Domain structure of flagellin

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The chemotaxis of bacteria such as *Salmonella* and *Escherichia coli* involves smooth swimming punctuated by periods of tumbling. In smooth swimming the flagellar filaments are left-handed, in tumbling they are right-handed with a different wavelength. The filaments are constructed from a globular protein, flagellin, by a process of self-assembly. The existing models assume that the flagellin molecule is bistable and longitudinal rows of subunits take one of the two possible conformations. Such a model explains the observed different morphology of the flagellum. We have studied *Salmonella* and *E. coli* flagellins in polymeric and monomeric forms by scanning microcalorimetry and circular dichroism. We have inferred that a flagellin molecule consists of several domains, two of which are structured at physiological temperatures and are in the monomeric form, while the others acquire a regular form only in the process of polymerization. This phenomenon may be the basis of a process during which the flagellin molecule, fitting into the flagellum, acquires a conformation analogous to that of the neighbouring molecule in the longitudinal row.

Flagellin *Domain structure* *Calorimetry* *Circular dichroism*

1. INTRODUCTION

Many species of bacteria, including *Escherichia* and *Salmonella*, swim in a medium by rotating their special organelle, the flagellum [1,2]. The main helical portion of the bacterial flagellum is constructed from molecules of a globular protein, flagellin. The flagellar filaments can be depolymerized into monomer flagellin, e.g., by an increase of temperature to 65°C. Reconstitution of the filaments can be done by adding short flagellum fragments (seeds) to the monomer flagellin solution [3]. Polymorphism of the flagellar filaments is well known [4]. Normal and curly configurations are the main ones for all polymeric structures. The normal flagellar filaments have the left-handed structure with a wavelength of 2.3 μm. The flagellar filaments of the curly configuration are right-handed with a wavelength of 1.1 μm. It was shown that in vitro the bacterial flagellar filaments can undergo reversible transitions between normal and curly types as a result of changes in pH values of the medium [5,6] or under the action of mechanical forces [7].

The normal-curly-normal transitions are very important for bacterial taxis. Peritrichous bacteria punctuate smooth swimming by periods of tumbling which allows them to change the direction of their motion. In smooth swimming the filament is normal and in tumbling it is curly. Models of the structural organization of flagellar filaments are based on the assumption that a flagellin molecule within the filament is dimorphous [8], i.e., it is capable of adopting one of the two different conformations.

It follows from the model that the flagellin molecule built-in into the flagellar filament must adopt a conformation similar to that of the neighbouring molecule in the longitudinal row. What structural features of the flagellin molecule can satisfy such a model? Recently we studied *Salmonella* flagellin by microcalorimetry and circular dichroism (CD) [9]. Similar investigations have been done now for *E. coli* flagellin. The common features of both flagellins are discussed.

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2. MATERIALS AND METHODS

E. coli strain K12J62 trp⁻pro⁻his⁻lac⁻Cya⁻ was used. *E. coli* cells were cultured at 32°C in T-broth containing 1% tryptone and 0.5% NaCl. Well-flagellated cells were obtained from the edge of a swarm in plates containing T-broth and 0.35% agar. When the absorption of the culture at 600 nm reached 1.3–1.5 the bacteria were harvested by centrifugation at 8000 × *g* for 25 min. The bacterial mass was resuspended in a mixture containing 0.15 M NaCl and 0.01 M phosphate buffer (pH 6.9). The flagellar filaments were separated by passing the bacterial mass through a glass syringe 15–20 times. The cells were centrifuged 3 times at 15000 × *g* for 10 min, after which the flagellar filaments were centrifuged at 80000 × *g* for 1.5 h and resuspended in 0.15 M NaCl and 0.01 M phosphate buffer (pH 6.9) to a final concentration of about 1 mg/ml. The concentration was determined by the micromethod in [10], assuming that the nitrogen concentration in the proteins is 16%. The flagellin solution was dialysed against two changes of buffer overnight. The flagellin obtained was electrophoretically pure. The M_r of *E. coli* flagellin was taken as 60000 [11]. The temperature dependences of $[\theta]_{222}$ were measured on a Jasco J-41A dichrograph. When estimating the value of the molar ellipticity the average weight of the flagellin residue was taken to be 115. Calorimetric measurements were done using a DASM-1M microcalorimeter [12]. Calorimetric curves were processed as in [13]. Heating rates in all experiments were 1°C/min. The same solutions were used for both calorimetric and CD measurements.

3. RESULTS

Heating *Salmonella* and *E. coli* flagellar filaments to 65°C is used for obtaining monomeric flagellin. After cooling to room temperature the flagellin remains in the monomeric state, and to carry out the reconstitution process it is necessary to add seeds to the solution. On the basis of this property of flagellin we used a solution of polymer flagellin as an initial sample in our calorimetric and CD measurements. The effects observed on repeated heating correspond to melting of the

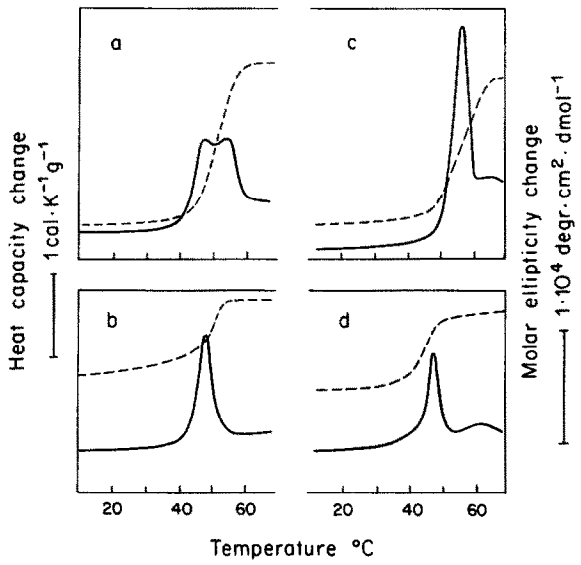

Fig.1. Melting curves of flagellins obtained by the calorimetric method (——) and curves of the dependence of the molar ellipticity at 222 nm on temperature (– – –): (a) polymeric form of *Salmonella*, (b) monomeric form of *Salmonella*, (c) polymeric form of *E. coli*, (d) monomeric form of *E. coli*; 0.01 M phosphate buffer containing 0.15 M NaCl (pH 7.0), concentration ~1 mg/ml, heating rate 1°C/min.

monomeric form of the flagellin. Fig.1 shows the melting curves of polymeric and monomeric forms of *Salmonella* and *E. coli* flagellins obtained by the calorimetric method and the curves of the dependence of the molar ellipticity change at 222 nm on temperature. The thermodynamic parameters of the melting process of the flagellin molecules are given in table 1. We shall not discuss the observed differences in the melting curves here, but shall indicate the features common to both the bacterial flagellins. First, the fact that the heat absorption peaks of the calorimetric curves and the changes in the ellipticity at 222 nm are in the same temperature range shows that every heat absorption peak indicates some change in the secondary structure of protein. Second, analysis of the calorimetric curves based on the sequential deconvolution algorithm [14] shows that monomeric forms of flagellins consist of at least two cooperative units or domains. It is also evident that polymeric forms of flagellins contain additional domains which are not present in the monomers.

Table 1

Thermodynamic parameters of flagellin denaturation

Origin of flagellin	Polymers (first scan) ($\Delta_d H$)		Monomers (second scan) $\Delta_d H$	
	$kJ \cdot mol^{-1}$	$J \cdot g^{-1}$	$kJ \cdot mol^{-1}$	$J \cdot g^{-1}$
Salmonella	2220 ± 120	43.5 ± 2.0	1340 ± 50	26.3 ± 1.0
E. coli	2750 ± 150	45.6 ± 2.5	1450 ± 200	24.2 ± 1.7

4. DISCUSSION

The process of flagellin polymerization has been intensively studied. Studying the kinetics of *Salmonella* flagellin polymerization in vitro, the author in [15] established that the linear dependence of polymerization rate on monomer concentration is disturbed at high concentrations of monomeric flagellin. He assumed that this can be connected with the necessity of some conformational change in the flagellin molecule in the process of its fitting into the polymer. Later it was shown [16] that polymerization of *Salmonella* flagellin causes significant conformational changes. The flagellin of *Proteus mirabilis* was studied by CD and adiabetic differential calorimetry. It was found that this flagellin undergoes two conformational changes on heat denaturation [17]. Discussing the problem of great changes in the ellipticity at 220 nm during polymerization, a suggestion was made that monomeric flagellin represents an intermediate state between thermally disordered and compact structures [18]. These data allow us to assume that during flagellin polymerization the molecule undergoes a conformational rearrangement, but it is not clear whether the rearrangement occurs in the whole molecule or only in some part of it. The results of the microcalorimetric studies and the dependences of $[\theta]_{222}$ on temperature for *Salmonella* and *E. coli* flagellins described here allow us to infer that the flagellin molecule of these bacteria consists of several domains, two of which are structured at physiological temperature and have the monomeric form, while the others adopt the regular form only in the process of polymerization. This phenomenon may be the basis of the process when the flagellin molecule fitting into the flagellar filament adopts a conformation similar to that of the neighbouring molecule in the longitudinal row.

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REFERENCES

[1] Berg, H.C. and Anderson, R.A. (1974) Nature 245, 380–382.
[2] Silverman, M. and Siman, M. (1974) Nature 249, 73–74.
[3] Asakura, S., Eguchi, G. and Jino, T. (1968) J. Mol. Biol. 35, 227–236.
[4] Asakura, S. (1970) Adv. Biophys. 1, 99–155.
[5] Kamiya, R. and Asakura, S. (1976) J. Mol. Biol. 106, 167–186.
[6] Kamiya, R. and Asakura, S. (1977) J. Mol. Biol. 108, 513–518.
[7] Macnab, R.M. and Ornston, M.K. (1977) J. Mol. Biol. 112, 1–30.
[8] Calladine, C.R. (1978) J. Mol. Biol. 118, 457–479.
[9] Fedorov, O.V., Khechinashvili, N.N., Kamiya, R. and Asakura, S. (1984) J. Mol. Biol. 174.
[10] Jaenicke, L. (1974) Anal. Biochem. 61, 623–627.
[11] Kondoh, H. and Hotani, H. (1974) Biochim. Biophys. Acta 336, 117–139.
[12] Privalov, P.L., Plotnikov, V.V. and Filimonov, V.V. (1975) J. Chem. Thermodyn. 7, 41–47.
[13] Privalov, P.L. and Khechinashvili, N.N. (1974) J. Mol. Biol. 86, 665–684.

[14] Freire, E. and Biltonen, R.L. (1978) Biopolymers 17, 463–469.
[15] Asakura, S. (1968) J. Mol. Biol. 35, 237–239.
[16] Uratani, J. and Asakura, S. (1972) J. Mol. Biol. 67, 85–98.
[17] Bode, W. and Blume, A. (1973) FEBS Lett. 36, 318–322.
[18] Bode, W., Hinz, H.-J., Jaenicke, R. and Blume, A. (1974) Biophys. Struct. Mech. 1, 55–64.