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FLAGELLAR ANTIGEN OF *VIBRIO ALGINOLYTICUS*

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SUMMARY Flagellar antigen of *Vibrio alginolyticus* was solubilized and some of its properties were studied. It was shown that flagellin of *Vibrio alginolyticus* consisted of a single protein, with similar immunological properties to that of U-II, one of the antigenic subunits of flagellin of *Vibrio parahaemolyticus*. The amino acid compositions of U-II and the flagellin of *Vibrio alginolyticus* were compared. The contents of most amino acids were similar, but differences in the contents of several amino acids were observed. The physicochemical properties of U-II and the flagellin of *Vibrio alginolyticus*, such as the S value and A_{280}/A_{260} ratio were similar. The presence of the same flagellar antigen in other strains of *Vibrio alginolyticus* was demonstrated.

INTRODUCTION

The biological and biochemical properties of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are very similar, and *Vibrio alginolyticus* was first classified as a subgroup of *Vibrio parahaemolyticus*. However, following the proposal of Sakazaki (1965), the species name "*Vibrio alginolyticus*" has generally been accepted.

Recently, we reported that the flagella of *Vibrio parahaemolyticus* contain two distinct subunits (Shinoda et al., 1970). The present study was to see whether the flagella of *Vibrio alginolyticus* also contain two subunits. It was found that they contain a single subunit, which is immunologically identical to one of the two subunits of *Vibrio parahaemolyticus*.

MATERIALS AND METHODS

1. *Strains*

Vibrio alginolyticus strains 6624, 6532, 6540, 6590, 6606 and 6640 were supplied from Dr. R. Sakazaki (National Institute of Health, Tokyo). *Vibro parahaemolyticus* RIMD-100 is a stock cultute in the Type Culture Collection of this Institute.

2. *Preparation of pure flagella*

Vibrio alginolyticus was grown on nutrient agar containing 3% sodium chloride at 37 C for 18 hr. Cells were harvested by washing them off the agar with 3% sodium chloride solution. Flagella were removed by homogenization in a Marusan Waring Blendor (8,000 rev/min for 5 min). The flagella were partially purified by repeated centrifugation and then subjected to preparative zone electrophoresis using a powdered copolymer of polyvinyl chloride and polyvinyl acetate (Pevikon C-870) as supporting medium. The method has been described in detail previously (Miwatani et al., 1970).

3. *Column chromatography*

Hydroxylapatite column chromatography and Sephadex G-100 gel filtration were carried out as

described previously (Shinoda et al., 1970).

4. *Examination of physicochemical properties*

Determinations of nitrogen content, sugar content and phosphorus content, analytical centrifugation and amino acid analysis were carried out as described previously (Shinoda et al., 1970).

5. *Gel diffusion test*

The method used for the gel diffusion test was described previously (Miwatani et al., 1969). Antiserum of flagellin was prepared as described in a previous paper (Shinoda et al., 1970).

RESULTS

1. *Hydroxylapatite column chromatography of flagellin*

The flagella of *Vibrio alginolyticus* were purified as described in MATERIALS AND METHODS. Electron microscopy showed that the preparation was as pure as that of *Vibrio parahaemolyticus* (Miwatani et al., 1970). Purified flagella of *Vibrio alginolyticus* 6624 were solubilized by keeping them in 6 M urea for 60 min at 37 C. Then the preparation was applied to a hydroxylapatite column. A typical elution profile with only one peak is shown in Fig. 1.

Since the elution profile of the flagellin of *Vibrio alginolyticus* was similar to that of U-II of *Vibrio parahaemolyticus* reported previously

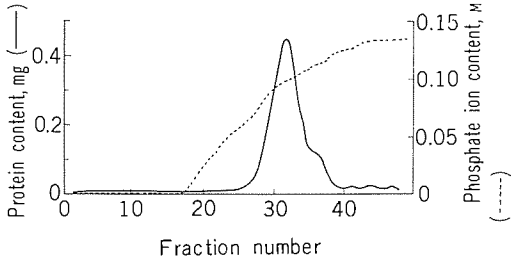


FIGURE 1. *Hydroxylapatite column chromatography of the flagellin of Vibrio alginolyticus.*

Hydroxylapatite column chromatography was carried out as described in the text. Protein content was determined by the method of Lowry et al. (1951). Phosphate ion content was determined by the method of Allen (1940).

(Shinoda et al., 1970), elution profiles of the flagellins of *Vibrio alginolyticus* and of *Vibrio parahaemolyticus* were compared. The results are shown in Fig. 2. Fig. 2A shows that there were two different subunits in the flagellin of *Vibrio parahaemolyticus*, confirming the results reported previously (Shinoda et al., 1970). On the other hand, a mixture of U-II of *Vibrio parahaemolyticus* and the flagellin of *Vibrio alginolyticus* gave a single peak on a similar column, suggesting that these two flagellins are identical (Fig. 2B).

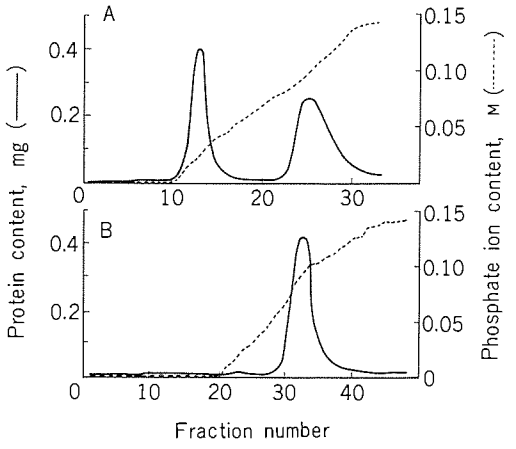


Figure 2. *Hydroxylapatite column chromatography of flagellins of Vibrio parahaemolyticus and Vibrio alginolyticus.*

Experimental conditions are as described in the legend of Fig. 1. (A) Flagellin of Vibrio parahaemolyticus; (B) a mixture of U-II of Vibrio parahaemolyticus and the flagellin of Vibrio alginolyticus.

2. *Gel filtration of flagellins*

To confirm that the flagellins of *Vibrio alginolyticus* and U-II of *Vibrio parahaemolyticus* are identical, these flagellins were subjected to gel filtration and results are shown in Fig. 3. Appropriate amounts of the flagellin of *Vibrio alginolyticus* and U-II were applied to separate Sephadex G-100 columns. The columns were eluted as described above. Typical chromatograms of the flagellin of *Vibrio alginolyticus* (Fig. 3A) and U-II (Fig. 3B) showed that they have similar molecular

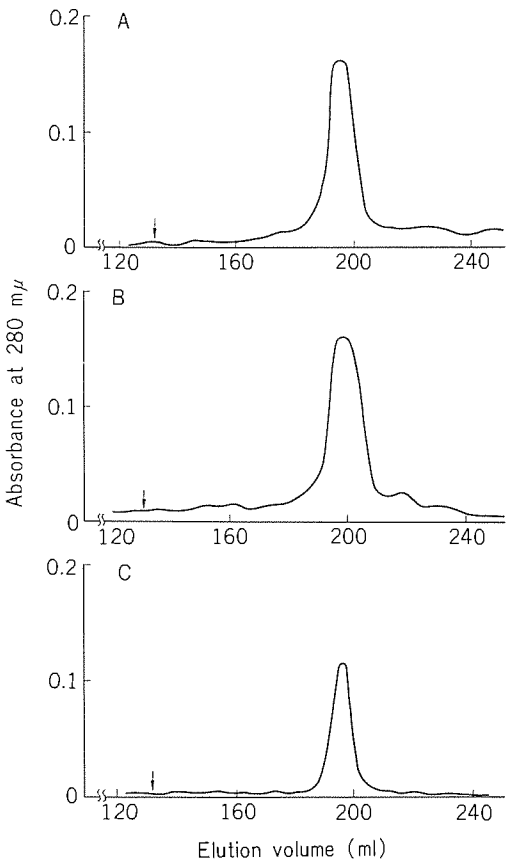


FIGURE 3. *Gel filtration of the flagellin of Vibrio alginolyticus and U-II of Vibrio parahaemolyticus.*

Sephadex G-100 gel filtration of U-II (A), the flagellin of Vibrio alginolyticus (B) and a mixture of U-II and the flagellin of Vibrio alginolyticus (C) was carried out as described in the text. The arrow indicates the void volume.

weights. The elution profile of a mixture of the flagellin of *Vibrio alginolyticus* and U-II, shown in Fig. 3C, indicates that these two flagellins are identical, having the similar molecular weight.

3. *Physicochemical properties of the flagellins*

Some physicochemical properties and the amino acid composition of the flagellin of *Vibrio alginolyticus* are presented in Tables 1 and 2. For comparison, data on U-II obtained previously (Shinoda et al., 1970) are also listed. Some physicochemical properties, such as the S values and A_{280}/A_{260} ratio, of U-II and the flagellin of *Vibrio alginolyticus* were similar. The contents of most amino acids were similar. However, the amounts of several amino acids were somewhat different in U-II and the flagellin of *Vibrio alginolyticus.*

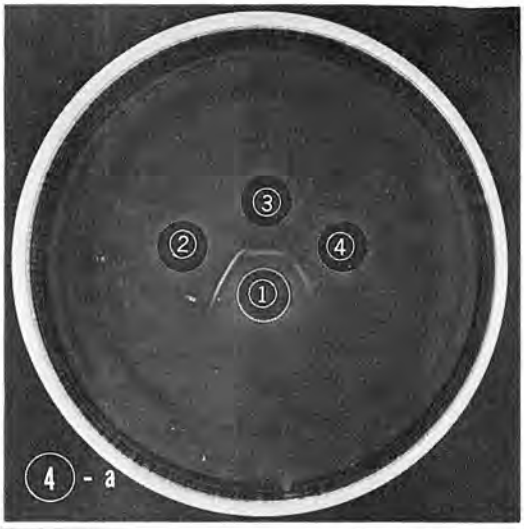
TABLE 1. *Physicochemical properties of the flagellin of Vibrio alginolyticus and U-II of Vibrio parahaemolyticus*

Property[a]	Flagellin of *V. alginolyticus*	U-II
Nitrogen content (%)	16.2	16.1
Sugar content (%)	0.05	0.02
Phosphorus content (%)	0.01	0.01
S value	2.6 S	2.7 S
A_{280}/A_{260}	1.58	1.59

a Properties were determined as described in the text.

4. *Immunological properties of the flagellins*

The identity of the flagellin of *Vibrio alginolyticus* with U-II of *Vibrio parahaemolyticus* was demonstrated immunologically. On agar gel diffusion (Fig. 4-a), the precipitation line between anti-flagellin of *Vibrio parahaemolyticus* and U-II fused with the precipitation line between anti-flagellin of *Vibrio parahaemolyticus* and the flagellin of *Vibrio altinolyticus.* The nonidentity of U-I and the flagellin of *Vibrio alginolyticus* is also demonstrated in this figure. The precipitation line between the anti-flagellin of *Vibrio parahaemolyticus* and the flagellin of *Vibrio alginolyticus* and that between the anti-flagellin of *Vibrio parahaemolyticus* and U-I intersected. (This is not very clear in the figure due to a technical difficulties of printing.) As shown in Fig. 4-b, no precipitation line was observed between the anti-flagellin of *Vibrio alginolyticus* and U-I of *Vibrio parahaemolyticus.* These results indicate that the flagellin of *Vibrio alginolyticus* and U-II are immunologically identical.



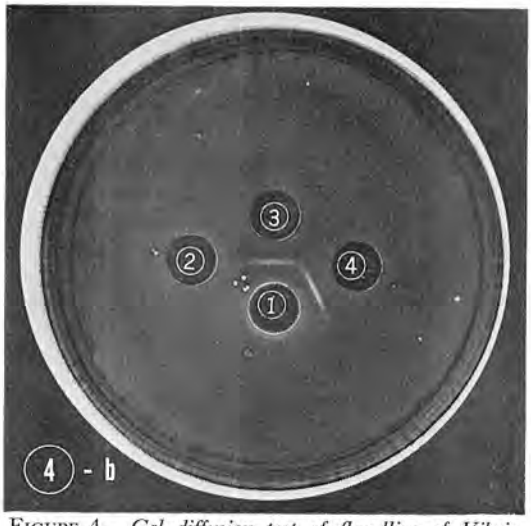


FIGURE 4. *Gel diffusion test of flagellins of Vibrio alginolyticus and Vibrio parahaemolyticus.*

Experimental conditions are described in the text. (a) Anti-flagellin of Vibrio parahaemolyticus was placed in well 1. U-I, the flagellin of *Vibrio alginoliticus and U-II were placed in wells 2, 3 and 4, respectively. (b) Anti-flagellin of Vibrio alginolyticus was placed in well 1. Other conditions are as described in (a).*

5. *Distribution of the flagellin in Vibrio alginolyticus*

The data in Fig. 5 show that the flagellin of *Vibrio alginolyticus* 6624 is also present in various strains of *Vibrio alginolyticus*. Partially purified flagella (PPF) of *Vibrio alginolyticus* 6532, 6540, 6590, 6606 and 6640 were prepared separately by the method described previously (Miwatani et al., 1970). They were solubilized by heat treatment and subjected to a gel diffusion test. It was found that the anti-flagellin of *Vibrio alginolyticus* 6624 formed a common precipitation line with the flagellins of 5 other strains and the homologous strain of *Vibrio alginolyticus*. Similar results were obtained using the anti-flagellin of *Vibrio alginolyticus* 6640.

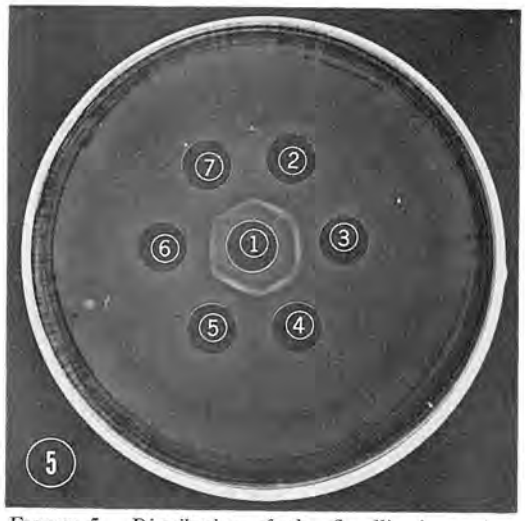


FIGURE 5. *Distribution of the flagellin in various strains of Vibrio alginolyticus.*

Conditions are as described in the text. Anti-flagellin of Vibrio alginolyticus 6624 was placed in the center well 1. Partially purified flagella of Vibrio alginolyticus 6590, 6624, 6532, 6606, 6540 and 6640 were placed in wells 2, 3, 4, 5, 6 and 7, respectively.

DISCUSSION

The flagellins of various bacteria, such as *Salmonella*, *Spirillum serpens* and *Proteus vulgaris*, have been reported to be a single protein (Weibull, 1950; Asakura et al., 1964; Martinez et al., 1967; Chang et al., 1969). Recently, we reported that the flagella of *Vibrio parahaemolyticus* contain two distinct subunits (Shinoda et al., 1970). Since *Vibrio alginolyticus* is closely related to *Vibrio para-*

haemolyticus (Fujino et al., 1965; Sakazaki, 1965), it was of interest to study the flagellin of *Vibrio alginolyticus*. Data obtained in this study show that the flagellin of *Vibrio alginolyticus* contains a single protein, which is immunologically identical to U-II of *Vibrio parahaemolyticus* reported previously (Shinoda et al., 1970). There were some differences in their amino acid compositions (Table 2.), but these did not affect spur formation in gel diffusion experiments.

Vibrio alginolyticus was previously regarded as a subgroup of *Vibrio parahaemolyticus*. However, following the classification of Sakazaki (1965), it has been named *Vibrio alginolyticus* and its identification is mainly based on biological characters. However, most biological and serological characters of these two organisms are similar. The present finding that the flagellin of *Vibrio alginolyticus* contains one of the two flagellins of *Vibrio parahaemolyticus* may provide a new method to identify these two organisms.

TABLE 2- *Amino acid compositions of the flagellin of Vibrio alginolyticus and U-II of Vibrio parahaemolyticus*

Amino acid	Flagellin of *V. alginolyticus*	U-II
Lysine	58.2	62.4
Histidine	3.2	4.4
Arginine	15.6	20.5
Aspartic acid	145.0	143.9
Threonine	57.7	59.6
Serine	64.7	74.8
Glutamic acid	136.3	136.6
Proline	0	0
Glycine	100.0	100.0
Alanine	90.2	94.7
Cystine	0	0
Valine	70.4	66.8
Methionine	3.7	6.3
Isoleucine	57.7	58.2
Leucine	60.2	61.2
Tyrosine	13.1	13.2
Phenylalanine	24.5	21.4
Amide nitrogen	94.6	95.2

Amino acid analysis was carried out as described in the text. Values are expressed taking the amount of glycine as 100.0 in each flagellin.

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