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The Predicted Secondary Structure of the N-Terminal Sequence of the *Lac* Repressor and Proposed Models for Its Complexation to the *Lac* Operator†

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ABSTRACT: Rules for the prediction of protein conformation (Chou, P. Y., and Fasman, G. D. (1974), *Biochemistry 13,* 211, 222) have been applied to the N-terminal sequence 1–60 of the *lac* repressor. This analysis predicts β structure at sequences 4–9 and 15–20, helices at 26–32, 38–45, and 52–57, and β turns at 48–51 and 14–17. Repressor mutants lacking operator binding capacity in which Pro replaces Ser-16 and Ala replaces Thr-19 (Weber, K., Platt, T., Ganem, D., and Miller, J. H. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 3624) have no effect on the prediction of β structure at residues 15 to 20, which suggests that the polar side chains of Ser-16, Tyr-17, Gln-18, and Thr-19 participate in intermolecular hydrogen bonding with complementary polar groups on the *lac* operator. The loss of operator binding capacity on replacement of Ala by Val at position 53 in the repressor results from a predicted secondary structural change from helix to β structure for residues 52–57 which can be transmitted to the N-terminal sequence via a β turn at residues 48–51. The basic residues at positions 33, 35, and 37 between the helical regions 26–32 and 38–45 probably bind to the phosphate groups on the operator on complexation. It is proposed that complex formation involves the interaction of either a β structure (residues 15–20) or a right-hand twisted antiparallel β-pleated sheet (residues 4–9 and 12–20) with operator DNA.

The regulation of transcription of the structural genes of the *lac* operon (Beckwith and Zipser, 1971) occurs on complex formation between the *lac* operator and *lac* repressor in the absence of inducer (Gilbert and Muller-Hill, 1967; Ptashne, 1967).

The isolated sequence of the *lac* operator is double-stranded and contains 27 base pairs with regions of twofold symmetry (Gilbert and Maxam, 1973) consistent with predictions from genetic studies (Sadler and Smith, 1971).

The *lac* repressor is a tetramer with identical subunits (Gilbert and Muller-Hill, 1966), each chain comprised of 347 amino acids (Muller-Hill et al., 1968) of known sequence (Beyreuther et al., 1973). Biochemical experiments (Platt et al., 1973) along with genetic studies (Muller-Hill et al., 1968; Davies and Jacob, 1968; Adler et al., 1972) strongly suggest that the amino-terminal region of the *lac* repressor is necessary for complex formation to the *lac* operator.

Results and Discussion

(1) Application of the Chou-Fasman Rules to N Terminus of Lac Repressor. The N-terminus (1–60) sequence of the *lac* repressor is presented in Figure 1. Under each amino acid are the helical potentials (α) in the first row and β-structure potentials (β) in the second row based on protein conformation prediction rules derived by Chou and Fasman (1974a,b).

The conformational parameters P_α, P_β, and P_t for amino acids in helices, β structures, and β turns, respectively (Chou and Fasman, 1974a,b), were utilized to predict helical regions between residues 8–13, 26–32, 38–45, and 52–57, β-structure regions between residues 4–9 and 15–20, and β turns involving tetrapeptides 14–17 and 48–51 (Table I).

(2) Repressor Mutants and Predicted Secondary Structure. Amino acid changes at positions 16 (Ser to Pro), 19 (Thr to Ala), and 53 (Ala to Val) are sufficient to eliminate operator-repressor binding (Weber et al., 1972). By contrast, four different amino acids are tolerated at position 26 with no effect on any functional properties of the *lac* repressor.

Chou-Fasman calculations of $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ for the mutant-16 *lac* repressor and mutant-19 *lac* repressor suggest that the region 15 to 20 remains as a β structure in both mutant repressors (Table II). Since the substitution from polar to nonpolar amino acids has no effect on the secondary structure between residues 15 and 20, hydrogen bond formation between the side chains of Ser-16 and Thr-19 with a complementary surface on the *lac* operator is an absolute requirement for complexation.

Fragment 52–57 with Ala at position 53 is predicted to be a helix [$\langle P_\alpha \rangle = 1.29$, $\langle P_\beta \rangle = 1.21$] while the same region with Val at position 53 is predicted to be a β structure [$\langle P_\alpha \rangle = 1.23$, $\langle P_\beta \rangle = 1.35$] (Table II). Since the region 52–57 is linked to the N-terminal end by a β turn at 48–51, any changes in secondary structure at 52–57 would dramat-

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	1	2	3	4	5	6	7	8	9	10	11	12
	Met	Lys	Pro	Val	Thr	Leu	Tyr	Asp	Val	Ala	Glu	Tyr
α	h	I	B	h	i	H	b	i	h	H	H	b
β	H	b	b	H	h	h	h	i	H	I	B	H
	13	14	15	16	17	18	19	20	21	22	23	24
	Ala	Gly	Val	Ser	Tyr	Gln	Thr	Val	Ser	Arg	Val	Val
α	H	B	h	i	b	h	i	h	i	i	h	h
β	I	i	H	b	h	h	h	H	b	i	H	H
	25	26	27	28	29	30	31	32	33	34	35	36
	Asn	Gln	Ala	Ser	His	Val	Ser	Ala	Lys	Thr	Arg	Glu
α	b	h	H	i	h	h	i	H	I	i	i	H
β	b	h	I	b	b	H	b	I	b	h	i	B
	37	38	39	40	41	42	43	44	45	46	47	48
	Lys	Val	Glu	Ala	Ala	Met	Ala	Glu	Leu	Asn	Tyr	Ile
α	I	h	H	H	H	h	H	H	H	b	b	I
β	b	H	B	I	I	H	I	B	h	b	h	H
	49	50	51	52	53	54	55	56	57	58	59	60
	Pro	Asn	Arg	Val	Ala	Gln	Gln	Leu	Ala	Gly	Lys	Gln
α	B	b	i	h	H	h	h	H	H	B	I	h
β	b	b	i	H	I	h	h	h	I	i	b	h

FIGURE 1: The amino acid sequence of the N terminus (1-60) of the *lac* repressor as elucidated by Beyreuther et al. (1973). The amino acids are characterized as H (strong former), h (medium former), I (weak former), i (indifferent), b (breaker), and B (strong breaker) of helices in the first row and β structure in the second row. Predicted secondary structural regions are enclosed in boxes.

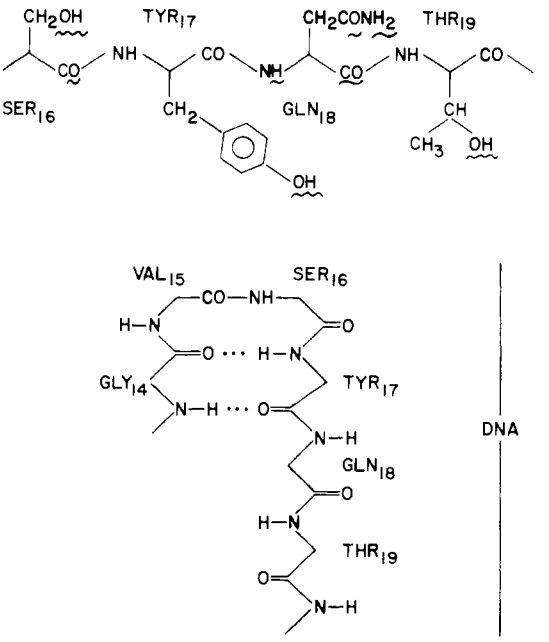

FIGURE 2: The secondary structure of the N-terminal sequence 14 to 19. A β turn is predicted at residues 15 and 16 with the rest of the sequence in a β structure.

Table I: Prediction of Helical, β-Structure, and β-Turn Regions for the N-Terminal Sequence 1–60 of the *lac* Repressor.[a]

Sequence	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	$\langle P_t \rangle$	Prediction
1–3	0.95	1.01		Coil
4–9	1.01	1.30		β structure
10–14	1.11	0.86		Coil
15–20	0.95	1.29		β structure[b]
21–25	0.92	1.11		Coil
26–32	1.15	1.00		Helix
33–37	1.06	0.77		Coil
38–45	1.39	1.00		Helix
46–47	0.67	0.97		Coil
48–51	0.78	0.94	1.19	β turn[c]
52–57	1.29	1.21		Helix

[a] Based on Chou–Fasman rules (Chou and Fasman, 1974a,b). [b] Residues 14 to 17 may occur as a β turn. $\langle P_\alpha \rangle$ = 0.79, $\langle P_\beta \rangle$ = 1.12, $\langle P_t \rangle$ = 1.20 and $\langle P_t \rangle = 0.3 \times 10^{-4}$. [c] $\langle P_t \rangle = 4 \times 10^{-4}$.

ically affect the N-terminal conformation of the *lac* repressor and affect its binding to the *lac* operator.

(3) Models for the Lac Operator–Lac Repressor Complex. Enzymatic digestion studies (Platt et al., 1973) have demonstrated cleavage at the N- and C-terminal ends of the *lac* repressor and suggest accessibility of these regions for interaction with the *lac* operator.

ELECTROSTATIC INTERACTIONS. The cluster of basic residues Lys-33, Arg-35, and Lys-37 between the helical regions 26 to 32 and 38 to 45 may form part of the binding site and participate in hydrogen bonds with phosphate groups on the operator. (For examples see Cotton et al., 1973; Aoki et al., 1971; Saenger and Wagner, 1972; Cotton and Hazen, 1971; Adams et al., 1973).

β-STRUCTURE-DNA INTERACTIONS. One model considers a β structure extending from residues 15 to 20 interacting with the base pair edges of the symmetrical hexanucleotide fragment in one of the grooves of the DNA structure (Figure 2). The side chains of Ser-16, Tyr-17, Gln-18, and Thr-19 can participate in hydrogen bond formation with the nucleic acid bases (for examples see Adams et al., 1973; Richards et al., 1972) and phosphate groups (for example, see Watenpaugh et al., 1973; Cotton and Hazen, 1971), as can the Gln-18 peptide NH and Ser-16 and Gln-

Table II: Prediction of Helical or β Structures for Normal and Mutant Sequences 15 to 20 and 52 to 57 in the *lac* Repressor.

							$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	Prediction
	15	16	17	18	19	20			
Native	Val	Ser	Tyr	Gln	Thr	Val	0.95	1.29	β structure
Mutant 16; Ser to Pro	Val	*Pro*	Tyr	Gln	Thr	Val	0.91	1.27	β structure
Mutant 19; Thr to Ala	Val	Ser	Tyr	Gln	*Ala*	Val	1.05	1.25	β structure
	52	53	54	55	56	57			
Native	Val	Ala	Gln	Gln	Leu	Ala	1.29	1.21	Helix
Mutant 53; Ala to Val	Val	*Val*	Gln	Gln	Leu	Ala	1.23	1.35	β structure

18 peptide carbonyl groups (for example, see Watenpaugh et al., 1973; Richards et al., 1972). Tyr-17 is available for stacking (for examples see Sundaralingam and Arora, 1972; Watenpaugh et al., 1973) and/or intercalation (for examples see Gabbay et al., 1973; Helene, 1971a,b; Novak and Dohnal, 1973).

ANTIPARALLEL β-PLEATED SHEET-DNA INTERACTIONS. Sections of the β structure extending from residues 4 to 9 and 15 to 20 can form an antiparallel β-pleated sheet if the chain can undergo reversal between residues 9 and 15. In an alternate model, a right-hand twisted antiparallel β-pleated sheet (see Chothia, 1974) can interact by hydrogen-bond formation and hydrophobic interactions with polar groups on the base pair edges in the major groove of the DNA double helix (see Carter and Kraut, 1974). The regions 4–9 and 15–20 contain five nonpolar and seven polar amino acids. Two tyrosines on antiparallel strands on the same side of the pleated sheet but not directly opposite each other may simultaneously intercalate between adjacent adenine base pairs in the *lac* operator sequence

AATT
TTAA

HELICES AND β STRUCTURE. Adler et al. (1972) have proposed a model for the complex in which a helical segment (17–33) on the repressor interacts with double-stranded operator in its deep groove. By contrast, this investigation, and a parallel study on the entire *lac* repressor (Chou et al., 1975), emphasize the important role that β-structural regions may play in protein–nucleic acid interactions.

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