Side-Chain Conformational Changes of Some Amino Acids and Dipeptides Having Aromatic Side Chains Induced by Complexation with Cycloamyloses

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Cycloamyloses, well-known also as cyclodextrins, are a series of cyclic oligosaccharides constructed from at least six units of α-(1→4)-linked D-glucose. The most important property of cycloamylose is its ability to admit a variety of guest molecules into its hydrophobic cavity without any covalent bonds being formed.[1-3] Because of this property the cycloamyloses serve as models for studying topochemical aspects and catalytic reactions of enzymes. There seem to be no severe restrictions, other than size matching and polarity, on the type of guest molecule to be included into the cycloamylose cavity, although some examples of stereospecific interactions have been reported.[2] There has been no experimental evidence which indicates a conformational change of guest molecule when it forms an inclusion complex with cycloamylose.

We report here the first observation of a conformational change of a guest compound on the occasion of complexation with cycloamylose by measurements of 200-MHz ^{1}H NMR spectra. We have chosen cyclohexaamylose (α-CD) and cycloheptaamylose (β-CD) as hosts and D- and L-phenylalanines (Phe), L-tyrosine (Tyr), L-phenylalanylglycine (L-Phe-Gly), and glycyl-L-phenylalanine (Gly-L-Phe) as guests.[4] The 200-MHz ^{1}H resonances of the α-CH and β-CH_2 protons of the Phe, Tyr, and Phe residues in both dipeptides are analyzable as ABX-type spin systems. The coupling constants are given in Table I.[6] The rotamer populations for the side chain, estimated with the use of the recent approach of Feeney[7] and the Pachler approximation,[8] are also given in Table I, with notation as given in Figure 1. In the free state the most stable rotamer is the gt one with the aromatic ring trans to the carboxyl (Phe, Tyr, and Gly-L-Phe) or carbonyl groups (L-Phe-Gly). These results are generally in agreement with the general observations that the aromatic side chain has a tendency to orient itself toward the amino terminal or to the NH group of the peptide backbone.[9]

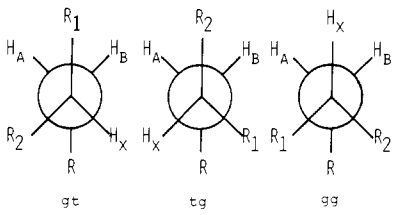

Figure 1. Rotamer notation. L-Phe; R = C_6H_5, R_1 = COOH, R_2 = NH_2; D-Phe; R = C_6H_5, R_1 = NH_2, R_2 = COOH; L-Tyr; R = C_6H_4OH, R_1 = COOH, R_2 = NH_2; Gly-L-Phe; R = C_6H_5, R_1 = COOH, R_2 = $NHCOCH_2NH_2$; L-Phe-Gly; R = C_6H_5, R_1 = $CONHCH_2COOH$, R_2 = NH_2. For D-Phe, the namings of gt and tg are inverted.

The complexation of the guest with β-CD effects considerable changes on the conformation of the side chain. The most stable rotamer, gt, in the free state is still the one in the complexed state. However, upon complexation, the gt rotamer becomes more dominant and the tg and gg rotamers contribute less to the side-chain conformation. The increase of gt rotamer population is large in Tyr, Phe, and L-Phe-Gly but not large in Gly-L-Phe, in which the gt rotamer is essentially the most stable one. The complexation with α-CD induces a slight and negligible change in the side-chain rotamer population of Phe. These findings can be explained by using space-filling (CPK) models. When the guest molecule forms an inclusion complex with β-CD by insertion of its aromatic ring into the CD's cavity from the secondary hydroxyl side,[1-3] the tg and gg rotamers become more and more unfavorable because the bulky carboxylate anion approaches and severely touches the rim of the β-CD torus. The repulsive electrostatic interactions between the ionized secondary hydroxyl groups and the guest's carboxylate keep the latter away from the former; i.e.,

Table I. ^{1}H Coupling Constants and Rotamer Populations for the Side Chains of Amino Acids and Dipeptides and Their Complexes with Cycloamyloses[a]

guest, host	coupling constnats, Hz			rotamer populations[b]		
	J_{AB}	J_{AX}	J_{BX}	gt	tg	gg
L-Tyr, none	13.8	7.2	5.2	0.45 (0.42)	0.23 (0.24)	0.32 (0.34)
L-Tyr, β-CD	13.8	9.2	4.4	0.69 (0.60)	0.20 (0.16)	0.11 (0.24)
D-Phe, none	13.6	7.5	5.6	0.48 (0.45)	0.30 (0.27)	0.22 (0.28)
D-Phe, α-CD	13.6	7.5	5.5	0.48 (0.45)	0.28 (0.26)	0.24 (0.29)
D-Phe, β-CD	13.3	8.9	4.6	0.65 (0.57)	0.22 (0.18)	0.13 (0.25)
L-Phe, none	13.5	7.4	5.6	0.47 (0.44)	0.29 (0.27)	0.24 (0.29)
L-Phe, α-CD	13.6	7.5	5.5	0.48 (0.45)	0.28 (0.26)	0.24 (0.29)
L-Phe, β-CD	13.3	8.9	4.6	0.65 (0.57)	0.22 (0.18)	0.13 (0.25)
L-Phe-Gly, none	13.3	7.1	6.3	0.42 (0.41)	0.37 (0.34)	0.21 (0.25)
L-Phe-Gly, β-CD	13.4	8.4	4.8	0.59 (0.53)	0.23 (0.20)	0.18 (0.27)
Gly-L-Phe, none	13.9	8.7	4.9	0.62 (0.55)	0.25 (0.21)	0.13 (0.24)
Gly-L-Phe, β-CD	14.0	9.1	4.6	0.67 (0.59)	0.23 (0.18)	0.10 (0.23)

[a] The notation is given in Figure 1. [b] The rotamer populations were obtained by the Feeney[7] and Pachler[8] (in parentheses) approximations.

(1) R. J. Bergeron, *J. Chem. Educ.*, **54**, 204 (1977).
(2) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry", Springer-Verlag, New York, 1978.
(3) R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, and D. A. Laufer, *J. Am. Chem. Soc.*, **103**, 1750 (1981).
(4) Amino acids and cycloamyloses were obtained from Nakarai Chemicals, Ltd., and dipeptides were purchased from Sigma Chemical Co. In preceding papers,[5] we have confirmed by means of ^{13}C NMR spectroscopy that these guest compounds form 1:1 inclusion complexes with α- and β-CDs by the insertion of their aromatic ring into the cavity of the host. The association constants for these complexations have been estimated to be larger than 10^2 M^{-1}.
(5) (a) Y. Inoue, Y. Katôno, and R. Chûjô, *Bull. Chem. Soc. Jpn.*, **52**, 1692 (1979); (b) Y. Inoue and Y. Miyata, *ibid.*, **54**, 809 (1981); (c) Y. Inoue, T. Okuda, and Y. Miyata, *Carbohydr. Res.*, in press.
(6) ^{1}H Fourier transformed NMR spectra were measured on a JEOL JNM FX-200 spectrometer operated at 200 MHz and at 30 °C. 1 N NaO^2H was used as solvent. The concentrations of guest and host were 0.1 and 0.12 M, respectively. In this condition, more than 95% of the guest molecule was complexed with host cycloamylose.[5] Thus the coupling constants of the guest observed in the host/guest mixture can be regarded as exclusively those of the complexed states.
(7) J. Feeney, *J. Magn. Reson.*, **21**, 473 (1976).
(8) K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964).
(9) For example: (a) K. Wüthrich A. de Marco, *Helv. Chim. Acta*, **59**, 2228 (1976); (b) H. Jäckle and P. L. Luisi, *Biopolymers*, **20**, 65 (1981).

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the gt rotamer becomes more probable. The introduction of the Gly residue to the amino or carboxyl side of L-Phe does not seem to affect the complexation-induced conformational change of the side chain, which provides additional confirmation that electrostatic interactions rather than steric ones play the dominant role.

In the case of complexation with α-CD, the penetration of the phenyl ring into the cavity is shallow[5] and the carboxyl group is sufficiently far away from the CD rim to avoid severe contact even in the tg and gg rotamers. We cannot find any enantiomeric differences for the complexation of L- and D-Phe with α- and β-CDs. This seems reasonable from examination of molecular models since no differences in structural features other than chirality are found. From this standpoint, it can be said that cycloamylose is a better, but not the best, model for enzymes.[10]

(10) We thank Professor R. Chûjô of this institute for helpful discussions. We are also grateful to Messers. K. Kosaka and K. Takahashi of JEOL for facilities for the 200-MHz NMR measurements. This work was partially supported by a Grant-in-Aid from the Ministry of Education, Science, and Culture, Japan (1980).

Selectivity in Binding a Phenanthridinium-Dinucleotide Derivative to Homopolynucleotides

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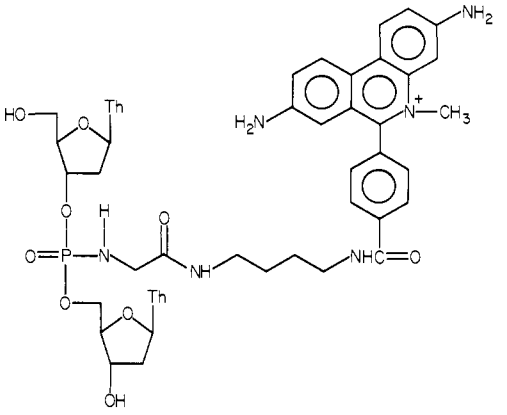
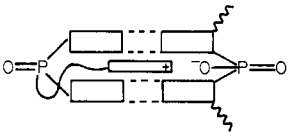
An oligonucleotide derivative possessing a structural feature that enhances binding of the oligomer to complementary sequences in a polynucleotide has interest as a potential site-specific inhibitor (or promoter) of enzymatic processes involving the polynucleotide. We describe in this Communication a model compound, **1**, designed to test the concept that a fragment capable of intercalating in the base pair pockets of DNA can serve as such a structural feature.

Selection of **1** for study was stimulated by the observations that the "melting temperature", T_m, of a double-stranded polynucleotide is increased when ethidium or related substances intercalate,[1] that ethidium intercalates even in dinucleotide pockets at sufficiently high concentrations,[2] and that an intercalator with two binding sites (i.e., a bis-intercalator) forms a much tighter complex with DNA than one with a single binding site (a mono-intercalator).[3] Molecular models indicate that the linker arm in **1** should permit the phenanthridinium moiety to fold back and insert into the pocket formed by the adjoined nucleoside bases and the complementary bases in a polynucleotide (see **2** for a schematic representation). In effect, the local concentration of the ethidium-like group is alway high in the vicinity of the dinucleotide. Compound **1** may therefore be viewed as a simple representative of an oligonucleotide derivative with two types of binding sites that could act cooperatively, the phenanthridinium ring and the pyrimidine bases. Alternatively, **1** is of interest as a model for a biologically active substance (the diaminophenanthridinium ring) with a covalently attached recognition system that could direct the active agent to a given nucleotide sequence.[4]



1



2

As outlined in Scheme I, compound **1** was obtained by constructing the nucleotide portion (**5**) and the phenanthridinium unit (**8**) and then linking these two fragments via an amide bond. For synthesis of **5**, 2-chlorophenyl phosphorodichloridite (0.9 equiv) in 2:1 THF–C_5H_5N was treated successively with triazole (3 equiv, 5 min), 5′-*O*-(phenoxyacetyl)thymidine (1 equiv, −78 °C, 20 min), and 3′-*O*-(di-*p*-methoxytrityl)thymidine (0.5 equiv, −78 to 0 °C, 30 min).[5] The resulting phosphite was converted without isolation[6] to phosphoramidate **4** by reaction with excess ethyl azidoacetate and water in THF (room temperature, 40 h)[7], and **4** was converted to **5** by treatment with excess 1,4-diaminobutane in dioxane (40 °C, 40 h). The phenanthridinium unit was prepared from 2-aminobiphenyl by adaptation of reported procedures.[3f,g] Nitration (H_2SO_4 and KNO_3, 5 °C, 4 h), aroylation with 4-cyanobenzoyl chloride (90 min in refluxing C_6H_5Cl), and cyclization ($POCl_3$, 2 h in refluxing $C_6H_5NO_2$) gave phenanthridine **7**. Methylation (Me_2SO_4, 180 °C in $C_6H_5NO_2$ for 1 h), hydrolysis of the nitrile (75% aqueous H_2SO_4, 130 °C, 90 min), reduction of the nitro groups (Fe–0.03M HBr, 3 h at reflux), and esterification with nitrophenol (DCC in 3:1 DMF–C_5H_5N, 6 h) yielded the active ester **8**. Compound **1**[8] was then obtained by reaction of equimolar

(1) (a) LePecq, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87. (b) Waring, M. J. *Biochem. J.* **1974**, *143*, 483. (c) Aktipis, S.; Martz, W. W. *Biochemistry* **1974**, *13*, 112. (d) Heller, M. J.; Tu, A. T.; Maciel, G. E. *Ibid.* **1974**, *13*, 1623. (e) Barton, J. K.; Lippard, S. J. *Ibid.* **1979**, *18*, 2661. (f) Phillips, D. R.; Roberts, G. C. K. *Ibid.* **1980**, *19*, 4795. (g) Kalisch, B. W.; van de Sande, J. H. *Nucleic Acids Res.* **1979**, *6*, 1881.

(2) (a) Reinhardt, C. G.; Krugh, T. R. *Biochemistry* **1978**, *17*, 4845. (b) Krugh, T. R.; Reinhardt, C. G. *J. Mol. Biol.* **1975**, *97*, 133. (c) Tsai, C.; Jain, S. C.; Sobel, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 628.

(3) (a) LePecq, J. B.; Lebret, M.; Barbet, J.; Roques, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915. (b) Barbet, J.; Roques, B. P.; Commbrisson, S.; LePecq, J. B. *Biochemistry* **1976**, *15*, 2642. (c) Canellakis, E. S.; Shaw, Y. H.; Hanners, W. E.; Schwartz, R. A. *Biochim. Biophys. Acta* **1976**, *418*, 277. (d) Fico, R.; Chen, T. K.; Canellakis, E. S. *Science* **1977**, *198*, 53. (e) Delbarre, A.; Roques, B. P.; LePecq, J. B. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1977**, *284*, 81. (f) Dervan, P. B.; Becker, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 1968. (g) Kuhlmann, K. F.; Charbeneau, N. J.; Mosher, C. W. *Nucleic Acids Res.* **1978**, *5*, 2629.

(4) Drugs that bind to DNA by intercalation include the trypanocidal agents ethidium bromide and aminoacridine, anticancer compounds adriamycin, daunomycin, and actinomycin, and the antimalarials chloroquine and quinacrine. Clearly, a recognition device that could direct an active pharmacological agent to a given DNA sequence or, closer to the present model, to a given RNA sequence offers interesting possibilities.

(5) Phosphite chemistry for oligonucleotide synthesis was followed up to the oxidation step. See: Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655. Melnick, B. P.; Finnan, J. L.; Letsinger, R. L. *J. Org. Chem.* **1980**, *45*, 2715.

(6) Some decomposition of the intermediate *o*-chlorophenyl phosphite was observed when an attempt was made to isolate this substance by preparative chromatography on silica gel; so the products of the reaction with dT(mmtr) were partitioned between CH_2Cl_2 and H_2O, the CH_2Cl_2 layer was concentrated, and the residual phosphite was treated directly with ethyl azidoacetate in THF.

(7) Use of an organic azide in forming phosphoramidate derivatives of dinucleoside phosphates was described by: Letsinger, R. L.; Heavner, G. A. *Tetrahedron Lett.* **1975**, 147. The P–N bond in **4** is stable in 80% aqueous HOAc (10 h, 25 °C) but is cleaved by CF_3COOH (100 °C, 20 min).

(8) λ_{max} (H_2O) 285 (ε 56 600), 495 nm (ε 5500).

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