latter agreement is somewhat surprising since the residual (R) for the X-ray structure of free hydroxyproline[8] was 0.148 (for the neutron structure[9] the value was 0.065) and since the coordination to calcium in the present case could be expected to change the geometry of the amino acid. The free molecule has the zwitterion form, which is in fact quite similar to the bonding situation in the metal-coordinated molecule.

The pyrrolidine rings are puckered. The planes C3-C2-N-C5 and C3-C4-C5 make angles of 36.1° (molecule A) and 37.6° (molecule B). In the free amino acid, the values are 17° (X-ray structure[8]) and 33.5° (neutron structure[9]).

The bidentate coordination gives rise to five-membered chelate rings Ca-O1-C1-C2-N which are close to being planar and which should also contain the atom O2. The ring for molecule B is more planar (maximum deviation: atom N = 0.08 Å; deviation of O2 = 0.06 Å) than that for molecule A (maximum deviation: atom O1 = 0.19 Å; deviation of O2 = 0.39 Å). The dihedral angle between the two chelate rings is 39.4°.

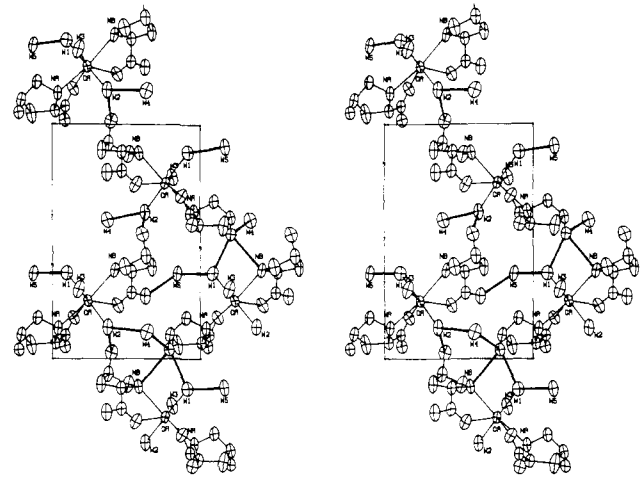

Figure 2. Structure viewed along the *a* axis. Hydrogen bonds are indicated by heavy lines.

(3) Hydrogen Bonding. The geometry of the hydrogen bonds in the crystal is given in Table IV and Figure 2. The arrangement that the structure adopts appears to be very reasonable:

(a) Fourteen hydrogen atoms are available for hydrogen bonding, and all but NA are involved in hydrogen bonds, if one can accept the rather poor geometry of the O3B···OW4 bond.

(b) The nitrogen atoms (already bonded to Ca, two carbon atoms, and their own hydrogen atom) cannot function as hydrogen bond acceptors, the atoms O1A and O1B can receive no more than one hydrogen bond, and the three oxygen atoms OW1-OW3 are unlikely acceptors because of their steric arrangement. Thus, the atoms O1A, O1B, O2A, O2B, O3A, O3B, OW4, and OW5 can, in principle, accept 14 hydrogen bonds. This is nearly what happens, but O3B accepts only one hydrogen bond rather than two, O1B accepts zero instead of one, and OW2 accepts one in spite of its coordination to calcium. The B hydroxyproline is appreciably less hydrogen bonded than the A molecule.

(c) The water molecules W1, W2, and W3 function primarily to complete the coordination sphere of the calcium ion. In addition, they use their hydrogen bonding capability to tie different corrdination complexes together, as can be seen from the symmetry operations in Table IV. Finally, these three water molecules are connected to water molecules W4 and W5, thus completing the framework that consists of 13 hydrogen bonds. It seems that OW4 might be connected, through HW42, to either O2B or O1B (Table IV). Even though the distance to O1B (2.861 Å) is shorter than that to O2B (2.997 Å), our preference is for a hydrogen bond to O2B, because of the poor geometry of the OW4-HW42···O1B bond.

Discussion

One remarkable feature of the structure is that it is composed of single, monomeric units of composition $Ca(C_5H_8NO_3)_2 \cdot 3H_2O$, tied together by two additional water molecules. Calcium amino acid complexes have a strong tendency to form dimers or chainlike

(7) See paragraph at end of paper regarding supplementary material.
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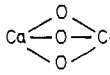
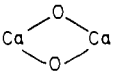
Table V. Geometry of Calcium in Complexes with Amino Acids (am. ac.)

compound	space gp	site symm of Ca	Ca surrounded by	type of linking of Ca polyhedra	Ca–Ca dist, Å	ref
			Octahedron (Coord 6)			
$Ca(Glu)_2 \cdot 4H_2O$	$P4_32_12$	2	4O (am. ac.); $2H_2O$	a	7.56	10
$Ca(Glu)Cl \cdot H_2O$	$P2_12_12_1$	1	5O (am. ac.); H_2O	c; dimer	3.84	11
$CaCl_2(GlyGly)_2$	$P\bar{1}$	$\bar{1}$	6O (am. ac.)	e; chain	4.49	12
$CaCl_2(sarcosine)_3$	*Pnma*	m	6O (am. ac.)	e; chain	4.61	13
			Pentagonal Bipyramid (Coord 7)			
$Ca(Hyp)_2 \cdot 5H_2O$	$P2_1$	1	2O (am. ac.); $3H_2O$; 2 N	a	6.21	this paper
$CaCl_2(GlyGlyGly)_3 \cdot 3H_2O$	$P\bar{1}$	1	5O (am. ac.); $2H_2O$	c; dimer	4.00	14
$CaBr_2(Gly)_3$	$Pbc2_1$	1	6O (am. ac.); Br	c; dimer	4.07	15
$CaI_2(Gly)_3 \cdot H_2O$	$P2_1/c$	1	5O (am. ac.); $2H_2O$	c; chain	3.90	16
$Ca(Tyr)(OH)^- \cdot 5H_2O$	$P2_1$	1	2O (am. ac.); OH^-; $3H_2O$; 1 N	e; chain	6.12	17
			Dodecahedron (Coord 8)			
$CaCl_2(Gly)_2 \cdot 4H_2O$	$P2_1/c$	1	5O (am. ac.); $3H_2O$	c; chain	4.07	18
			Square Antiprism (Coord 8)			
$Ca(Glu) \cdot 3H_2O$	$P3_121$	1	5O (am. ac.); $2H_2O$; 1 N	d; chain	4.79	19
			Tricapped Prism (Coord 9)			
$Ca(Ser)_2 \cdot 2H_2O$	$P2_1$	1	5O (am. ac.); $2H_2O$; 2 N	b; chain	3.74	20

[a] *a* = monomer. *b* = face sharing,  ; chain. c = edge-sharing,  ; dimer or chain. d = corner-sharing; Ca–O–Ca; chain. e = carboxyl-bridge, Ca–O–C–O–Ca; chain.

polymers, as is shown in the listing of some of these complexes in Table V. It can be seen that aside from the structure presented here, there is only one other (calcium diglutamate·$4H_2O$) that consists of monomeric units (type a in Table V). The Ca–Ca distances in these two complexes are very long: 6.21 Å in the present structure, 7.56 Å in the diglutamate. Most of the other structures contain either chains or dimers which have some form of sharing of the atoms of the coordination polyhedron around the calcium atom. As a result, the Ca–Ca separation in the other complexes is much shorter, ranging from 3.74 to 4.79 Å (except in the tyrosine complex). These latter complexes can be further differentiated by considering the exact type of linkage of the calcium polyhedra, types b–e in Table V.

There is one instance of type b, face-sharing, with a Ca–Ca distance of 3.74 Å. This distance is very short, even shorter than that determined in body-centered metallic calcium of 3.80 Å.[21] Type c, edge-sharing of the calcium polyhedra, gives rise to either dimeric units or to infinite chains along one of the crystallographic axes. The five instances of this type have Ca–Ca distances ranging from 3.84 to 4.07 Å, with an average of 3.98 Å. The corner-(or apex-)shared type d is not very prevalent. The one structure of this type has a Ca–Ca distance of 4.79 Å. Finally there are three examples of structures of type e, which contain a bidentate carboxyl group that bridges two neighboring polyhedra. Since in this type the orientation of the calcium atom in relation to the carboxyl group can vary greatly, there is a large range of Ca–Ca separations, from 4.49 Å in the glycylglycine complex, to 6.12 Å, in the tyrosine complex. In the latter structure, the bridge is extended almost to its maximum length of about 6.9 Å, which one obtains by adding two Ca–O distances of 2.4 Å each and the O–O separation of 2(C–O) sin 60° = $(2.4)(3)^{1/2}/2$ = 2.1 Å.

The listing in the Table V shows seven complexes that contain halide ions and five that do not. It is of interest to observe that all the halide-containing structures belong to either type c or type e, i.e., they have a pronounced tendency toward chain or dimer formation. In addition, the halide ions do not form part of the coordination polyhedra of calcium, with only one exception: $CaBr_2(Gly)_3$ consists of edge-shared dimers in which each pentagonal bipyramid has one apex occupied by a bromide ion, while the other two bromide ions are farther away. It is clear from this behavior that a lattice consisting of positively charged calcium polyhedra separated by negative halide ions (in other words an ionic lattice) is energetically more favorable than a lattice that contains electrically neutral groups which are held together by hydrogen bonds and van der Waals forces. In contrast, the complexes that contain no halide ions have no choice other than the bonding by hydrogen bonds, and in order to accomplish this, they are seen to contain a larger number of water molecules: $4H_2O$ on the average in the five complexes without halide ion, compared to $1.3H_2O$ on the average in the complexes containing halide ions.

There is great variation in the coordination number of the calcium ion and in the geometry of its environment, as is well-known. For the complexes listed, table V shows four different coordination numbers, and five different geometries of which the octahedral and pentagonal bipyramidal are by far the most common. The strong preference of calcium for coordination by oxygen is also evident: the 12 complexes have a total of 84 coordination sites, of which 77 are occupied by oxygen atoms, 6 by nitrogen atoms, and 1 by a bromide ion. It can be seen that coordination to nitrogen does not occur in any of the complexes with octahedral geometry and that none of the halide-containing complexes show nitrogen coordination. The latter is readily explained: all the halide-containing crystals are reported to have been prepared by evaporation of aqueous solutions containing the calcium halide and the amino acid, sometimes in stoichiometric ratios, sometimes with excess CaX_2. No pH values for the solutions are reported except for the case of $CaCl_2(Glu) \cdot H_2O$ where it was adjusted to 7.5 prior to evaporation, by adding HCl. For all these solutions, the pH is probably less than 8.0. In such an aqueous medium, the amino acids occur as zwitterions; the amino

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groups are protonated and not capable of coordinating to the Ca^{2+} ion. In the crystals, the charge of the Ca^{2+} ion is balanced by that of the halide ions. The $Ca(Glu)_2 \cdot 4H_2O$ crystals were also obtained by recrystallization of commercially available material from water solution,[10] and again the pH must have been relatively low. On the other hand, crystals of the four remaining complexes were obtained from solutions of pH 10 or higher, usually because free $Ca(OH)_2$ was involved in the preparation. At such high pH values, the NH_2 is not protonated and thus available for coordination to the calcium ion. It is these four complexes that indeed exhibit nitrogen coordination.

Registry No. $Ca(C_5H_8NO_3)_2 \cdot 5H_2O$, 97860-73-6.

Supplementary Material Available: Tables of parameters of the hydrogen atoms, thermal parameters of non-hydrogen atoms, least-squares planes, and observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

Stereochemical Course of an Enzyme-Catalyzed Allene–Acetylene Isomerization

John M. Schwab*[1] and Daniel C. T. Lin

Contribution from the Department of Chemistry, The Catholic University of America, Washington, D.C. 20064. *Received March 11, 1985*

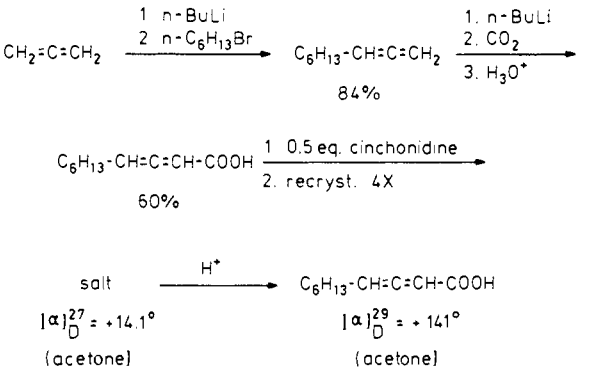
Abstract: An allene–acetylene isomerase (AAI) isolated from hog liver interconverts the *N*-acetylcysteamine thio ester of 3-decynoic acid (3-decynoyl-NAC) and (+)-2,3-decadienoyl-NAC. It is thus far the only enzyme that has been isolated that produces (or utilizes) an allenic compound and that is not inactivated by that same allene. Although physical and kinetic characteristics of the enzyme have previously been obtained, details of its mechanism of action remain unknown. The stereochemical course of the AAI-catalyzed propargylic rearrangement has now been determined, by synthesis of 2,3-[2-^{2}H]- and 2,3-[4-^{2}H]decadienoyl-NAC and enzymatic conversion of these substrates to 3-[2-^{2}H$_1$]decynoyl-NAC. Derivatization of the chirally labeled acetylenes, followed by ^{2}H NMR analysis, has shown that protonation occurs on the *si* face at C-2 of the allene. X-ray crystallographic analysis of a derivative has revealed that (+)-2,3-decadienoic acid possesses the *S* configuration. The enzyme-mediated propargylic rearrangement is therefore a suprafacial process, apparently involving a single active-site base.

Although nature elaborates a substantial variety of allenic natural products,[2] the enzymology of allene biosynthesis is virtually unexplored. To date, knowledge of biological allene formation is confined exclusively to those allenes that are formed transiently from acetylenic "suicide" substrates.[3]

In 1975 Miesowicz and Bloch reported[4] the isolation from hog liver of an enzyme capable of converting the (2-mercaptoethyl)amine (*N*-acetylcysteamine; NAC) thio ester of 3-decynoic acid (3-decynoyl-NAC) into the corresponding allenic thio ester, 2,3-decadienoyl-NAC. This is precisely the process that transpires in the course of the mechanism-based ("suicide") inactivation of β-hydroxydecanoylthioester dehydrase.[5-9] Dehydrase is inactivated almost instantaneously by 2,3-decadienoyl-NAC;[6] remarkably, the allene–acetylene isomerase (AAI) is unaffected by this allenic thio ester. Moreover, the allene is the predominant species at equilibrium. AAI is thus far the only enzyme that has been isolated that produces (or utilizes) an allenic compound and that is not inactivated by that same allene.

AAI's physical properties and substrate specificity have been rigorously characterized.[10] However, understanding of its mechanism of action has been hampered owing to the enzyme's failure to react in a specific manner with any of a number of reagents.[11]

Scheme I. Synthesis and Resolution of 2,3-Decadienoic Acid



We now report the results of experiments demonstrating that the AAI-catalyzed propargylic rearrangement is a suprafacial process, thus implicating a single active-site base.[12,13] In the course of these studies the absolute configurations of the allenic inactivators of β-hydroxydecanoylthioester dehydrase have also been determined.[7]

Results

Elucidation of the overall steric course of the AAI-catalyzed propargylic rearrangement required determination of (a) the

(1) Present address: Department of Medicinal Chemistry and Pharmacognosy, Pharmacy Building, Purdue University, West Lafayette, IN 47907.
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