BIKEN JOURNAL, Vol. 14, 75-76, 1971

SHORT COMMUNICATION

A COMMON ANTIGENIC SUBSTANCE OF *VIBRIO PARAHAEMOLYTICUS*. II. SOME PHYSICOCHEMICAL PROPERTIES

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(Received November 10, 1970)

We reported previously that an antigenic substance (A-substance) differing from the O, K, and flagellar antigens is present in *Vibrio parahaemolyticus*. It was found in all strains of *Vibrio parahaemolyticus* examined, but not in other species of the genus Vibrio, such as *Vibrio cholerae* and *Vibrio alginolyticus* (Miwatani et al., 1969).

This paper reports some physicochemical properties of the A-substance. The samples were isolated from *Vibrio parahaemolyticus* EB 101 and purified as described previously (Miwatani et al., 1969). Purified A-substance was demonstrated to be a single component by analytical centrifugation, gel diffusion and immunoelectrophoresis.

Some physicochemical properties of the purified A-substance are summarized in Table 1. The sedimentation coefficient was determined by analytical centrifugation. A solution of purified A-substance (5 mg/ml) in 0.01 M phosphate buffer (pH 7.0) was centrifuged in a Hitachi Analytical Centrifuge, model UCA-1A at 55,430 rev/min and the sedimentation coefficient was calculated. The molecular weight was determined by the gel diffusion method as described by Andrews (1964). Five mg of sample were applied to a Sephadex G-200 column (4×100 cm) with 5 mg each of human gamma globulin (Nutritional Biochemicals Co.), bovine serum albumin (Sigma Chemical Co.) and egg albumin (Nutritional Biochemicals Co.) and the column was eluted with 0.01 M phosphate buffer, pH 7.0. The elution volume of each substance was plotted against its molecular weight (Fig. 1) and the molecular weight of the A-substance was estimated to be about 120,000. The nitrogen and sugar contents were determined as described previously (Shinoda et al., 1970) by the methods by Shiffman et al. (1964) and Morris (1948), respectively. Five mg of pure A-substance were used for each determination.

Results of amino acid analysis of the A-substance are presented in Table 2. Five mg of purified A-substance were hydrolyzed by heating in 2 ml of 5.5 N HCl at 110 C for 24 hr in a sealed tube. Then the mixture was dried by evaporation and dissolved in 2 ml of 0.2 N citrate buffer (pH 2.2). A 0.5 ml aliquot was examined in a Yanagimoto, model LC-50, amino acid analyzer.

The stability of the antigenicity of the A-substance was tested in several ways and the A-substance was assayed by gel diffusion as described previously (Miwatani et al., 1969). Samples of a solution of 100 μg/ml of A-substance in 0.01 M phosphate buffer, pH 7.0, were subjected to various treatments and then formation of a precipitation line between the

TABLE 1. *Physico-chemical properties of the A-substance*

Sedimentation coefficient	6.6 S
Molecular weight	120,000
Absorption maximum	278 mμ
$A_{280\,m\mu}/A_{260\,m\mu}$	1.58
Nitrogen content	16.1%
Sugar content	< 0.1%

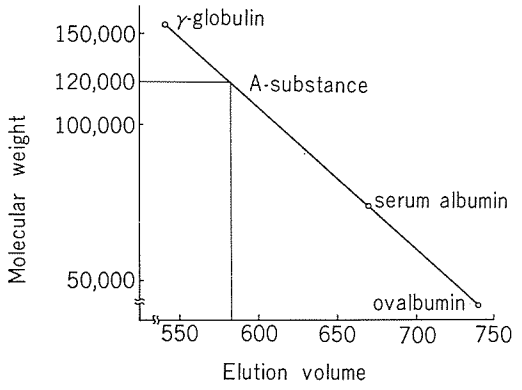

FIGURE 1. *Plot of the elution volumes against the molecular weights of various proteins and the A-substance. Human gamma globulin, bovine serum albumin, egg albumin and the A-substance were chromatographed on a Sephadex G-200 column as described in the text.*

samples and antiserum of the A-substance was examined. Treatment with Nagarse (40 Proteolytic Units of Nagarse, Nagase Sangyo Co.) destroyed the antigenicity of the A-substance

TABLE 2. *Amino acid composition of the A-substance*

Tryptophan	—
Lysine	6.4
Histidine	2.4
Arginine	4.3
Aspartic acid	13.1
Threonine	3.5
Serine	2.1
Glutamic acid	14.1
Proline	4.4
Glycine	10.0
Alanine	11.5
Cystine	—
Valine	8.0
Methionine	0.03
Isoleucine	5.6
Leucine	9.4
Tyrosine	—
Phenylalanine	4.2

Amino acid analysis was carried out as described in the text. Values are expressed as percentages of the total amino acid residues (the molecular weight is assumed to be 120,000).

so that no precipitation line was formed, while treatment with heat (70 C for 10 min or 100 C for 5 min) did not destroyed the antigenicity. These findings, together with physicochemical properties such as the nitrogen and sugar contents and the absorption spectrum, suggest that the A-substance is a heat stable protein.

The authors would like to express their thanks to Dr. Y. Takeda for his help in preparing this manuscript.

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