An IgM Waldenström with Specificity against Phosphorylcholine†

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ABSTRACT: Anti-phosphorylcholine specificity has recently been shown to occur with relatively high incidence among IgA myeloma proteins secreted by oil-induced plasma cell tumors in the BALB/c strain of mice. A similar screening of human myeloma sera indicates that in man activity for phosphorylcholine is very rare. Among 904 human sera containing IgG, IgA, and IgM M-components only one reacted with phosphorylcholine-containing antigens. This serum was obtained from a patient with macroglobulinemia Waldenström. The active homogeneous protein could be isolated by affinity chromatography using a Sepharose-phosphorylcholine immunoadsorbent. It was an IgM immunoglobulin; the light chains were of the κ type. The association constant for the reaction with phosphorylcholine was homogeneous and equalled 6.4×10^4 l. mol^{-1} at 25° and 8.1×10^4 l. mol^{-1} at 2°, indicating that the binding reaction is exothermic. The valences of the pentamer IgM, the 7S IgM subunit produced by reduction with cysteine, and the Fab fragment obtained by cleavage with papain were 10, 2, and 1, respectively. By all criteria available for antibody-like binding such as high specificity, restriction of the binding sites to the Fab part of the molecule and correct stoichiometry this IgM exhibits the fundamental characteristics associated with conventionally induced antibodies.

Attempts to define the structure and the topography of the antibody combining site have been hampered by the heterogeneity of conventionally induced antibodies. Chemically homogeneous immunoglobulins (M-components) produced by plasma cell tumors in man and mice have been reported to combine with antigens or haptens (reviewed by Metzger (1969), Potter (1971) and Seligmann and Brouet (1973)). Such antigen binding monoclonal immunoglobulins are of great value in overcoming the structural ambiguities resulting from antibody heterogeneity. However, since the true immunogen when dealing with M-components exhibiting binding activity is unknown and in view of the possibility of unspecific interactions between hydrophobic regions on the Fab fragment of immunoglobulins and aromatic derivatives (Parker and Osterland, 1970) caution is warranted in interpreting the specificity of such reactions and their relevance to antigen–antibody reactions.

IgA M-components derived from plasma cell tumors induced in the inbred BALB/c strain of mice have been reported to precipitate the pneumococcus C polysaccharide (PnC)[1] with high frequency (Potter and Lieberman, 1970). Leon and Young (1971) demonstrated that the immunodominant group on the PnC identified by the IgA myeloma proteins is phosphorylcholine, which is a nonhydrophobic ligand. By all measurable criteria the reactions of these myeloma proteins with the antigen PnC or the hapten phosphorylcholine parallel authentic antigen–antibody reactions. Moreover, the identity of idiotypic determinants of some of these IgA's with those of mouse antibodies in the population induced by PnC (Cohn *et al.*, 1969; Claflin *et al.*, 1974) and with naturally occurring antibodies to PnC (Lieberman *et al.*, 1974) argues for a close analogy to conventional antibodies.

The phosphorylcholine binding M-components thus prove to be a valuable tool for the characterization of the structural basis of antibody specificity. Yet in man binding activity against phosphorylcholine has been detected but in a single case (Riesen *et al.*, 1973). This paper reports on this monoclonal human immunoglobulin which is an IgM Waldenström.

Experimental Procedure

Material

Serum FR was obtained from patient FR, an 82 year old man, suffering from macroglobulinemia Waldenström. The patient was first admitted to the hospital following a heart attack at the age of 81. He died at the age of 82 from pneumonia. Post mortem findings were compatible with the diagnosis of Waldenström's macroglobulinemia. There were no signs of hyperlipidemia nor xanthomatosis, which would indicate an interaction of the M-component with the patient's own serum lipoproteins or lipids.

Methods

Screening Procedure. Screening was performed by double diffusion in agar gels in the presence and absence of 0.01 M EDTA. The PnC and a polysaccharide isolated from the species of *Lactobacillus acidophilus* occurring in the normal intestinal flora of BALB/c mice (Potter, 1970) were used for the detection of precipitating activity. Both antigens have previously been shown to precipitate the mouse anti-phosphorylcholine IgA myeloma proteins (Potter and Lieberman, 1970). The concentration of the antigens was 2 mg/ml.

Isolation of Phosphorylcholine Reactive Protein. An immunoadsorbent consisting of phosphorylcholine coupled to Sepharose 4B was prepared by the method of Chesebro and Metzger (1972). Essentially *p*-nitrophenylphosphorylcholine was synthesized from choline iodide (Fluka) and *p*-ni-

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[1] Abbreviations used are: PnC, Pneumococcus C polysaccharide; Nbs_2, 5,5′-dithiobis(nitrobenzoic acid).

trophenyl phosphorodichloridate (Aldrich). The nitro group was reduced and diazotized. The *p*-diazoniumphenylphosphorylcholine was then reacted with glycyltyrosine (Fluka) which had previously been coupled to CNBr activated Sepharose 4B (Pharmacia) using the procedure of Cuatrecasas (1970). Serum of patient FR which precipitated the PnC and the *Lactobacillus* antigen was applied to a column packed with the phosphorylcholine immunoadsorbent. The column was washed with pH 8.0 borate-buffered saline until the effluent had an absorbance of less than 0.05 at 280 mμ. The specifically bound protein was eluted with 10^{-3} M phosphorylcholine (Fluka) in borate-buffered saline. The phosphorylcholine borate-buffered saline buffer was then exchanged for borate-buffered saline by filtration through Sephadex G-25.

Determination of the Concentration of IgM. The concentration of IgM was estimated by the radial immunodiffusion method introduced by Mancini *et al.* (1965) using Partigen plates (Behring-Werke, Marburg/Lahn) or spectrophotometrically on isolated preparations based on the extinction coefficient $E_{280}(1\%)$ of 12.5 according to Metzger (1970).

Sedimentation Measurements. The sedimentation coefficients were kindly determined by Professor v. Tavel (Theodor-Kocher Institute, University of Berne) on a Spinco analytical ultracentrifuge. The observed sedimentation coefficients were corrected to 20° and water according to Jahnke and Scholtan (1960). The protein concentration was 0.8%.

Preparation of 7S Subunits (IgM_s). The subunits were produced by reduction with 0.02 M cysteine or with 0.02 M dithiothreitol (Sigma) in 0.1 M Tris-NaCl buffer (pH 8.6) at room temperature for 60 min, followed by alkylation with a 10% molar excess (over reagent-SH) of iodoacetamide (Fluka). IgM_s was then purified by Sephadex G-200 gel filtration.

Analysis of SH. IgM (FR) was reduced with 0.02 M dithiothreitol in 0.1 M Tris-NaCl (pH 8.6) or with 0.1 M mercaptoethanol in 0.1 M Tris-NaCl (pH 8.2). The liberated sulfhydryl groups were determined by the 5,5′-dithiobis(nitrobenzoic acid) (Nbs_2, Merck) reagent of Ellman (1959), following the method described by Miller and Metzger (1965). Essentially the reduced samples were precipitated by 5% trichloroacetic acid, washed five times with trichloroacetic acid and redissolved in 5 M guanidine hydrochloride containing 10^{-4} M EDTA, cleared from trace turbidity by ultracentrifugation at 25000*g*, and reacted with Nbs_2 at pH 9.1. A molar extinction coefficient of 13,600 at 412 nm was used for the reduced Nbs_2.

Inhibition of Precipitation by Haptens. IgM (FR) was reacted with the *Lactobacillus* antigen by double diffusion in agar gels for 20 hr. The gels were then immersed in solutions containing the inhibitors at various concentrations. Inhibition of precipitation was indicated by redissolution of the precipitin lines.

Preparation of Proteolytic Fragments. F(c)μ fragment of IgM (FR) was produced at 56° by trypsin digestion according to Plaut and Tomasi (1970). Fab fragment was prepared as described by Mihaesco and Seligmann (1968) by enzymatic cleavage of IgM with mercuripapain for 24 hr at 37°. Upon filtration through Sephadex G-200 the second peak contained the Fab fragment. This fraction reacted with anti-κ antiserum, however, not with anti-μ.

Equilibrium dialysis experiments were carried out in borate-buffered saline (pH 8.0) at 25° and at 2° in 0.1-ml dialysis cells (Bolab. Inc.) using phoshoryl[*methyl*-^{14}C]-choline (specific activity 50 Ci.mol) (New England Nuclear). The protein concentration was routinely between 3 and 6 mg/ml.

Table I: Screening of Human Sera Containing M-Components for Binding Activity against the Pneumococcus C Polysaccharide (PnC) and the *Lactobacillus* Antigen.

M-Components	Number of Cases	Positive Reaction in Absence of EDTA		Positive Reaction in the Presence of EDTA	
		With PnC	With *Lactobacillus* Antigen	With PnC	With *Lactobacillus* Antigen
IgG	665	21	21	0	0
IgA	169	2	2	0	0
IgM	70	3	3	1	1
Total	904	26	26	1	1

Farr Test. Serum (FR) (25 μl) containing 8 mg of M-component/ml was incubated with 25 μl of phosphoryl-[*methyl*-^{14}C]choline for 2 hr; 50 μl of saturated $(NH_4)_2SO_4$ were then added, the precipitate was centrifuged and the supernatant counted in a liquid scintillation counter (Nuclear Chicago).

The binding data were plotted according to $r/c = Kn - Kr$ (Eisen, 1964).

Results

Screening for Active M-Components. The results of the screening of human sera containing M-components for precipitating activity against the pneumococcus C polysaccharide and the *Lactobacillus* antigen are summarized in Table I. In order to exclude the possibility of falsely positive reactions with the C-reactive protein, which is known to precipitate the pneumococcus C polysaccharide in the presence of Ca^{2+} (Volanakis and Kaplan 1971) the sera were also screened in agar containing 0.01 M EDTA. In only one case was a precipitin line formed also when Ca^{2+} was complexed by EDTA. The M-component in this serum (serum of patient FR) was an IgM, type κ.

Purification of IgM (FR). Serum of patient FR was passed over the phosphorylcholine-Sepharose immunoadsorbent and a single protein (IgM, κ) was eluted with hapten. Purity of the isolated IgM was assessed by immunoelectrophoresis using antisera to whole normal human serum, IgM, κ and λ light chains (Figure 1). The concentration of the purified IgM was at 40 mg/ml. The yield of the eluted protein was 80–90% based on the concentration of IgM in the serum (8 mg/ml) as estimated by the radial immunodiffusion method and on spectrophotometrical determination of the protein concentration eluted from the immunoadsorbent.

Sedimentation studies of the IgM preparation isolated by the immunoadsorbent indicated the presence of three different components: one major component (80%) sedimenting at 18.8 $S_{20,w}$ and two minor components (20%) sedimenting at 7.1 $S_{20,w}$ and 27.2 $S_{20,w}$, respectively.

Reduction of IgM (FR). In four different determinations analysis of the sulfhydryl groups liberated after reduction of 19S IgM (FR) with dithiothreitol or with mercaptoethanol in aqueous solution yielded 50.5 ± 1.4 mol of SH/mol of

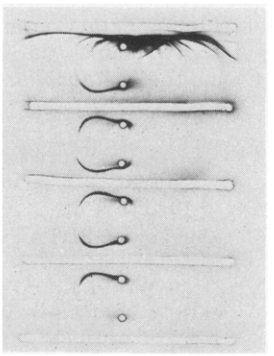

FIGURE 1: Immunoelectrophoretic analysis of IgM (FR) isolated by immunoadsorption: (1) anti whole human serum; (2) serum FR; (3) anti IgM; (4) IgM FR (isolated); (5) anti κ (6) anti λ.

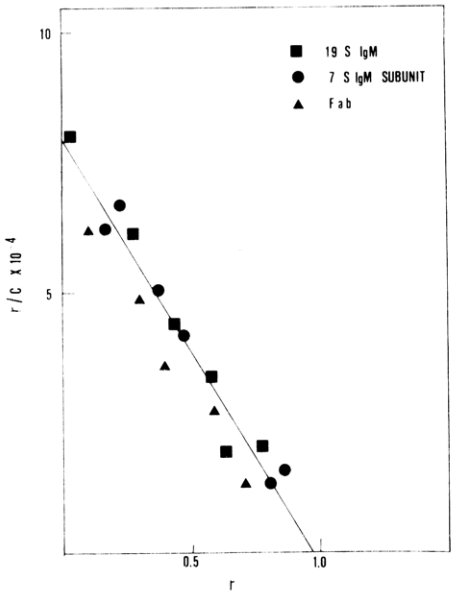

FIGURE 2: Scatchard plot of the binding of phosphoryl[*methyl*-^{14}C]choline by 19S IgM (FR), 7S IgM (FR) subunit, and Fab fragment studied by equilibrium dialysis. r is defined as moles of hapten bound per mole of heavy–light chain pair of each molecular species; c is the concentration of unbound ligand.

IgM, which is in accordance with the findings of Miller and Metzger (1965) and Schrohenloher and Mestecky (1973).

Inhibition of Precipitation by Choline Derivatives and N-Acetylgalactosamine. The isolated IgM (FR) precipitated the PnC and the *Lactobacillus* antigen. In the inhibition of precipitation studies, the precipitin line between IgM (FR) and the *Lactobacillus* antigen was redissolved by phosphorylcholine at concentrations $>10^{-5}$ M, and by choline chloride and acetylcholine at concentrations $>10^{-4}$ M. *N*-Acetylgalactosamine proved not to inhibit up to a concentration of 10^{-2} M.

Binding Studies. The results from equilibrium dialysis experiments with phosphoryl[*methyl*-^{14}C]choline are given in Figures 2 and 3. The straight line obtained by plotting r/c *vs.* r indicates that the combining sites of the IgM under study are homogeneous with respect to binding affinity for phosphorylcholine. Structural homogeneity is indicated by the fact that a unique amino acid sequence in the N-terminal 40 residues of the light chain from protein FR was found (Riesen and Jaton, unpublished). In order to determine the valence of the pentamer 19S IgM the preparation obtained from the immunoadsorbent was additionally

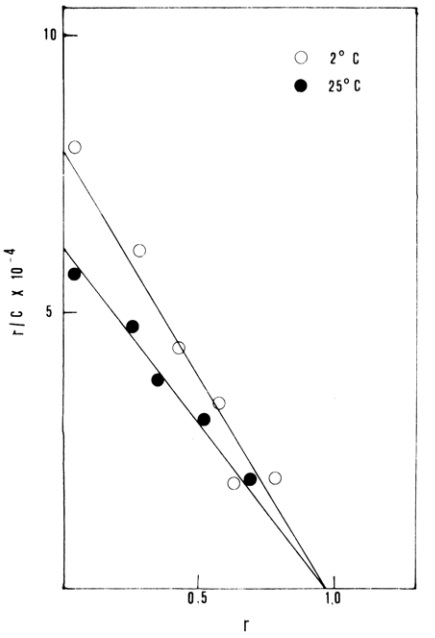

FIGURE 3: Scatchard plot of the binding of phosphoryl[*methyl*-^{14}C]choline by 19S IgM at 2 and 25° studied by equilibrium dialysis. r and c are defined as in Figure 2.

Table II: Association Constants and Valences for 19S IgM (FR), Its 7S Subunits, and Its Fab Fragments with Phosphoryl[*methyl*-^{14}C]choline.

	$K \times 10^{-4}$ l. mol^{-1}		
	2°	25°	Valence
IgM	8.1 ± 0.4	6.4 ± 0.4	9.6 ± 0.5
IgM_s	8.0 ± 0.4		2.0 ± 0.1
Fab μ	7.8 ± 0.4		0.9 ± 0.1

passed over Sephadex G-200. In Figure 2 the data from experiments with the 19S IgM, the 7S IgMs, and the Fab fragment are shown. Table II summarizes the association constants and the valences observed with the mentioned protein fractions. The results indicate that the pentamer IgM has 10, the 7S subunit, prepared by cysteine reduction, 2, and the Fab fragment, 1 combining sites per molecule, respectively. In samples reduced with dithiothreitol the valence was 1.6. A similar observation has been made by Ashman and Metzger (1969) and is probably due to dissociation into free chains, which occurs with dithiothreitol reduction, whereas with cysteine reduction the chains remain disulfide linked (Miller and Metzger, 1965). With the $(Fc)_5\mu$ fragment no significant binding of phosphorylcholine was noted. The crude isolated IgM preparation containing the 7S, the 19S, and the 27S components gave a valence of 8. A valence of 8 was furthermore obtained from binding data with the whole serum of patient FR using the Farr test. The association constant calculated from these experiments was within the error of the value obtained with the isolated IgM by equilibrium dialysis.

Figure 3 illustrates the data obtained from equilibrium dialysis experiments with the pentamer IgM studied at 2 and at 25°. The association constants were $8.1 \pm 0.4 \times 10^4$ l. mol^{-1} at 2° and $6.4 \pm 0.4 \times 10^4$ l. mol^{-1} at 25°. The higher value at 2° indicates that the binding reaction is exo-

thermic. ΔH calculated from association constant measurements as $\Delta H = -R[\ln K(T_2) - \ln K(T_1)]/(1/T_2) - (1/T_1)$ gave a value of -1.7 ± 0.8 K cal mol^{-1}. ΔS obtained from $\Delta G = \Delta H - T\Delta S$ was found to be 16 ± 2 cal deg^{-1} mol^{-1}. ΔG calculated from $\Delta G = -RT \ln K$ was -6.55 ± 0.04 kcal mol^{-1} at 25°.

Discussion

Several human M-components have been reported to combine with antigens or haptens (reviewed by Metzger (1969), Potter (1971), and Seligmann and Brouet (1973)). A great number of these proteins seem to react with complex antigens which are structurally not well defined or with the hapten dinitrophenyl. This ligand is a strongly hydrophobic chemical, which may interact in an unspecific way with hydrophobic regions on the Fab fragment (Parker and Osterland, 1970). The significance of the interactions of myeloma proteins with this compound is still unclear. The crucial question, *i.e.*, whether the observed interactions of human myeloma proteins are true antigen–antibody interactions and which determinant on the antigen is involved in the binding reaction, could therefore not be answered in the majority of the reported cases.

IgM (FR), described above, reacts with antigens in a manner typical of antigen–antibody systems. It precipitates the pneumococcus C polysaccharide and a polysaccharide derived from the strain of *Lactobacillus acidophilus*. This precipitation is specifically inhibited by small molecules, structurally related to these two antigens, such as phosphorylcholine, acetylcholine, or choline chloride. With *N*-acetylgalactosamine, which is a potential determinant as it occurs on the pneumococcus C polysaccharide (Gottschlich and Liu, 1967), no inhibition was observed. The hapten inhibition studies thus indicate that IgM (FR), like the BALB/c mouse IgA myeloma proteins, recognizes phosphorylcholine as the immunodominant group on the pneumococcus C polysaccharide. Comparison of primary structure of IgM (FR) and of the BALB/c mouse myeloma proteins exhibiting anti-phosphorylcholine specificity will therefore be of great interest. Especially since the sequence analysis data of the first hypervariable regions of five phosphorylcholine binding mouse IgA myeloma proteins have revealed that with the exception of a single substitution in one protein these immunoglobulins all have identical heavy chain sequences throughout the first hypervariable region, suggesting that the heavy chain plays a dominant role for this antigen binding specificity (Barstad *et al.*, 1974).

The association constant for the reaction with phosphorylcholine, 6.4×10^4 (25°), parallels values obtained with the mouse IgA myeloma proteins (Chesebro and Metzger, 1972). Association constants of conventionally induced sheep antibodies to phosphorylcholine–protein conjugates are in the range of 10^5 (Riesen, unpublished). van't Hoff calculation using the association constants at 2 and 25° indicates that entropic factors provide the driving force for the binding of phosphorylcholine to IgM (FR). This is in agreement with other types of antibody–hapten and antibody–antigen systems (Karush, 1962; Epstein *et al.*, 1956; Singer, 1967) and with data obtained from an IgM Waldenström with anti-dinitrophenyl activity (Ashman and Metzger, 1969). On the other hand the reaction of anti-dinitrophenyl antibodies with hapten or of mouse myeloma proteins with dinitrophenyl or with phosphorylcholine was reported to be driven by enthalpic factors (Eisen and Siskind, 1964; Barisas *et al.*, 1971, 1972; Johnston *et al.*, 1974; Pollet and Edelhoch, 1973).

The valence of IgM has been subject to controversy (for detailed review see Metzger, 1970). A valence of 10 as observed for IgM (FR) is in agreement with theoretical considerations based on a 20-chain model, consisting of five subunits (Miller and Metzger, 1965). Decavalent IgM has indeed been experimentally confirmed by several workers (Ashman and Metzger, 1969; Merler *et al.*, 1968; Young *et al.*, 1971; Edberg *et al.*, 1972; Kim and Karush, 1974). On the other hand, IgM exhibiting only five binding sites was observed on interactions with high molecular weight antigens (Franklin *et al.*, 1957; Lindquist and Bauer, 1966; Schrohenloher and Barry, 1968). The pentavalency in these cases was explained by steric hindrance due to the high molecular weight of the antigen (Stone and Metzger, 1967).

Conflicting results, however, have been obtained from experiments with induced heterogeneous IgM antibodies against low molecular weight ligands (Onoue *et al.*, 1965; Voss *et al.*, 1969; Mukkur, 1972; Mukkur and Tewari, 1973) indicating a valence only half as large as expected for the 19S IgM, the 7S subunits, and the Fab fragments. Later reports suggested that if one used IgM antibodies with an average association constant greater than 10^6 it was possible to detect two sets of binding sites (Onoue *et al.*, 1968; Kishimoto and Onoue, 1971; Oriol *et al.*, 1971), one with low and one with high affinity. Accordingly the pentavalency of some IgM antibodies was explained by the assumption that in case of K values smaller than 10^6 the second set of sites would be below detectability (Kishimoto and Onoue, 1971; Mukkur, 1972).

The data on IgM M-components and on induced homogeneous IgM antibodies (Kim and Karush, 1974) indicate that homogeneous IgM antibodies show ten binding sites per molecule which are functionally equivalent and independently reactive. With heterogeneous IgM antibodies, however, the situation seems to be more complex and one has to await further results in order to answer the problem of heterogeneity and the possible existence of two sets of binding sites of different affinity or the possibility of an interaction among identical sites of the same molecule (Oriol and Rousset, 1974).

The reported data obtained with IgM (FR) suggest that the reaction of this protein with phosphorylcholine meets all criteria for antibody-like binding such as high specificity, correct stoichiometry, appropriate affinity, and restriction of the binding site to the Fab fragment.

Compared to myeloma proteins produced by chemically induced plasma cell tumors in the BALB/c strain of mice the frequency of human myeloma proteins with specificity against phosphorylcholine seems to be rather low, since only one of 904 M-components was active. Among 300 mouse myeloma proteins derived from an independently induced tumor six have been found to react with phosphorylcholine-containing antigens, which have been isolated from bacteria found in the intestinal tract (Potter *et al.*, 1973). This remarkably high frequency in mice was interpreted by the assumption that neoplastic transformation following intraperitoneal injection of mineral oil mainly affects cells from the gastrointestinal tract, which are committed to the production of antibodies against antigenic material present in the gastrointestinal microorganisms. It has also been shown that normal BALB/c serum contains 8–64 μg/ml of anti-phosphorylcholine antibody of the T-15 type (Lieberman *et al.*, 1974). Attempts to detect antibody activity among

human M-components by screening against a variety of antigens have led to conflicting results. Several investigators were successful in demonstrating activity against various antigens; however, the noted frequencies of such active M-components varied considerably. Whereas Yoo and Franklin (1971) were unable to demonstrate activity after screening 275 myeloma sera against 20 antigens, consisting of hapten–protein conjugates and bacterial antigens, another study indicated that about 10% of human myeloma sera react with β-lipoproteins (Riesen *et al.*, 1972). In three of these cases it could be demonstrated that the binding activity resided in the M-component. These differences may be either attributed to different methodological attempts, or more important to the selection of the antigen. The data available to date (Metzger, 1969; Potter, 1971; Seligmann and Brouet, 1973) indicate that a considerable proportion of human myeloma proteins behave like autoantibodies. From the pathogenic point of view this high incidence of autoimmune phenomena associated with multiple myeloma or macroglobulinemia Waldenström compared to other activities seems to be noteworthy. Persistent antigenic stimulation has indeed repeatedly been postulated as a possible mechanism for the induction of neoplastic processes. Cells maintained in active proliferation for some time might be more vulnerable to neoplastic transformation. However, more results from systematic screening of human myeloma proteins against endogenous and exogenous antigens are necessary to get further insights into the stimulus leading to uncontrolled cell growth.

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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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