CHANGES IN POLARIMETRIC PARAMETERS ASSOCIATED WITH THE POLYMERIZATION OF FLAGELLIN INTO FLAGELLAR FILAMENTS[1]

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Filaments of bacterial flagella are composed of protein subunits, the flagellins, whose molecular weight ranges between 30,000 and 50,000 daltons depending on the bacterial species from which they are obtained. X-ray diffraction patterns (Astbury et al., 1955; Burge, 1961; Swanbeck and Forslind, 1964; Champness and Lowy, 1968) and infrared absorption spectra (Beighton et al., 1958) of such flagella suggest the presence of α-helix. Recently, the investigation of flagellin structure by polarimetry has provided additional evidence for the presence of α-helix (Koffler et al., 1966; Klein et al., 1967; 1967a; 1968). This paper, is a report on changes in polarimetric parameters that occur when flagellin molecules undergo a transition between the solubilized and polymerized states. These changes appear to result from changes in helical structure as well as changes due to aggregation.

Materials and Methods

Native flagella were isolated from Proteus vulgaris, Bacillus pumilus 236, B. licheniformis NRS 243, Bacillus sp. X1, and B. stearothermophilus

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[1]This material was part of a lecture presented by one of us (HK) at the Conference on Interactions between Subunits of Biological Macromolecules, organized by the Commission on Molecular Biophysics, International Union for Pure and Applied Biophysics, held June 24-27th, 1968, in Cambridge, England.

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2184 and purified by the method of Abram and Koffler (1964). Flagellin was prepared by disintegration of flagella at pH 2.0 (Klein et al., 1968). Further purification of flagellin was achieved by reaggregation of flagellin into flagella-like filaments followed by centrifugation and subsequent acid disintegration of the filaments (Abram and Koffler, 1964).

Sonication of flagellar filaments was performed with a Biosonik II High Intensity Ultrasonic Probe. Approximately five ml of a one per cent suspension of filaments was subjected to six ten-second bursts of ultrasound interspersed with two-minute intervals of cooling at 0°C. Filamentous fragments were separated from solubilized flagellins by centrifugation.

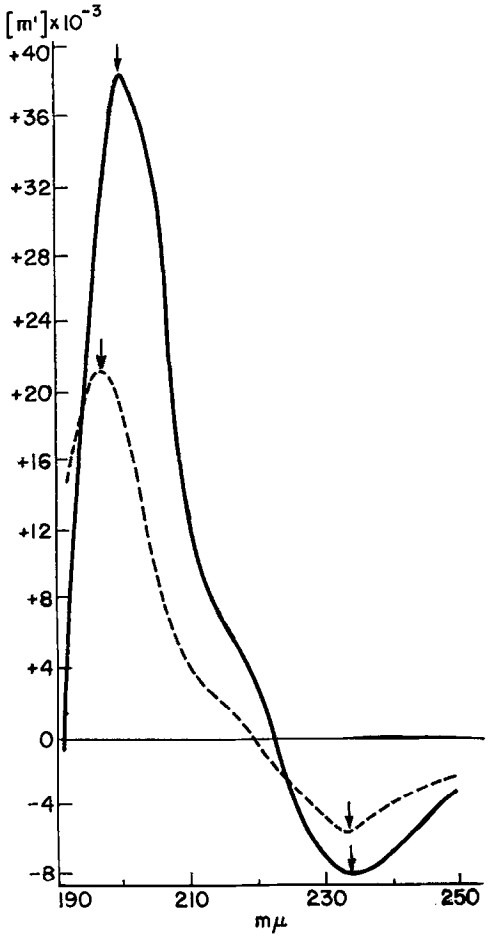

Figure 1. Optical rotatory dispersion curves for B. pumilus flagellin (-----) at pH 4.3 and reconstituted flagellar filaments (———) at pH 6.2.

Optical rotatory dispersion (ORD) measurements were made mainly with the Bendix Polarmatic 460C recording spectropolarimeter. One cm thermostatically controlled cells were used. The data in Figures 1 and 2 regarding ORD properties and circular dichroism (CD) were obtained with the Cary 60 spectropolarimeter. Both instruments were continuously flushed with nitrogen gas and measurements were made at 24°C. The concentrations of flagellin in soluble and polymerized form generally were 60-90 μg/ml and 40-60 μg/ml, respectively. Estimations of α-helix content were based on the methods of Simmons et al. (1961), Moffitt and Yang (1956), and Shechter and Blout (1964a, b). In the second method the statistical procedure of Sogami et al. (1963) was followed.

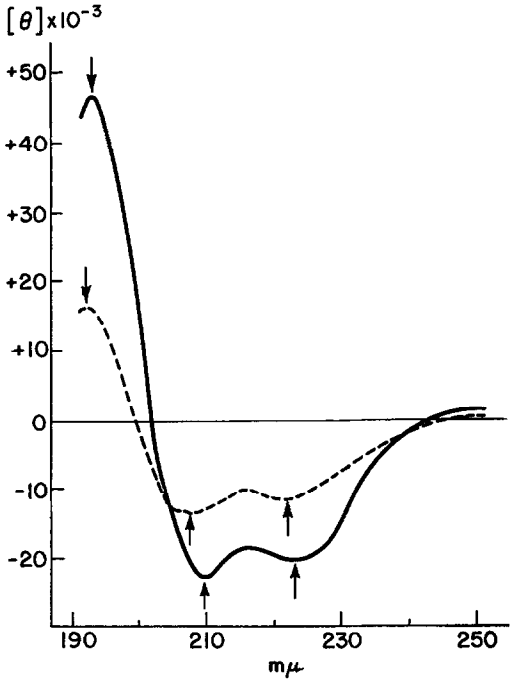

Figure 2. Circular dichroic curves for B. pumilus flagellin (-----) at pH 4.3 and reconstituted flagellar filaments (——) at pH 6.2.

Results and Discussion

The magnitudes of rotation at the 233 mμ trough for flagellins and flagellar filaments are listed in Table 1. There is a consistently large

Table 1

Optical Rotation of Flagellin and Flagellar Filaments

at the 233mμ Trough

Source	Solvent	Flagellin[a]			Flagellar Filaments[b]		
			$-[m']_{233}$	Percent helix[c]		$-[m']_{233}$	Percent helix[c]
Proteus vulgaris	H_2O	(6)[d]	4420±190	24	(8)	7590±170	53
	0.1 N KCl	(1)	4610	26	(1)	7790	54
Bacillus pumilus	H_2O	(9)	5100±290	30	(4)	7680±160	54
	0.1 N KCl	(3)	5000±130	29	(2)	8690±240	63
	H_2O	(2)[e]	5360±190	32	(6)[f]	8700±340	63
Bacillus licheniformis	H_2O	(7)	5110±410	30	(7)	9510±630	70
Bacillus sp. X1	H_2O	(2)	4190±240	22	(2)	7260±140	50
Bacillus stearothermophilus	H_2O	(5)	3950±250	21	(1)	5100	30
	H_2O	(4)[e]	4500±170	25	(2)[f]	7510±90	52

a. 60-90 μg/ml; pH 4-10; 24°C

b. 40-60 μg/ml; pH 6-7; 24°C

c. Based on a value of -11,800° used by Simmons et al. (1961) for protein (paramyosin) with 100% α-helix

d. Values in parentheses indicate number of samples examined

e. From reconstituted flagellar filaments (see Materials and Methods)

f. Reconstituted filaments

increase in rotational strength when flagellins from several bacterial species are polymerized to flagellar filaments. This change does not appear to be influenced by ionic strength. Analyses of the dispersion curves (Table 2), and of the 233 mμ trough values (Table 1) indicate that the α-helix content of the flagellins is increased approximately by a factor of two as the subunits are polymerized. Cotton effects and CD curves of flagellin and reconstituted flagellar filaments from B. pumilus are shown in Figures 1 and

2, respectively. The effect of polymerization on the spectral properties of flagellin results in a marked increase in rotational strengths accompanied by shifts of the peaks and troughs toward the red end of the spectrum. The observations of Asakura and associates (1968) studying flagellin of Salmonella typhimurium agree with us in that CD measurements at 222 mμ for the polymeric form are 1.7 times greater than corresponding values for monomeric flagellin.

Similar increases in intensity and shifts were reported by Cassim and Yang (1967) when poly-L-glutamic acid (PGA) aggregates in the pH range where the molecule is presumably but not necessarily fully helical (Cassim and Taylor, 1965). They proposed that the spectral changes are due to aggregation rather than an increase in helical content. Earlier, Schuster (1965) and Tomimatsu et al. (1966) reported that the Moffitt parameter, b_o, which is normally expected to increase negatively with increases in the magnitude of the 233 mμ trough, is not significantly affected when PGA aggregates, even though large rotational increases occur at the 233 mμ trough. In fact, Cassim and Taylor (1965) found the value for b_o to become slightly less negative under these conditions. Schuster (1965) also noted that the a_o parameter, which is usually inversely related to b_o in helix-coil transitions (Table 3), shows large negative changes during aggregation.

The structure of flagellin is essentially unfolded at pH 2 and helical (to the extent of 21-32 per cent) in the range of pH 4 to 10 (Tables 2 and 3). As the flagellin molecule becomes more helical, b_o increases negatively and a_o increases positively, changes that are consistent with those observed for the usual helix-coil transitions in proteins. In contrast to the observations for PGA, these data show that during the aggregation of flagellin b_o becomes considerably more negative, a change that suggests an increase in helix content; the value for a_o changes relatively slightly. Perhaps in the case of flagellin a large negative change for a_o due to aggregation, as observed by Schuster (1965) for PGA, is essentially neutralized by a positive change for the value of a_o due to an increase in helix content. The flagellin

Table 2

Alpha-helix Content of Flagellin and

Sonicated Flagellar Filaments of B. pumilus

Preparation	Percent Helix calculated by the following methods:			
	233mμ Trough	Moffitt-Yang	Shechter-Blout	
	H_{233}	$H_{b_o}(\lambda_o=212)$	$H^{H_2O}_{225}$	$H^{H_2O}_{193}$
Flagellin (pH 2)	12	13	19	27
Flagellin (pH 10)	30	29	31	36
Flagellar filaments, sonicated (pH 6)	65	55	58	55

Table 3

Moffitt-Yang Parameters (at $\lambda_o=212$) for Flagellin

and Sonicated Flagellar Filaments of B. pumilus

Preparation	$-a_o$	$-b_o$
Flagellin (pH 2)	478	79
Flagellin (pH 10)	281	182
Flagellar filaments, sonicated (pH 6)	322	344

molecule contains much less α-helix than PGA and unlike PGA under the conditions used by Cassim and Yang (1967) has the potential of assuming additional helical conformation as the subunits aggregate to form filaments. Therefore, it appears plausible that both aggregation and an increase in

helical content contribute to the changes in the various polarimetric parameters that take place during the assembly of flagellins into flagellar filaments. Further studies are needed to examine the speculation that the helix content of flagellin does indeed increase during polymerization.

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