which may indicate that B cells are a mixture of cells equipped with different sets of such enzymes. Variation in the oligosaccharide patterns of IgG myeloma proteins by Mizuochi et al. (1982) seems to support this possibility. If it is the case, IgG molecules may be classified by their sugar chain structures, although microheterogeneity does exist in the whole IgG molecule. It might be worth investigating whether or not such a subclass of IgG functions differentially.

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Model for Interactions of Amino Acid Side Chains with Watson-Crick Base Pair of Guanine and Cytosine: Crystal Structure of 9-(2-Carbamoylethyl)guanine and 1-Methylcytosine Complex†

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ABSTRACT: As a model of interaction between the guanine–cytosine base pair and carbamoyl group, the crystal structure of 9-(2-carbamoylethyl)guanine–1-methylcytosine complex has been studied by X-ray method. The crystal data are a = 8.540 (1) Å, b = 12.693 (3) Å, c = 14.249 (2) Å, β = 94.02 (1)°, space group $P2_1/c$, Z = 4, d_m = 1.50, d_c = 1.49 g cm^{-3}, and R = 0.10 for 1035 reflections. The bases form a Watson–Crick pair, and the carbamoyl group is hydrogen bonded with O(6) of guanine and N(4) of cytosine in the adjacent pairs. A structural correlation has been found between the hydrogen-bonding pattern and the secondary structural fitting of α-helical segment with B-form DNA.

Recent determination of the three-dimensional structures of DNA binding proteins has furnished a model of their docking or matching with double-stranded DNA (McKay & Steitz, 1981; Anderson et al., 1981; Pabo et al., 1982), but "fine tuning" in the secondary structural fitting is still uncertain. In such mutual recognition, the component–component interaction should play a crucial role, and among several types of the interactions the hydrogen bonds may be the leading part, especialy when high specificity is required.

Detailed information for these interactions would be hardly obtained even if structures of complex crystals between both biomolecules could be solved at low resolution. One approach

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to the problem is to use suitable model crystals that contain both the components because protein crystallography has indicated the general trend that the packing density and mean-square displacement of the protein interface with other protein or substrate are similar to those of crystals of small molecules (Richards, 1974; Frauenfelder et al., 1979).

Studies of this kind have been done for several combinations and extended to the ternary model systems of adenine–thymine–carbamoyl group (Takimoto et al., 1982) and of adenine–uracil–carboxyl group (Fujita et al., 1983). But Watson–Crick pairing has never been obtained. Recently, we have succeeded in preparing the new ternary model systems of guanine–cytosine–carboxyl group (Fujita et al., 1984a) and guanine–cytosine–carbamoyl group and revealed that the complementary bases form a Watson–Crick base pair.

This paper describes crystal structure of the latter system, 9-(2-carbamoylethyl)guanine–1-methylcytosine complex, and discusses the interactions of the side chains of asparagine and glutamine with the Watson–Crick guanine–cytosine base pair in the secondary structural matching between α-helical segment and B-form DNA.

Experimental Procedures

Materials. The reaction of N^2-acetylguanine with β-propiolactone in the presence of 4-(dimethylamino)pyridine resulted in 2-carboxyethylation of the former. After purification on a silica gel column, the product was deacetylated with NaOH to give 9-(2-carboxyethyl)guanine. Esterification of 9-(2-carboxyethyl)guanine with HCl-saturated MeOH and then ammonolysis with NH_3-saturated MeOH gave 9-(2-carbamoylethyl)guanine. Its crystalline complex with 1-methylcytosine was obtained from a solution containing equimolar amounts of both components, the solvent being a 1:1 mixture of dimethyl sulfoxide and acetic acid. Colorless platelike crystals were deposited from the solution at 40° C. Density was measured by flotation in a mixture of *n*-hexane and tetrachloromethane.

X-ray Work. Measurement of diffraction was carried out on a Rigaku four-circle diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.54184 Å). The unit-cell dimensions were obtained by least squares of high-angle reflections. Five reference reflections monitored showed no significant intensity deterioration during the data collection. Corrections were made for Lorentz and polarization factors but not for the absorption effects (μ = 9.56 cm^{-1}). Weak reflections below background were regarded as zero reflections. The standard deviations were estimated by the equation $\sigma^2(F_0) = \sigma_p^2(F_0) + qF_0^2$, where q was derived from measurement of the monitored reflections and $\sigma_p(F_0)$ is due to counting statistics (McCandlish & Stout, 1975). The crystal data and experimental details are summarized in Table I.

Structure Determination. The structure was solved with use of MULTAN 78 (Main et al., 1978), and their atomic parameters were refined by the full-matrix least-squares method. The quantity minimized was $\sum\omega(|F_0| - |F_c|)^2$ with $\omega = 1/[\sigma^2(F_0)]$. In the refinement, the zero-reflections with $|F_c| > F_{lim}$ were included by assuming $|F_0| = F_{lim}$ with $\omega = \omega(F_{lim})$, F_{lim} being the observed threshold value. In the later stage of refinement, all the hydrogen atoms were found on a difference map and included in the calculation of structure factors. The R value was 0.10 for 1035 reflections, and the maximum shift in the atomic parameters was 0.6σ. The final atomic parameters are given in Table II. The atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974). (See paragraph at end of paper regarding supplementary material.)

Table I: Crystal Data and Details of Experiment and Analysis for $C_{13}H_{17}N_9O_3$

Crystal Data	
fw	347.3
space group	$P2_1/c$
a (Å)	8.540 (1)
b (Å)	12.693 (3)
c (Å)	14.249 (2)
β (deg)	94.02 (1)
V (Å^3)	1545.5
Z	4
d_m (g cm^{-3})	1.50
d_c (g cm^{-3})	1.49
μ (Cu Kα) (cm^{-1})	9.56
Details of Experiment and Analysis	
radiation	Cu Kα (λ = 1.54184 Å)
crystal size (mm^3)	0.2 × 0.1 × 0.05
scan mode	ω scan
scan width (deg)	1.6 + tan θ
scan speed (min^{-1})	2 (in ω)
2θ range (deg)	3 – 110
no. of unique reflections	1940
no. of zero reflections	850
q	0.011
R	0.10
no. of reflections for R	1035
maximum shift of parameters	0.6σ

Table II: Fractional Coordinates and Isotropic Temperature Factors[a]

atom	x	y	z	B (Å^2)
C(1′)	0.473 (1)	−0.417 (9)	0.3637 (9)	3.5 ⟨16⟩
N(1′)	0.395 (1)	−0.3152 (6)	0.3471 (6)	2.4 ⟨12⟩
C(2′)	0.480 (1)	−0.224 (1)	0.3659 (7)	2.9 ⟨16⟩
O(2′)	0.6214 (8)	−0.2321 (5)	0.3965 (6)	3.4 ⟨17⟩
N(3′)	0.413 (1)	−0.1276 (6)	0.3513 (6)	2.4 ⟨10⟩
C(4′)	0.262 (1)	−0.1208 (9)	0.3177 (8)	3.0 ⟨15⟩
N(4′)	0.2014 (9)	−0.0256 (6)	0.3043 (6)	2.8 ⟨12⟩
C(5′)	0.171 (1)	−0.2153 (9)	0.2980 (8)	3.1 ⟨13⟩
C(6′)	0.243 (1)	−0.3079 (9)	0.3139 (8)	3.2 ⟨13⟩
N(1)	0.6117 (9)	0.0536 (6)	0.4007 (6)	2.6 ⟨10⟩
C(2)	0.766 (1)	0.0406 (9)	0.4378 (7)	2.6 ⟨8⟩
N(2)	0.8147 (9)	−0.0610 (6)	0.4459 (7)	3.5 ⟨28⟩
N(3)	0.8623 (9)	0.1192 (6)	0.4612 (6)	2.3 ⟨11⟩
C(4)	0.792 (1)	0.2140 (8)	0.4447 (7)	2.0 ⟨8⟩
C(5)	0.640 (1)	0.2358 (8)	0.4109 (8)	2.6 ⟨9⟩
C(6)	0.539 (1)	0.1511 (8)	0.3846 (8)	2.8 ⟨13⟩
O(6)	0.4050 (8)	0.1552 (5)	0.3483 (5)	3.1 ⟨15⟩
N(7)	0.6142 (9)	0.3442 (6)	0.4052 (6)	2.3 ⟨13⟩
C(8)	0.750 (1)	0.3854 (8)	0.4357 (9)	3.3 ⟨24⟩
N(9)	0.8616 (9)	0.3101 (6)	0.4617 (6)	2.1 ⟨8⟩
C(10)	1.025 (1)	0.3298 (8)	0.5049 (8)	2.3 ⟨8⟩
C(11)	1.149 (1)	0.3069 (8)	0.4360 (7)	2.9 ⟨10⟩
C(12)	1.145 (1)	0.3792 (9)	0.3510 (8)	2.8 ⟨19⟩
N(12)	1.231 (1)	0.3438 (8)	0.2830 (7)	4.0 ⟨14⟩
O(12)	1.0688 (8)	0.4618 (5)	0.3456 (5)	3.5 ⟨16⟩

[a] The B values are the equivalent isotropic temperature factors calculated from anisotropic thermal parameters with the equation $B = 8\pi^2(U_1 + U_2 + U_3)/3$, where U_1, U_2, and U_3 are principal components of the mean-squares displacement matrix U. Values in broken brackets are anisotropicity defined by $(\sum(B - 8\pi^2U_i)^2/3)^{1/2}$, and those in parentheses are estimated SD; they refer to last decimal places.

Results and Discussion

Molecular Structure. The bond distances and angles are shown in Figure 1. Molecular dimensions of purine, carbamoyl, and pyrimidine moieties are in good agreement with those in 9-ethylguanine (Destro et al., 1974), glutamine (Koetzle & Frey, 1973) and asparagine (Ramanadham et al., 1972), and 1-methylcytosine (Rossi & Kistenmacher, 1977), respectively. The torsion angle of the methylene chain in 9-(2-carbamoylethyl)guanine is 176.0 (9)° for C(5)-C(4)–N(9)–C(10), 75 (1)° for C(4)–N(9)–C(10)–C(11), 65 (1)° for N(9)–C(10)–C(11)–C(12), and 193.4 (4)° for C(10)–C-

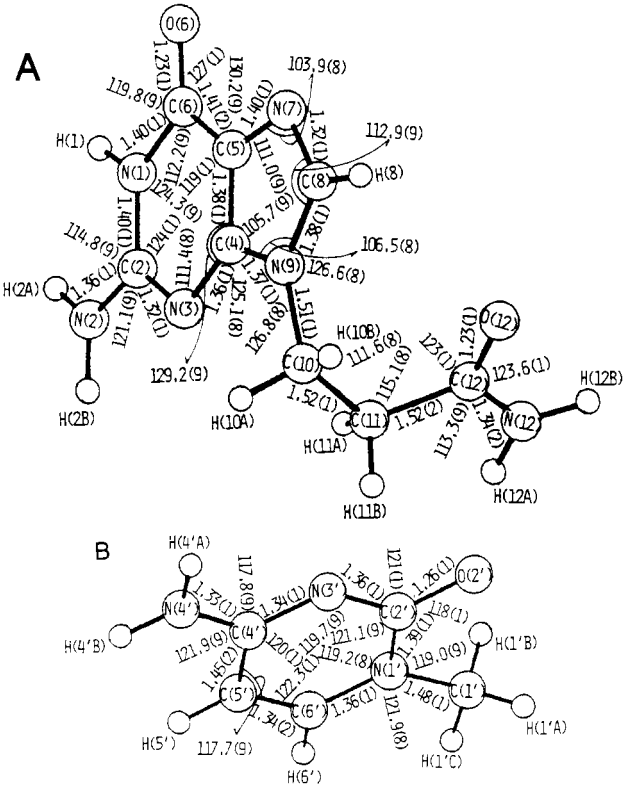

FIGURE 1: Bond distances (Å) and angles (deg) of (A) 9-(2-carbamoylethyl)guanine and (B) 1-methylcytosine. Standard deviations referring to last decimal places are given in parentheses.

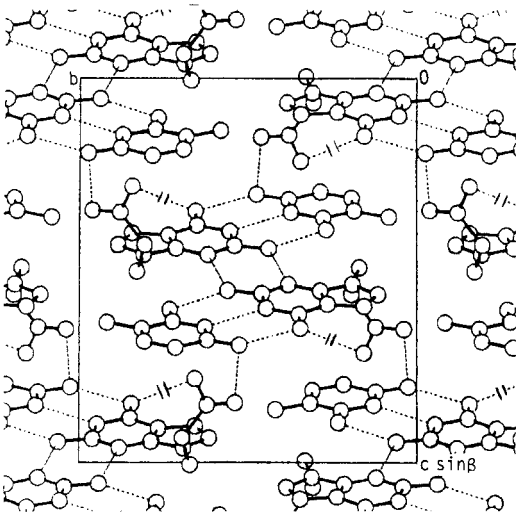

FIGURE 2: Crystal structure of 9-(2-carbamoylethyl)guanine–1-methylcytosine complex viewed down the *a* axis.

(11)–C(12)–N(12). The purine and pyrimidine moieties are planar within 0.012 and 0.004 Å, respectively, and their dihedral angle is 1.4 (3)°.

Crystal Structure. As shown in Figure 2, the guanine moiety and 1-methylcytosine form a Watson–Crick base pair through the hydrogen bonds of N(1)···N(3′) [2.92 (1) Å], N(2)···O(2′) [2.79 (1) Å], and O(6)···N(4′) [2.92 (1) Å]. These hydrogen-bond distances are similar to those found in the crystalline complexes of 9-ethylguanine–1-methylcytosine (O'Brien, 1976), deoxyguanosine–5-bromocytidine (Haschemeyer & Sobell, 1965), and 9-(2-carboxyethyl)guanine–1-methylcytosine (Fujita et al., 1984a).

The carbamoyl group interacts with two sites of the Watson–Crick pair through hydrogen bonds (Figure 3 and Table III). One site is O(6) of guanine at (1 + *x*, *y*, *z*), the N···O

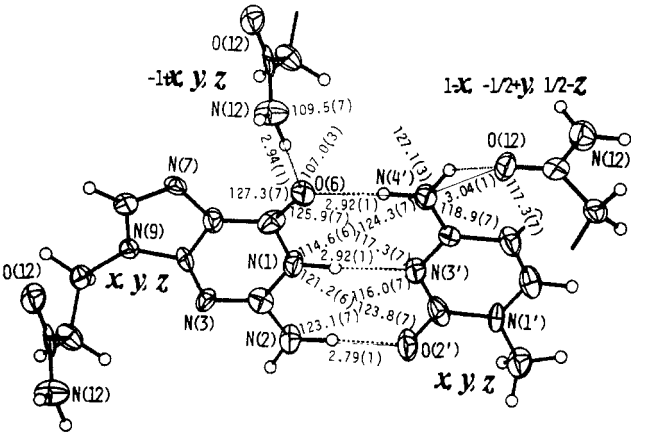

FIGURE 3: Binding mode and its geometrical data found in the ternary model system of guanine, cytosine, and the carbamoyl group. Thermal ellipsoids with 50% probability are also shown for non-hydrogen atoms. Other hydrogen-bond geometries are given in Table III.

Table III: Hydrogen-Bond Distances and Angles[a]

	distances (Å)		angles (deg)
H(2A)···O(2′)	1.79 (1)	N(2)–H(2A)···O(2′)	175.3 (5)
H(2B)···N(3)[b]	2.07 (1)	N(2)–H(2B)···N(3)[b]	175.3 (5)
N(2)···N(3)[b]	3.07 (1)	C(2)–N(2)···N(3)[b]	121.7 (7)
		O(2′)···N(2)···N(3)[b]	114.8 (4)
		N(2)···N(3)–C(2)[b]	116.8 (4)
		N(2)···N(3)–C(4)[b]	131.5 (6)
H(1)···N(3′)	1.92 (1)	N(1)–H(1)···N(3′)	175.1 (5)
H(4′A)···O(6)	1.92 (1)	N(4′)–H(4′A)···O(6)	176.2 (5)
H(4′B)···O(12)	2.18 (2)	N(4′)–H(4′A)···O(12)[c]	143.1 (5)
O(6)···H(12A)[d]	1.97 (1)	O(6)···H(12A)–N(12)[d]	162.0 (5)

[a] Standard deviations are given in parentheses, referring to the last decimal place. Other parameters are shown in Figure 3. [b] 2 − *x*, 1 − *y*, 1 − *z*. [c] −1 + *x*, *y*, *z*. [d] 1 − *x*, $-^1/_2 + y$, $^1/_2 - z$.

distance being 2.94 (1) Å. The other site is N(4′) of cytosine at (1 − *x*, 1/2 + *y*, 1/2 − *z*), the N···O distance being 3.04 (1) Å. In addition, the carbamoyl group is close to O(2′) of cytosine at (2 − *x*, 1/2 + *y*, 1/2 − z). The N(12)···O(2′) distance, 3.09 (1) Å, is within the range of hydrogen-bond distances, but the position of the hydrogen atoms is much deviated from linearity. In the model crystal, 1-(2-carbamoylethyl)cytosine, the carbamoyl group interacts with this site, O(2) of cytosine, through the N–H···O(2) hydrogen bond (Fujita et al., 1984b), the distance being 2.985 (4) Å and the N–H···O(2) angle being 170 (4)°. Therefore, the interaction between the carbamoyl group and O(2′) of the present model crystal may be a hydrogen bond distorted by the other hydrogen bonds.

The Watson–Crick base pairs are connected, as usually observed, with each other by N(2)-H···N(3) hydrogen bonds around the inversion center at (1, 1/2, 1/2).

Structural Correlation between Secondary Structural Fitting and Elementary Binding Patterns between Nucleotide Base and Amino Acid Side Chain. Structural determination of the specific DNA repressors has led to the binding model with double-stranded B-form DNA; its principal feature is the accomodation of α-helical segment in the major groove and β-ribbon in the minor groove (Anderson et al., 1981). Such a secondary structural fitting, however, would not be enough to accomplish mutual recognition. In addition to this, the elementary hydrogen bonds between nucleotide bases and amino acid side chains should be properly formed to stabilize the complex further. It is interesting to examine whether the secondary structural fitting imposes steric restrictions on the hydrogen-bonding patterns.

Table IV: Structural Correlation between Secondary Structural Fitting and Binding Mode of Asparagine and Glutamine Residues on α-Helical Segment with Guanine–Cytosine Base Pair in B-Form DNA

interaction	χ_1	χ_2	DNA strand[a]	binding site
Asn···G-C	215 ± 35		C ↑↑ 5′	guanine O(6) or N(7) cytosine N(4)
Gln···G-C	225 ± 75	180 ± 20	C ↑↑ 5′	guanine O(6) or N(7)
	190 ± 40	195 ± 35	N ↑↑ 5′	guanine O(6) or N(7)
	240 ± 10	290 ± 10	C ↑↑ 5′	guanine O(6)
			N ↑↑ 5′	guanine O(6)

[a] The 5′-end of DNA strand is in the same direction as the C-terminal (C ↑↑ 5′) or the N-terminal (N ↑↑ 5′) of the α-helical segment.

Table V: Structural Correlation between Secondary Structural Fitting and Binding Mode of Asparagine and Glutamine Residues on α-Helical Segment with Adenine–Thymine Base Pair in B-Form DNA

interaction	χ_1	χ_2	DNA strand[a]	binding site
Asn···A-T	230 ± 10		C ↑↑ 5′	adenine N(6) and N(7)
Gln···A-T	200 ± 20	170 ± 10	N ↑↑ 5′	adenine N(6) and N(7)

[a] The 5′-end of DNA strand is in the same direction as the C-teminal (C ↑↑ 5′) or the N-terminal (N ↑↑ 5′) of the α-helical segment.

We have sought, by using the molecular model and computer graphics, suitable secondary structural fitting between right-handed B-form DNA and α-helical segment that is compatible to the elementary binding pattern in the present study. The B-form DNA and α-helical segment were set up with the typical structural parameters (Arnott & Hukins, 1972; Schultz & Schirmer, 1979). As for the asparagine and glutamine residues on the α-helical segment, the conformation of the carbamoyl group was assumed to be a syn-planar arrangement for C^{β}—C^{α}—C=O because this conformation has been exclusively observed in the crystals of α,β-saturated amide (Leiserowitz & Schmidt, 1965). The remaining variables were χ_1 of Asn and χ_1 and χ_2 of Gln, as rotation around the C–C bond.

The results of examination are given in Table IV. Glutamine residue, for example, with $\chi_1 = 225 \pm 75°$ and $\chi_2 = 180 \pm 20°$ can be hydrogen bonded with O(6) or N(7) of guanine when the 5′-end of the nucleic acid strand is in the same direction as the C-terminal of the α-helical segment. From this table, it is obvious that the secondary structural fitting considerably limits the possible hydrogen bonding. Two of the successful models are illustrated in Figure 4. The following characteristics emerge. (i) The asparagine residue can bind with both guanine and cytosine bases only when the C-terminal of the α-helical segment and the 5′- end of the nucleic acid strand are in the same direction. (ii) The glutamine residue can bind only with guanine base in both directions of nucleic acid strand.

A similar consideration was applied to the interaction of asparagine and glutamine residues with adenine–thymine Watson–Crick base pair. Both residues can selectively bind with adenine base through the double hydrogen bonding, N(6)–H···O(carbonyl) and N(7)···H–N(amino).[1] The C-terminal of the α-helical segment containing the asparagine residue and the 5′-end of the nucleic acid strand are in the same direction. For the glutamine residue, on the other hand, the C-terminal and 5′-end are in the opposite direction. As can be seen from Table V, the hydrogen bonding with adenine–thymine pair is restricted to a greater extent. These steric restrictions are summarized in Figure 5; Asn and Gln on the α-helix in the major groove of DNA can bind with the circled bases of each strand.

[1] This hydrogen-bonding pattern has been found in the crystal of 9-ethyladenine–parabanic acid–oxaluric acid complex (Shieh & Voet, 1976).

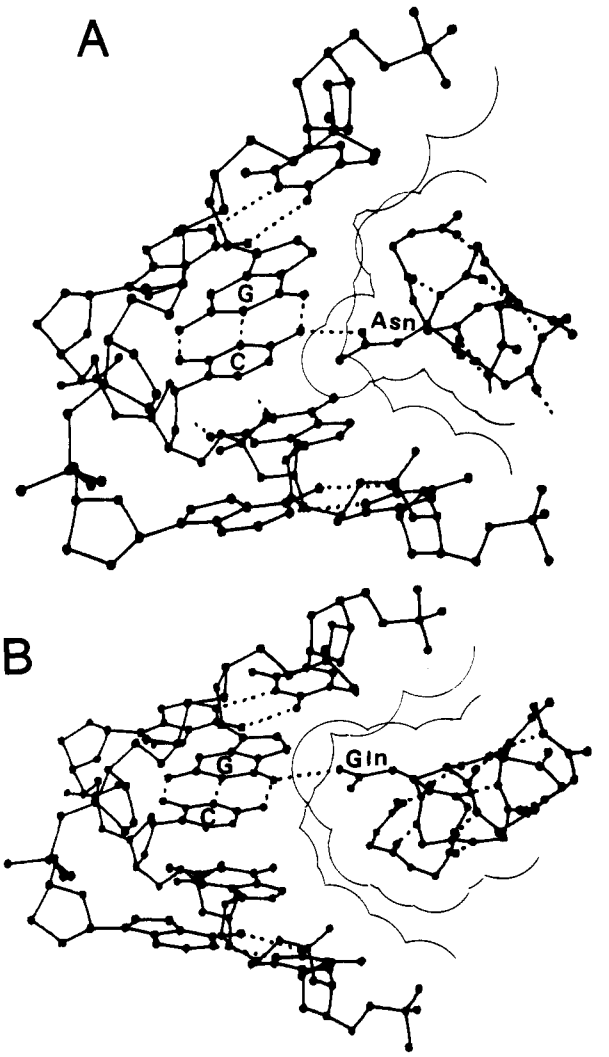

FIGURE 4: Successful binding models compatible with the secondary structural fitting between α-helical segment and B-form DNA. (A) Asparagine or (B) glutamine residue on α-helical segment is hydrogen bonded with guanine–cytosine pair.

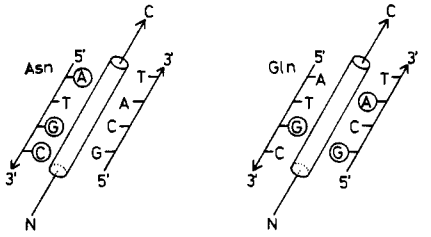

FIGURE 5: Steric restrictions imposed by the secondary structural fitting on the binding of asparagine and glutamine residues with base pairs. Asparagine (left) and glutamine (right) residues can be hydrogen bonded only with the nucleotide bases in circles.

Applications. Taking account of the steric restrictions discussed in the preceding section, we have investigated the specific interaction between the α-helix of cro repressor and the operator site, OR3 (Johnson et al., 1978). The operator binding domain of cro repressor is an α–helical segment of some 10 amino acid residues (Anderson et al., 1981). We have noticed in the partial amino acid sequence that the amino acid residues with functional groups are found at every four residues. These residues, Gln-27, Asn-31, and His-35, are located on the same side of the α-helix. When the α-helix fits into

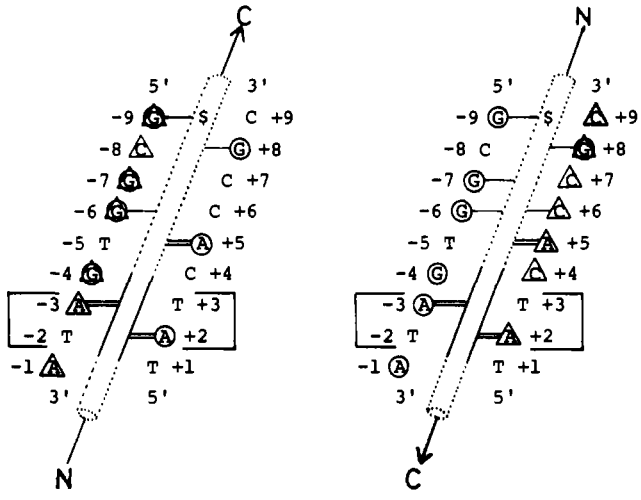

FIGURE 6: Specific hydrogen bonds between the operator-binding α-helical segment of cro repressor and the operator site, OR3, allowable under the steric restrictions. Bases in circles make hydrogen bonds with Gln and those in triangles with Asn. The number of the horizontal lines corresponds to that of hydrogen bonds. The nucleotide base sequence has an approximate 2-fold symmetry at the position denoted with a dollar sign. Local numbering is given for the bases in one strand, and the ith base constitutes base pair with the $-i$th base in the other strand.

the major groove of DNA as suggested in the binding model (Anderson et al., 1981), its helical axis is tilted by 32° to the base pairs, and the translation between the ith and $(i + 4)$th α-carbons is about 3.2 Å along the helical axis of B DNA, nearly equal to the spacing between base pairs, 3.4 Å. So, Gln-27, Asn-31, and His-35 can simultaneously bind with three successive base pairs.

When the α-helix fits into the major groove, nucleotide bases capable of hydrogen bonding with Gln-27 and Asn-31 were sought; the bases for Gln-27 are in circles and those for Asn-31 are in triangles in Figure 6, where the horizontal line represents a hydrogen bond. As seen from the Figure, the maximum number of hydrogen bonds of successive Asn and Gln can be formed at the nucleotide bases closed in brackets.

However, the number of specific hydrogen bonds and the position of nucleotide bases are the same for two alternative axial directions of α-helix. Such ambiguity may be removed by considering the interaction of His-35. Studies on the binary models indicate the characteristic hydrogen bonds between protonated histidine and O(6) and N(7) of guanine (S. Fujita, A. Takenaka, and Y. Sasada, unpublished results). It is suggested that His-35 binds only with guanine bases just above the bases in brackets (See Figure 6). The direction of the α-helix is in agreement with that proposed from the three-dimensional structure of the oligomeric cro repressor (Anderson et al., 1981; Ohlendorf et al., 1982). As the successive three nucleotide bases are the symmetric part, the cro repressor dimer may stably bind with OR3.

According to the interaction scheme thus obtained, a molecular model was constructed with a little deformation of α-helix. In the interaction model proposed by Ohlendorf et al. (1982), Gln-27, Ser-28, and Lys-32 are hydrogen bonded with just the same bases as ours. This corresponds to the anticlockwise rotation of the α-helix by 90° around the helical axis. A merit of our model is that the strong basic residue, Lys-32, can be hydrogen bonded with phosphate of the DNA backbone.

Supplementary Material Available

Four tables depicting observed and calculated structure factors, anisotropic temperature factors of non-hydrogen atoms, coordinates of hydrogen atoms, and least-squares planes and deviations of atoms from purine and pyrimidine rings (13 pages). Ordering information is given on any current masthead page.

Registry No. Gln, 56-85-9; Asn, 70-47-3; guanine, 73-40-5; cytosine, 71-30-7; adenine, 73-24-5; thymine, 65-71-4; N^2-acetylguanine, 19962-37-9; β-propiolactone, 57-57-8; 9-(2-carboxyethyl)guanine, 84628-21-7.

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