Conformational Studies of the Synthetic Hydrophobic Sequences in Signal Peptides of Secretory Proteins

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Abstract: The conformation of chemically synthesized hydrophobic sequences of secretory proteins was studied in aqueous and organic solvents by circular dichroism (CD) spectroscopy. All the peptides studied adopt β-structures in aqueous solvents and α-helical conformations in organic solvents. The α-helical conformation of the peptides is dramatically stabilized in more lipophilic solvent systems. This finding strongly suggests that signal peptides may be present as α-helices in the lipophilic environment of the membranes penetrated in secretion.

Most nascent secretory proteins have an N-terminal signal peptide containing strongly hydrophobic amino acids, which is cleaved during secretion by a membrane-bound protease.[1] Signal peptides are believed to play a role in initiating introduction of the nascent peptides into membranes in the course of their secretion.[2] The detailed mechanism of incorporation of the peptide chain into the membrane, however, remains controversial.[3] The observation that signal peptides, without exception, include hydrophobic sequences 10-15 amino acids long may hold the key to this question. Conformationally, those signal peptides which contain a large proportion of L-leucine would be expected to be helical on the basis of the high helix-forming potential of this amino acid residue.[4] The α-helical conformation has indeed been predicted[5] for the central region of a number of signal peptide sequences by the Chou-Fasman method.[4] If signal peptides do in fact adopt the helical conformation, which enhances hydrophobicity by presenting only hydrophobic side chains to the environment, part of the driving force for membrane pepetration may be provided by thermodynamic stabilization of the helices by a lipid medium.[6-8] Thus, determination of the conformation of signal peptides would be important in understanding their role in protein secretion.[9] We have synthesized several hydrophobic sequences taken from signal peptides and studied their conformation in solution by circular dichroism spectroscopy.

Results

The N-terminal signal peptides examined in this study are listed in Table I, with the hydrophobic sequences synthesized by us in italics. Initially we considered synthesizing only the hydrophobic sequences themselves. We decided, however, that they might not be a proper model for deducing the role of the hydrophobic sequences in signal peptides. Since the hydrophobic sequences in signal peptides are preceded by an N-terminal peptide 3-8 amino acids long (see Table I), the peptides investigated in this study include an N-terminal peptide 3 amino acids long, which is independent of the hydrophobic sequence in terms of its structural preferences. Thus, we have synthesized hydrophobic peptides with an N-terminal triglycyl moiety which does not support the formation of any secondary structures.[4]

Conformations of the synthetic peptides were examined in water, methanol, 1-butanol, and 1-heptanol which contain portions of 1,1,1-trifluoroethanol (TFE) or 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP). The aqueous solution was used to approximate the cytosolic medium, and the alcoholic solution was used to approximate the lipophilic intramembranous medium. The last system consists of 1-heptanol, whose long hydrophobic chain might most resemble the intramembranous environment.[10]

Figure 1 represents the CD spectra of a hydrophobic peptide Nps-Gly-Gly-Gly-Ile-Phe-Gly-Phe-Leu-Leu-Leu-Leu-Phe-Pro-Gly-OEt from preimmunoglobulin L-chain MOPC-41κ (Mouse)[11] in various solvents. In a very polar solvent, HFIP, the peptide showed a CD spectrum with negative ellipticity which is characteristic of unordered structure in oligopeptides.[12-14] A change in the CD profile was observed in the less polar solvent, 10% HFIP-methanol. In this solvent, the CD spectrum has two negative extremes at 203 and 220 nm, with $[\theta]_{203} = -12\,000$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -6000$ deg cm^2 dmol^{-1}, which indicate a helical conformation.[15] The helix content is estimated to be 15% by the Greenfield-Fasman method.[16] In a lipophilic solvent, 10% HFIP-1-butanol, the CD band of the amide $\pi \rightarrow \pi^*$ transition[17] moved to 206 nm with increasing ellipticity $[\theta]_{206} = -14\,500$ deg cm^2 dmol^{-1} and the trough of the amide n $\rightarrow \pi^*$ transition at 220 nm also increased to $[\theta]_{220} = -10\,500$ deg cm^2 dmol^{-1} (31% α-helix). In a more lipophilic solvent, 10% HFIP-1-heptanol, the peptide showed a CD spectrum similar to that in 10% HFIP-1-butanol, with a slight increase of the ellipticity $[\theta]_{206} = -15\,800$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -11\,400$ deg cm^2 dmol^{-1} (33% α-helix). These indicate that the formation of α-helical structure in this peptide is dramatically promoted by a lipophilic environment. In contrast to CD spectra obtained in HFIP-alcohol solvent mixtures, the CD spectrum of the same peptide obtained in 30% HFIP-water had a single trough at 217 nm with $[\theta]_{217} = -9000$ deg cm^2 dmol^{-1} and a very weak shoulder at 203 nm. This spectrum indicates that the peptide adopts predominantly β-structure with a very small proportion of helix.[16] The formation of β-structure was facilitated as the proportion of HFIP in water decreased until the peptide finally precipitated from 10% HFIP-water. The IR spectrum of the precipitated sample shown in Figure 2 has bands at 1694 and 1632 cm^{-1} and 1534 cm^{-1} in the amide I and II region, respectively. These bands are characteristic of antiparallel β-sheet structure in peptides[18] which strongly suggests that the precipitation of the peptide in aqueous solution is associated with the formation of β-structure.

Figure 3 represents the CD spectra of another hydrophobic peptide Nps-Gly-Gly-Gly-Leu-Phe-Leu-Phe-Leu-Ala-Leu-Leu-Leu-Ala-OEt from pretrypsinogen (Dog)[19] in the same solvents.

(1) Davis, B. D.; Tai, P.-C. *Nature (London)* **1980**, *283*, 433-438.
(2) Blobel, G.; Dobberstein, B. *J. Cell. Biol.* **1975**, *67*, 852-862.
(3) Wickner, W. *Annu. Rev. Biochem.* **1979**, *48*, 24-45.
(4) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1979**, *13*, 211-222.
(5) Austen, B. M. *FEBS Lett.* **1979**, *103*, 308-313.
(6) Von Heijne, G.; Blomberg, C. *Eur. J. Biochem.* **1979**, *97*, 175-181.
(7) Von Heijne, G. *Eur. J. Biochem.* **1980**, *103*, 431-438.
(8) Engelman, D. A.; Steitz, T. A. *Cell* **1980**, *23*, 411-422.
(9) Rosenblatt, M.; Beaudette, N. V.; Frasman, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3983-3987.
(10) Sugihara, T.; Blout, E. R.; Wallance, B. A. *Biochemistry* **1982**, *21*, 3444-3452.
(11) Burstein, Y.; Schechter, I. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 716-720.
(12) Goodman, M.; Naider, F.; Rupp, R. *Bioorg. Chem.* **1971**, *1*, 310-328.
(13) Becker, J. M.; Naider, F. *Biopolymers* **1974**, *13*, 1747-1750.
(14) Bonora, G. M.; Toniolo, C. *Biopolymers* **1974**, *13*, 1067-1078.
(15) Holwarth, G.; Doty, P. *J. Am. Chem. Soc.* **1965**, *87*, 218-228.
(16) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108-4116.
(17) Tinoco, I., Jr.; Woody, R. W.; Bradley, D. F. *J. Chem. Phys.* **1963**, *38*, 1317-1325.
(18) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, 83, 712-719.

0002-7863/84/1506-5715$01.50/0

Table I. The Amino Acid Sequences of Signal Peptides in Secretory Proteins

origin	amino acid sequence
preimmunoglobulin L-chain MOPC-41κ	MetAspMetArgAlaProAlaGln*IlePheGlyPheLeuLeuLeuLeuPheProGly*ThrArgCysAsp (−1 +1)
pretrypsinogen	X AlaLys*LeuPheLeuPheLeuAlaLeuLeuLeuAla*TyrValAlaPhePro (−1 +1)
pre-β-lactoglobulin	MetLysCys*LeuLeuLeuAlaLeuGlyLeuAlaLeuAla*CysGlyValGlnAlaIle (−1 +1)

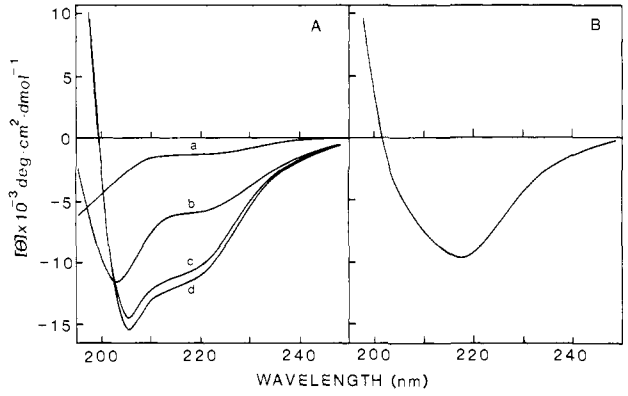

Figure 1. The CD spectra of the hydrophobic sequence from preimmunoglobulin L-chain MOPC 41-κ in HFIP and 10% HFIP-alcohols (A) and in 30% HFIP-water (B): a, HFIP; b, HFIP-MeOH; c, HFIP-BuOH; d, HFIP-heptanol.

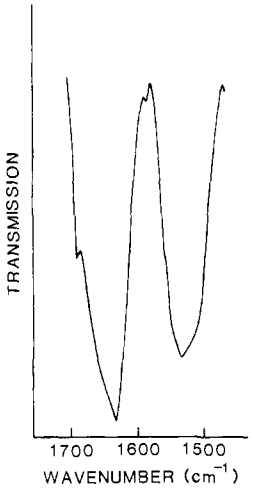

Figure 2. The IR spectrum of the solid sample of the hydrophobic sequence from preimmunoglobulin L-chain MOPC 41-κ precipitated from 10% HFIP-water.

In the HFIP–alcoholic solutions, the peptide showed a CD spectra having two negative extremes at 203–205 and 220 nm, which indicate the presence of α-helical conformation. The ellipticity of the extremes increased gradually with increasing lipophilicity of the solvent, to reach $[\theta]_{205} = -16\,000$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -10\,900$ deg cm^2 dmol^{-1} (34% α-helix) in 10% HFIP–1-heptanol. On the other hand, in 30% HFIP–water, the peptide showed a CD spectrum having a single trough at 214 nm with $[\theta]_{214} = -12\,500$ deg cm^2 dmol^{-1}, typical of β-structure. In aqueous solution with a lower proportion of HFIP, precipitation of the peptide was observed. These results clearly show that the conformational preference of this hydrophobic peptide taken from pretrypsinogen is quite similar to that of the peptide from preimmunoglobulin L-chain MOPC-41κ.

Figure 4 represents the CD spectra of Nps-Gly-Gly-Gly-Leu-Leu-Leu-Ala-Leu-Gly-Leu-Ala-Leu-Ala-OEt from pre β-lactoglobulin (Sheep)[20] in HFIP–alcoholic solution. For this peptide,

(19) Devillers-Thiery, A.; Kindt, T.; Scheele, G.; Blobel, G. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 5016–5020.

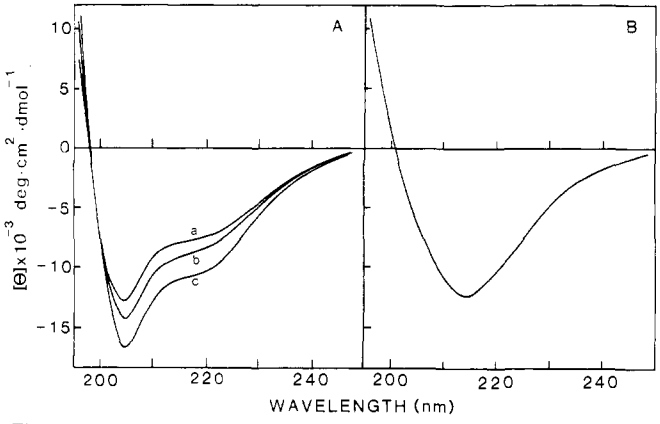

Figure 3. The CD spectra of the hydrophobic sequence from pretrypsinogen in 10% HFIP-alcohols (A) and in 30% HFIP-water (B): a, HFIP-MeOH; b, HFIP-BuOH; c, HFIP-heptanol.

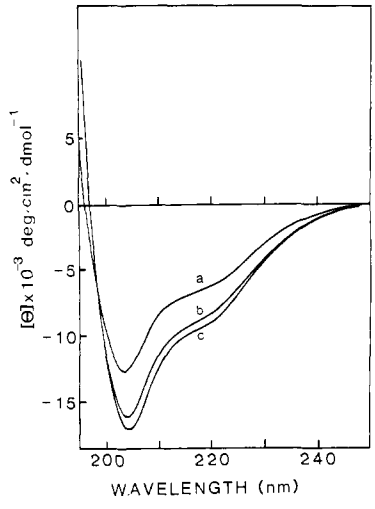

Figure 4. The CD spectra of the hydrophobic sequence from pre β-lactoglobulin in 10% HFIP-alcohols: a, HFIP-MeOH; b, HFIP-BuOH; c, HFIP-heptanol.

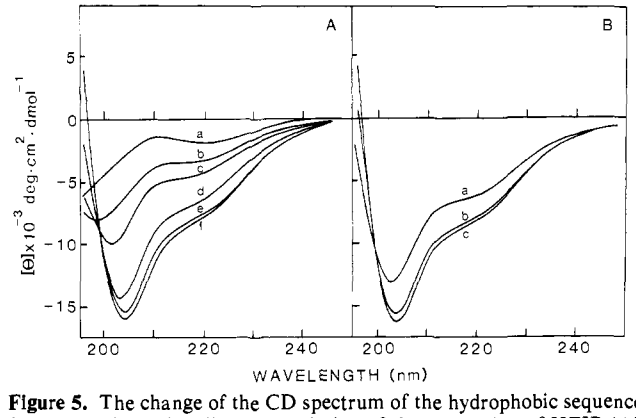

Figure 5. The change of the CD spectrum of the hydrophobic sequence from pre β-lactoglobulin with variation of the proportion of HFIP (A) or TFE (B) to butanol: A,a, 100% HFIP; A,b, 90% HFIP; A,c, 80% HFIP; A,d, 60% HFIP; A,e, 50% HFIP; A,f, below 40% HFIP; B,a, 100% TFE; B,b, 50% TFE; B,c, below 40% TFE.

the CD spectrum in aqueous solution could not be obtained because of immediate precipitation of the peptide after preparation of the solution. In the alcoholic solution, this peptide too showed CD spectra having two negative extremes at 203–204 and 220 nm, and the highest values of the ellipticity were $[\theta]_{204} = -17\,900$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -9000$ deg cm^2 dmol^{-1} (35% α-helix) in 10% HFIP–1-heptanol.

Figure 5 represents the change in the CD spectrum of the hydrophobic peptide from pre β-lactoglobulin with variation in the proportion of HFIP or TFE to 1-butanol. In HFIP, the peptide showed the CD spectrum typical of unordered structure. In 80% HFIP–1-butanol, two negative extremes appeared at 201 and 220 nm in the CD spectrum, although the magnitudes of the ellipticities were small. This shows that the peptide begins to adopt an α-helical conformation in solution as the result of a small decrease of polarity. The magnitudes of the extremes increased markedly with decrease in the proportion of HFIP to become constant at $[\theta]_{204} = -17\,000$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -8500$ deg cm^2 dmol^{-1} (33% α-helix) below 40% HFIP–1-butanol. On the other hand, in TFE, which is less polar than HFIP, the peptide showed a CD spectrum having two negative extremes at 203 and 220 nm with $[\theta]_{203} = -13\,000$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -6800$ deg cm^2 dmol^{-1} (17% α-helix). This indicates that the peptie adopts an α-helix in TFE. The ellipticity of the extremes increased with decrease in the proportion of TFE to become constant below 40% TFE-1–butanol. It should be noted that the highest ellipiticities of the extremes in TFE–1-butanol, $[\theta]_{204} = -17\,200$ deg cm^2 dmol^{-1} and $[\theta]_{220} = -8500$ deg cm^2 dmol^{-1}, are the same as those in HFIP–1-butanol. The same result was obtained in HFIP–1-heptanol and TFE–1-heptanol systems.

The conformational examintaion of three hydrophobic peptides taken from signal peptides has shown interesting characteristics of the sequences. First, all the peptides have a common structural feature. Although they do not adopt secondary structure in HFIP, they have a strong tendency to form secondary structure in less polar solvent systems. The secondary structure adopted by the hydrophobic sequences, however, differs markedly depending on the solvent. In aqueous HFIP solution, the peptides adopt a β-sheet structure. In alcoholic solution, they assume an α-helical conformation. Second, the helical conformation is dramatically stabilized in lipophilic environments provided by higher alcohols. As illustrated by Figure 5, the hydrophobic sequences adopt stable α-helices in solvents with a range of lipophilicity resembling that of the intramembranous environment. This fact strongly suggests that the hydrophobic sequences may be in α-helical conformation in the membranes.

Discussion

Signal peptides of the secretory proteins share a common structural feature in that they contain a sequence of strongly hydrophobic amino acids in their central region. This structural feature may be crucial to the function of the signal peptides. The hydrophobic sequences suggest that the peptides may adopt stable secondary structures in the membranes. Although a wide variety of amino acid sequences has been found for the hydrophobic moieties of the signal peptides, they are characterized by frequent occurrence of the L-leucyl residue. In fact, the hydrophobic peptides examined in this study are 40–60% L-leucine. Since L-leucine has a high helix-forming potential, $P_\alpha = 1.21$, as well as a high parameter for the formation of β-structure, $P_\beta = 1.30$,[4] peptides containing a large proportion of this amino acid would be expected to be highly structured. Preferred secondary structure for the peptides is predicted by the Chou-Fasman method.[4] The hydrophobic sequence from pre-β-lactoglobulin has a higher parameter for α-helix formation, $\langle P_\alpha \rangle = 1.21$, than that for β-structure formation, $\langle P_\beta \rangle = 1.10$. The sequence from pretrypsinogen has a comparable value for the formation of both conformations, $\langle P_\alpha \rangle = 1.24$ and $\langle P_\beta \rangle = 1.22$. On the other hand, the hydrophobic sequence from preimmunoglobulin L-chain MOPC-41κ has a higher potential for β-structure formation, $\langle P_\beta \rangle = 1.18$, than that for α-helix formation, $\langle P_\alpha \rangle = 1.00$. According to this prediction, the prefered conformation is the α-helix, either α-helix or β-structure, and β-structure for the first, second, and last peptide sequence, respectively. This result indicates that variation in the conformation would be expected for the hydrophobic sequences of different signal peptides.[5]

In spite of the predicted variation of the conformation, a common conformational preference for α-helix formation has been experimentally observed for the hydrophobic sequences. It is especially interesting that the hydrophobic sequence from preimmunoglobulin L-chain MOPC-41κ adopts the α-helical conformation although β-sheet structure has been predicted for this peptide. This inconsistency between the predicted and the observed conformations may result from application of the predictive method for protein conformation to short peptide sequences, in which short-range interactions of the peptide chains are dominant. Thus the results obtained in this study suggest that all the hydrophobic sequences in signal peptides might be in α-helical conformations in the membranes and in β-sheet structures in aqueous cytosolic environments. In fact, the entire sequence of the signal peptide of preproparathyroid hormone[9] shows the same conformational preference as the hydrophobic sequences in this study.

A number of models have been proposed for the spontaneous traverse of the secretory proteins through membranes.[6-8] According to these models, the energy for transmembrane passage of the proteins may be provided by the conformational transition of the hydrophobic sequences of their signal peptides to α-helices in the lipophilic environment of membranes. This is consistent with our observation that the hydrophobic sequences change their conformation from β-sheet structure in aqueous solution to the α-helix in lipophilic media.

Table II. Analytical Data of the Hydrophobic Peptides

peptide	R_f[a]	anal. calcd, found C	H	N
Nps-Gly-Gly-Gly-Ile-Phe-Gly-	0.14	60.49,	7.13,	13.23,
Phe-Leu-Leu-Leu-Leu-Phe-Pro-		60.	7.	12.93
Gly-OEt		30	25	
Nps-Gly-Gly-Gly-Leu-Phe-Leu-	0.43	59.84,	7.55,	13.21,
Phe-Leu-Ala-Leu-Leu-Leu-		59.	7.	13.24
Ala-OEt		71	76	
Nps-Gly-Gly-Gly-Leu-Leu-Leu-	0.34	55.54,	7.74,	14.87,
Ala-Leu-Gly-Leu-Ala-Leu-		55.	7.	14.45
Ala-OEt		52	85	

[a] Eluent: HFIP–tetrahydrofuran 2:1.

Experimental Section

Synthesis of Peptides. The peptides were synthesized by a solution method for peptide synthesis involving the condensation of short peptide fragments which had been prepared stepwise with dicyclohexylcarbodiimide in the presence of *N*-hydroxysuccinimide.[21-23] The most critical problem in preparing a pure product with hydrophobic side chains is dissolving the peptide fragments to be condensed with each other, because peptides with hydrophobic side chains have generally poor solubility in many organic solvents suitable for peptide synthesis.[24] We succeeded in obtaining a solution of the peptides by changing the solvent depending on the peptide chain length: *N*,*N*-dimethylformamide for short peptides, dimethyl sulfoxide for peptides of moderate length, and hexamethylphosphoramide for long peptides.[25] Condensation of peptide fragments was able to proceed in a clear solution. The product was isolated by dilution of the reaction mixture with water and purified by repeated reprecipitation from HFIP with methanol. A high purity of the final product was established by thin-layer chromatography, and analysis with a Jasco Trirotar high-performance liquid-chromatograph system controlled by a microcomputer. The R_f values and elemental analyses of the

(20) Mercier, J. C.; Haze, G.; Gaye, P.; Hue, D. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 1236–1245.

(21) Weygand, F.; Hoffmann, D.; Wunsch, E. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1966**, *21B*, 426–428.

(22) Wunsch, E.; Dress, F. *Chem. Ber.* **1966**, *99*, 110–120.

(23) Zimmerman, J. E.; Anderson, G. W. *J. Am. Chem. Soc.* **1967**, *89*, 7151–7152.

(24) Pillai, V. N. R.; Mutter, M. *Acc. Chem. Res.* **1981**, *14*, 122–130.

(25) Katakai, R.; Shida, H.; Takada, T. *Biopolymers*, in press.

products are summarized in Table II.

Measurements. CD spectra were obtained with a Jasco J-20 CD spectrophotometer controlled by a Jasco DP-X data processor at a 0.1 mg/mL concentration of peptide at room temperature. The ellipticity, $[\theta]$, is expressed in deg cm^2 dmol^{-1} of amino acid residue by using mean residue molecular weight. The calibration was based on $[\theta]_{304}$ = 11 200 deg cm^2 dmol^{-1} for 0.05% androsterone in dioxane.[26] The IR spectrum was obtained for a KBr disk with a Jasco A-702 spectrophotometer controlled by a Jasco A-330 data processor.

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Registry No. Nps-Gly-Gly-Gly-Ile-Phe-Gly-Phe-Leu-Leu-Leu-Leu-Phe-Pro-Gly-OEt, 91295-37-3; Nps-Gly-Gly-Gly-Leu-Phe-Leu-Phe-Leu-Ala-Leu-Leu-Leu-Ala-OEt, 91295-38-4; Nps-Gly-Gly-Gly-Leu-Leu-Leu-Ala-Leu-Gly-Leu-Ala-Leu-Ala-OEt, 91311-02-3.

(26) Tuzimura, K.; Konno, T.; Megro, H.; Hatano, M.; Murakami, T.; Kashiwabara, K.; Saito, K.; Kondo, Y.; Suzuki, T. M. *Anal. Biochem.* **1977**, *81*, 167-174.

Crystalline-State Reaction of Cobaloxime Complexes by X-ray Exposure. 7. Structure Analysis of Multistage Racemization

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Abstract: A crystal of [(*R*)-1-(methoxycarbonyl)ethyl](pyridine)bis(dimethylglyoximato)cobalt(III) methanol solvate changed its cell dimensions without degradation of the crystallinity at 293 K. After 140 h, the change became so slow that the three-dimensional intensity data were collected. The cell dimensions were varied from a = 9.289 (3) Å, b = 21.059 (6) Å, c = 11.499 (4) Å, β = 94.16 (3)°, and V = 2243 (1) Å^3 to a = 9.296 (3) Å, b = 20.09 (1) Å, c = 11.39 (1) Å, β = 94.27 (8)°, and V = 2121 (3) Å^3. The space group $P2_1$ remained unaltered. The structure analyses before and after the change revealed that the solvent methanol molecules completely went out from the crystal and the conformation of the (methoxycarbonyl)ethyl (mce) group was drastically changed to fill the void space due to the loss of solvent. The absolute configuration of the mce group, however, was conserved. Then the crystal was warmed to 343 K to accelerate the rate of the change. The cell dimensions began to vary again. The change finally stopped after 180 h. The space group was converted from $P2_1$ to $P2_1/n$. The structure at the final stage at 293 K agreed with that reported previously (Kurihara, T.; Ohashi, Y.; Sasada, Y. *Acta Crystallogr., Sect. B* **1982**, *B38*, 2484-2486). The chiral mce group was converted to a disordered racemic structure. Comparison of the three structures at the initial, intermediate, and final stages clearly revealed the atomic process of the multistage racemization of the mce group in the crystalline state.

We have found that the chiral 1-cyanoethyl (cn) group, $-C^*H(CH_3)CN$, bonded to the cobalt atom, in the crystals of several bis(dimethylglyoximato)cobalt (cobaloxime) complexes is racemized by X-ray exposure without degradation of the crystallinity.[2-8] Since the rate of the racemization is closely related with the packing of the cn group in the crystal structure, we have defined the cavity for the cn group and proved that the reactivity has a positive correlation with the volume of the cavity.[7] The chiral 1-(methoxycarbonyl)ethyl (mce) group, $-C^*H(CH_3)CO_2CH_3$, in the crystal of [(*R*)-1-mce](4-chloropyridine)cobaloxime (*R*-mce-Clpy), on the other hand, was racemized only at high temperatures. The mode of the racemization was very complicated; the inversion of configuration of the mce group was accompanied by the rotation around the central C–CO_2CH_3 bond.[9] The crystal of [(*R*)-1-mce](pyridine)cobaloxime (*R*-mce-py, Figure 1) also revealed the crystalline-state racemization. Since the rate of the change in the unit-cell dimensions was too rapid to collect the three-dimensional intensity data, only the final racemic structure was determined.[10] It seemed at first sight strange that the

Table I. Crystal Data and Details of Refinement at the Three Stages

	initial		intermediate	final[a]
formula	$C_{17}H_{26}CoN_5O_6 \cdot CH_4O$	$C_{17}H_{26}CoN_5O_6 \cdot CH_4O$	$C_{17}H_{26}CoN_5O_6$	$C_{17}H_{26}CoN_5O_6$
fw	487.40	487.40	455.36	455.36
temp, K	223	293	293	293
space group	$P2_1$	$P2_1$	$P2_1$	$P2_1/n$
a, Å	9.249 (7)	9.289 (3)	9.296 (3)	9.288 (1)
b, Å	20.82 (2)	21.059 (6)	20.09 (1)	19.952 (2)
c, Å	11.45 (1)	11.499 (4)	11.39 (2)	11.397 (1)
β, deg	94.06 (9)	94.16 (3)	94.27 (8)	94.10 (2)
V, Å^3	2201 (3)	2243 (1)	2121 (3)	2106.6 (5)
Z	4	4	4	4
R factor	0.053	0.132	0.104	
N_F[b]	3843	3422	3029	
N_p[c]	691	668	560	
C[d]	0.00386	0.00390	0.02820	

[a] Values taken from ref 10. [b] Number of observed reflections. [c] Number of parameters. [d] Value in the weighting scheme.

(1) (a) Tokyo Institute of Technology. (b) Niigate College of Pharmacy.
(2) Ohashi, Y.; Sasada, Y. *Nature (London)* **1977**, *267*, 142-144.
(3) Ohashi, Y.; Sasada, Y.; Ohgo, Y. *Chem. Lett.* **1978**, *457*-460.
(4) Ohashi, Y.; Yanagi, K.; Kurihara, T.; Sasada, Y.; Ohgo, Y. *J. Am. Chem. Soc.* **1981**, *103*, 5805-5812.
(5) Ohashi, Y.; Sasada, Y.; Ohgo, Y. *Chem. Lett.* **1978**, 743-746.
(6) Ohashi, Y.; Yanagi, K.; Kurihara, T.; Sasada, Y.; Ohgo, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6353-6359.
(7) Ohashi, Y.; Uchida, A.; Sasada, Y.; Ohgo, Y. *Acta Crystallogr., Sect. B* **1983**, *B39*, 54-61.
(8) Kurihara, T.; Uchida, A.; Ohashi, Y.; Sasada, Y.; Ohgo, Y.; Baba, S. *Acta Crystallogr., Sect. B* **1983**, *B39*, 431-437.
(9) Kurihara, T.; Ohashi, Y.; Sasada, Y.; Ohgo, Y. *Acta Crystallogr., Sect. B* **1983**, *B39*, 243-250.
(10) Kurihara, T.; Ohashi, Y.; Sasada, Y. *Acta Crystallogr., Sect. B* **1982**, *B38*, 2484-2486.

0002-7863/84/1506-5718$01.50/0