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Architectonics of a bacterial flagellin filament subunit

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Flagellins of two *Escherichia coli* strains and their tryptic fragments were studied by different methods. Probabilities of secondary structure formation were also calculated for all flagellins with a known primary structure. The obtained data permit one to suggest a model for the flagellin molecule consisting of a central part responsible for antigenic properties and terminals responsible for polymerization. The central part is variable in length from a few amino acid residues to three-four hundred depending on the bacterial species. The terminal parts consist of about 160 amino acid residues from the N-end and 100 from the C-end.

Flagellin; Immunology; Polymerization; Predicted structure; (*Escherichia coli*)

1. INTRODUCTION

Bacterial flagellar filaments are one of the main types of surface antigens. Different strains of bacteria are characterized by a various antigen specificity of the flagellum, whereas the polymerizing properties of these flagellins remain unchanged because they can be co-polymerized. The regions of polypeptide chains responsible for subunit contacts are rather conservative evolutionally, since flagellin co-polymerization occurs even in various species of bacteria [1]. Genetic studies of *Salmonella* have shown that flagellar shape mutations are mapped in the terminal regions and that the antigenic region is located between them [2]. Later, primary structures of several flagellins have been determined and it was shown that the terminal region of the molecule in *E. coli* and *Salmonella* have a high homology [3].

Previously we have reported general regularities of limited proteolysis and a microcalorimetric investigation of flagellins of two *E. coli* strains [4]. In this paper we present the results of structural and functional studies of these flagellins. This allows us to describe the architectonics of flagellin, i.e. to isolate the regions playing different functional roles, to determine their mutual arrangement and to estimate the structure of each region.

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2. MATERIALS AND METHODS

The preparation of flagellin and tryptic fragments has been described in a previous paper [4]. These fragments were formed by sequential splitting from the molecule ends and denoted as FK44 and FK34 for strain K12J62 and FB50 and FB37 for strain B38. The obtained fragments were transferred into 0.01 M Na-phosphate buffer, pH 7.0, 0.15 M NaCl. The concentrations of flagellins and their fragments were determined from the nitrogen content [5]. Antiserum was raised in rabbits by injections of mixed polymer flagellins from strains B38 and K12J62, 0.2 mg of protein per injection, using the method described in [6]. Immunodiffusion precipitation [7] was carried out on the gel containing 1% agarose in 0.15 M NaCl/10 mM Tris-HCl buffer, pH 7.2. The reaction proceeded for 3 days at room temperature. Then the gel was washed with 0.15 M NaCl for a day and stained with Coomassie G-250.

The polymerization of monomer flagellin on seeds and the control were done as described in [1]. Homogeneity of seeds and the formation of polymers were tested on a JEM-100c electron microscope (JEOL, Japan). The method of negative staining with 2% uranyl acetate was used.

3. RESULTS AND DISCUSSION

We have determined which part of the flagellin molecule is exposed on the polymer surface. Im-

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munodiffusion precipitation has shown that there are antibodies to both flagellins in the antiserum (fig.1). Merging of the precipitation lines with the formation of spurs indicates the partial identity of antigenic determinants of two flagellins, the flagellin of strain K12 having an additional type of determinant. The precipitation lines formed by each flagellin and its fragments smoothly merge showing a complete identity of their antigenic determinants. Thus, all the antigenic determinants of the polymeric form of flagellin are located on the part of the molecule which is the most stable to proteases and it must be exposed on the polymer surface.

The terminal fragments of the polypeptide chain do not have polymer antigenic determinants and it can be assumed that they are located within the polymer and play an important role in polymerization. We decided to examine whether fragments FK44 and FB50 can participate in polymerization.

While flagellins of both strains formed polymers upon ammonium sulfate desalting, their fragments FK44 and FB50 did not polymerize under the same conditions. Appropriate conditions for polymerization of flagellins on seeds have been chosen. Polymerization of flagellin of strain B38 required higher concentrations than K12J62, up to 10 mg/ml; if the concentration was smaller, polymerization did not proceed. Addition of the corresponding fragments to the reaction mixture of polymerizing flagellin did not affect the rate of polymerization (fig.2), all fragments remain in the supernatant. We concluded that splitting of the unstable fragments results in the loss of polymerization abilities.

Primary structures of flagellins of several bacterial species are available at present [3,8–10]. Using the program for prediction of secondary structures [11], we compared probabilities of secondary structure formation in all the studied flagellins (fig.3). In *Salmonella* and *E. coli* bacteria there is an almost complete analogy of the predicted structures of the terminal regions but there are differences both in dimensions and the secondary structure of the middle region. The same pattern is observed for flagellins of such evolutionary distant bacteria as *Bacillus subtilis* and *Caulobacter crescentus*.

Our data favour the following model for the flagellin molecule. The central part of the polypeptide chain has a variable structure, which can be from a few to three-four hundred amino acid residues long, depending on the bacterial species. This part is rather stable to proteases [4] and determines the surface properties of the bacterial flagellum, namely its antigenic specificity.

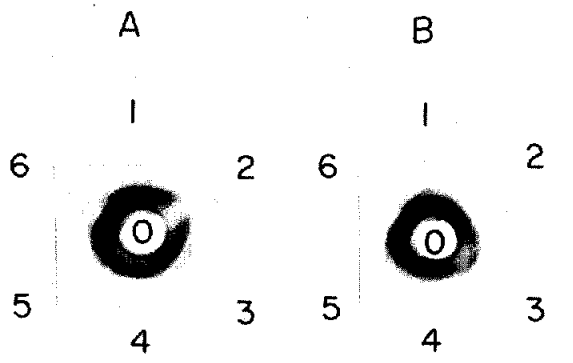

Fig.1. Immunodiffusion analysis of flagellins and their tryptic fragments using rabbit antiserum to polymer flagellins. The concentration of all reagents was 1 mg/ml. (A) 0, antiserum; 1,5, strain K12J62 flagellin; 2, low-molecular-mass fragment (10 kDa); 3, FK34 fragment; 4, FK44 fragment; 6, strain B38 flagellin. (B) 0, antiserum; 1,5, strain B38 flagellin; 2,4, fragment FB37; 3, fragment FB50; 6, strain K12J62 flagellin.

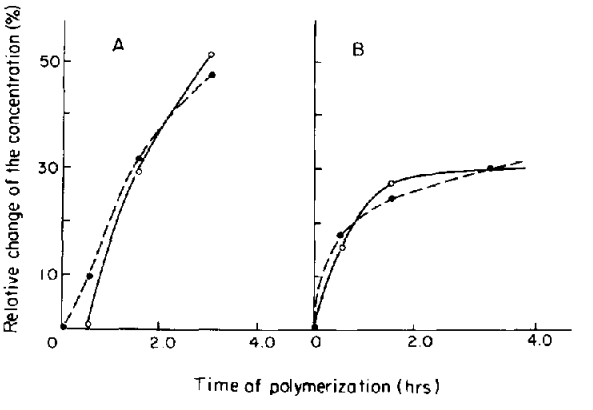

Fig.2. Curves of flagellin polymerization on seeds. Time dependence of relative change of monomer flagellin concentration in the supernatant determined from absorption at 277.4 nm. (A) Inhibition of polymerization of flagellin from strain K12J62 by fragment FK44: (●---●) final concentrations were 3.8 mg/ml (seed), 5.3 mg/ml (monomer), 1.7 mg/ml (fragment); (○—○) without the fragment. (B) Inhibition of polymerization of flagellin from strain B38 by fragment FB50: (●---●) final concentrations were 1.25 mg/ml (seed), 9.8 mg/ml (monomer), 1.2 mg/ml (fragment); (○—○) without the fragment.

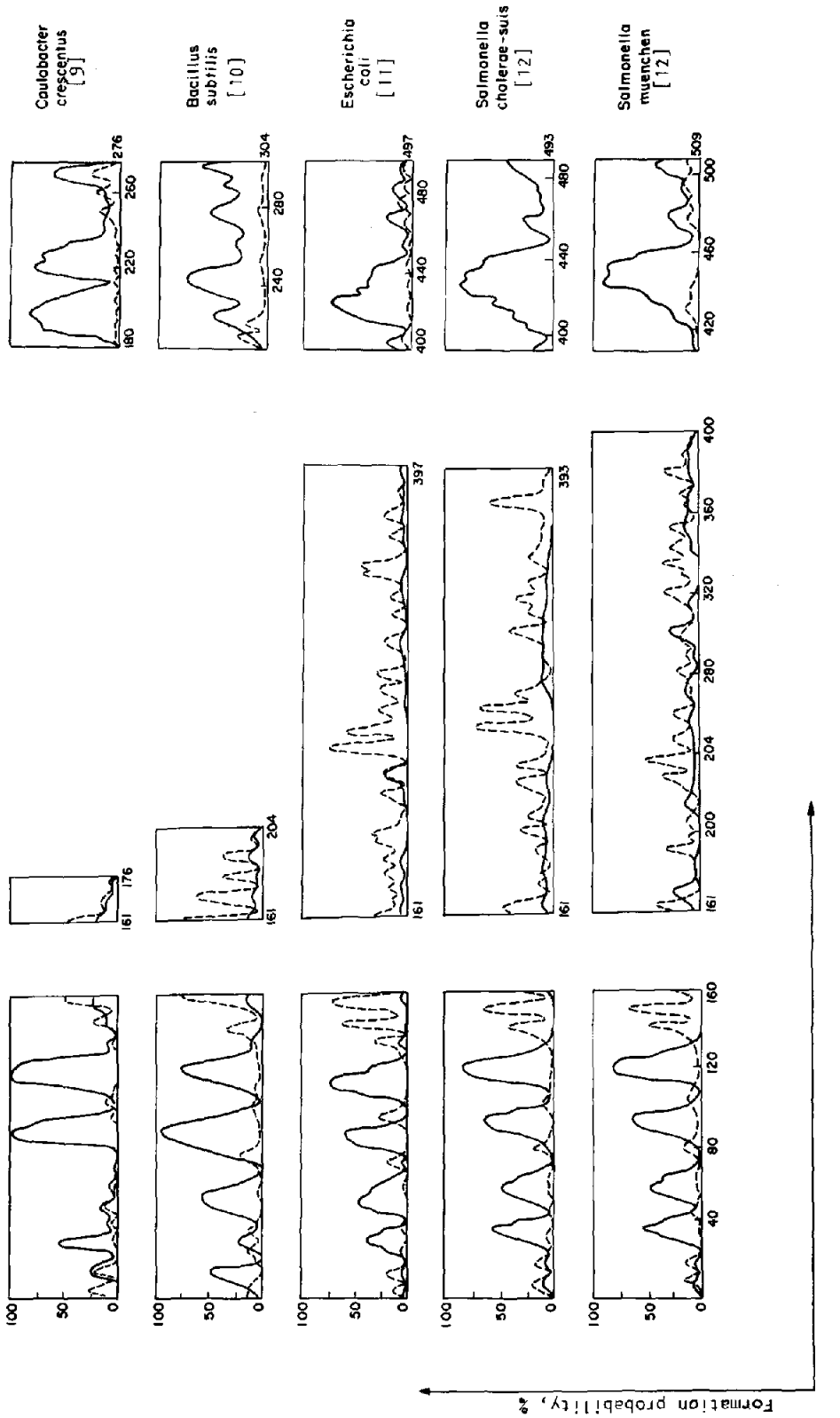

Fig.3. Predicted secondary structure of different flagellins: (——) α-helix, (– – –) β-structure.

The two other parts of the molecule consisting of about 160 amino acid residues from the N-end and 100 residues from the C-end are responsible for the polymerization properties of flagellin. Within the polymer this part of the molecule is turned in towards the flagellum. The final folding of the polypeptide chain in these fragments and/or the selection of a desirable conformation occurs during incorporation of the molecule into the polymer, since it is known that the shape of the seed determines the form of the reconstituted flagellum, i.e. the incorporated molecule must adopt a conformation analogous to that of the adjacent molecule in the longitudinal row [12] and polymerization proceeds in two stages described by Asakura [13]. There should also be two ways of terminal fragment folding resulting in two conformers during assembly to provide for the flagellum polymorphism.

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