ON THE CHEMISTRY OF FLAGELLA OF SPIRILLUM SERPENS

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Received June 7, 1963

Flagellar preparations obtained from three genera of bacteria (Bacillus, Proteus, and Salmonella) purified primarily by differential centrifugation have been analyzed chemically (Kobayashi, et al., 1959; Ambler and Rees, 1959). Based on these analyses, it has been reported that the flagella of bacteria consist exclusively or almost exclusively of protein (Weibull, 1960). This report describes preliminary experiments on the chemical nature of the flagella of Spirillum serpens which suggest that radical differences in the chemistry of these organelles exist in diverse bacterial genera.

Purified suspensions of flagella from S. serpens were prepared with the aid of a DEAE cellulose column according to a procedure recently developed (Martinez, 1963). The molar ratios of the amino acids in these flagella are different from those reported for other genera; additionally, the flagella contain an unidentified basic amino acid. Ambler and Rees (1959) have identified N-methyl-lysine in the flagella of Salmonella. Since the unknown amino acid of the Spirillum migrates in ion-exchange chromatography identically with N-methyl-lysine, it is possible that spirilla may also contain this amino acid in their flagella. However, the molar ratio (based solely on nynhydrin color equivalents) of this unknown compound is only approximately 1/8 that of lysine whereas in Salmonella lysine and the N-methylated analogue occur in roughly equimolar ratios.

Weibull (1960) has reported that purified flagella contain not more than 0.0001-0.2% carbohydrate. Analyses of our purified flagella by a modification of the anthrone procedure revealed the presence of 1.5% carbohydrate expressed as glucose equivalents (1.3-1.9% range). The relative constancy of the carbohydrate:protein ratio in a number of flagellar preparations suggested that the carbohydrate might be chemically associated with the protein of the organelles. It has been demonstrated (Kobayashi, et al., 1959) that flagella are dissociated to their protein components by a variety of treatments (acid, heat, etc.). We therefore applied these treatments to our preparations to determine whether the carbohydrate moiety can be separated from the flagellar protein; no such separation was achieved (Table 1). Further, the elution pattern from a Sephadex G 50 column of a heated flagellar preparation shows one peak containing all the protein and the carbohydrate placed on the column. Experiments are in progress which will determine the nature of the carbohydrate in question and its chemical relationship to the structure of flagella.

Treatment	µg sugar/ml
Untreated control	27.2
Heat dissociated (60^{o}/30 min.)	35.0
Acid dissociated (N HCl/25^{o}C/20 min.)	35.4

Table 1. Sugar content of native and dissociated flagella. Purified flagellar preparations of S. serpens (1.9 mg protein; 31 µg sugar) were dissociated by acid and heat treatment and dialyzed for 18 hr. The contents of the dialysis bag were brought to 1.0 ml and analyzed by the anthrone procedure.

In addition, there is evidence that intact flagella may contain RNA associated with a "hook-like" structure at the base of the organelle. Electron microscopic examinations of crude flagellar suspensions have shown the presence of hooks on some flagella. After chromatography of crude flagellar suspensions on DEAE cellulose and elution with a salt gradient the hook-like structures are no longer found. By this procedure there is

a separation of the flagella from an RNA-containing peak. The flagella are eluted at 0.42 M NaCl and the RNA between 0.65 and 0.70 M. Experiments have been carried out in which crude flagellar suspensions containing hooks and purified preparations devoid of hooks have been reacted with homologous flagellar antiserum and the antigen-antibody complex has been analyzed for

Table 2. Association of RNA with S. serpens flagella. Flagellar suspensions were treated with one equivalent of homologous antiserum in a total volume of 1 ml and agglutination was carried out at 37°C for 1 hr. The Ag-Ab complexes were washed twice with buffered saline and analyzed for RNA by the orcinol test and absorbancy.

Agglutinogen	µg RNA/ml in Ag-Ab complex
Crude flagellar suspension (6.68 mg protein)	142.0
Crude flagellar suspension RNase treated (10 µg/20'/37°C) (6.68 mg protein)	160.0
Purified flagella (350.0 µg)	< 0.4
Purified flagella (350.0 µg) + RNA from same column run (280 µg)	< 0.4
Purified flagella (350.0 µg) + extracted RNA* (360 µg)	< 0.4
Extracted RNA* + flagellar antiserum	< 0.4

* Kirby (1956)

RNA after washing. Table 2 presents the data from such an experiment using S. serpens flagella. The experiments show: 1) homologous flagellar antiserum agglutinates RNA in crude flagellar suspensions but not after purification of the flagella by ion-exchange chromatography; 2) this flagellar-associated RNA is resistant to hydrolysis by pancreatic RNA'ase (10 µg enzyme/ml for 20' at 37°C); 3) nonspecific adsorption of RNA by purified flagella could not be demonstrated under a variety of conditions. It appears that intact flagella possess protected but readily dissociable RNA.

References

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