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Purification and Properties of an Anti-B Hemagglutinin Produced by *Streptomyces* sp.†

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ABSTRACT: An anti-B hemagglutinin was purified to homogeneity from the culture filtrate of a strain of *Streptomyces* sp. by affinity chromatography. The *Streptomyces* hemagglutinin was adsorbed to insolubilized gum arabic and eluted with 1 *M* NaCl containing 1 *M* D-galactose. The purified hemagglutinin is thought to be homogeneous judging from sodium dodecyl sulfate–polyacrylamide gel electrophoresis at pH 7.2, disc gel electrophoresis at pH 4.3, isoelectric focusing, and ultracentrifugation. The molecular weight was estimated to be 11,000 from results of gel filtration in 6 *M* guanidine hydrochloride (Gdn·HCl), sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and sedimentation equilibrium analysis. The amino acid analyses revealed that the hemagglutinin contained large amounts of alanine, glycine, and valine, 47% of the total amino acid residues, and no phenylalanine. Carbohydrate analysis demonstrated that the hemagglutinin might not be a glycoprotein. The circular dichroic (CD) spectrum of the protein is quite different from those of usual proteins in having a large positive peak at 226 nm (θ = 10,000) and a negative band at 212 nm (θ = −2600). The hemagglutinin showed a typical precipitation curve with gum arabic, and agglutinated human blood group B erythrocytes 256 times as strongly as A or O erythrocytes. These activities were not affected by pH (from 4 to 12). The anti-B activity was further confirmed by serological tests. The hemagglutination-inhibition studies indicated that D-galactose was inhibitory, but α-D-galactosides were not necessarily better inhibitors than β-D-galactosides. L-Rhamnose was the best inhibitor among the monosaccharides tested, and L-arabinose and D-fucose were also inhibitory.

Since blood group specific hemagglutinins were found in the extracts of certain plant seeds by Renkonen (1948) and Boyd and Regura (1949), numerous agglutinins have been purified to homogeneity from plants and animals (Sharon and Lis, 1972; Oppenheim et al., 1974). Many anti-A and some anti-H hemagglutinins occur in plants; however, hemagglutinins with anti-B activity had not been found until the *Streptomyces* agglutinin was reported in 1973 (Fujita et al., 1973). A lack of anti-B hemagglutinins has restricted the use of hemagglutinins as serological reagents.

Recently, Hayes and Goldstein (1974) purified and characterized an anti-B hemagglutinin from extracts of *Bandeiraea simplicifolia* seeds. Though plant agglutinins generally bind to *N*-acetyl-D-galactosamine more strongly than D-galactose (Etzler and Kabat, 1970; Galbraith and Goldstein, 1972; Poretz et al., 1974), the *B. simplicifolia* agglutinin binds to D-galactose 15 times more strongly than *N*-acetyl-D-galactosamine. The *Streptomyces* agglutinin specifically agglutinates blood-group B erthrocytes and appears to bind to D-galactose 60 times more strongly than *N*-acetyl-D-galactosamine, based on the results of hemagglutination-inhibition tests with a partially purified sample. Thus, the *Streptomyces* agglutinin is probably more specific to blood group B erythrocytes than *B. simplicifolia* agglutinin and will be very useful as a serological reagent.

This report describes the purification of the *Streptomyces* hemagglutinin and characterization of the purified hemagglutinin concerning its purity, specificity, and some of its physicochemical properties.

Materials and Methods

Cultivation. Streptomyces 27S5 was kindly supplied by Professor H. Yonehara, Institute of Applied Microbiology, University of Tokyo. The bacteria were grown in a 30-l. jar fermenter under the same conditions as described previously (Fujita et al., 1973). Each jar containing 15 l. of medium was inoculated with 400 ml of a log phase culture. Growth was continued for 4 days at 27°. At the end of this period, the hemagglutinating activity reached a plateau of about 2 hemagglutination titer, and the culture broth was harvested by filtration.

Assays. Assays of hemagglutinating activity were performed with a microtiter apparatus (Cooke Engineering Co., Alexandria, Va.) using a 2% human A, B, O, or AB erythrocyte suspension and 0.15 *M* NaCl as diluent. Hemagglutination was conducted for 90 min at room temperature. The activity was expressed as titer, the reciprocal of the highest twofold dilution exhibiting positive hemagglutination. For inhibition studies, purified hemagglutinin (titer,

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2-4) was thoroughly mixed with varying concentrations of saccharides to be tested, and then a 2% human B erythrocyte suspension was added to the mixture. The degree of inhibition was expressed as a final concentration of the maximum dilution at which the saccharide could inhibit hemagglutination.

Quantitative precipitation analyses were performed in test tubes (1 × 10.5 cm) in a total volume of 200 μl. The final concentration of NaCl in the reaction mixture was 0.15 *M*. Various amounts (0–100 μg) of gum arabic and 20 μg of the hemagglutinin were mixed and incubated at 37° for 15 min. The resulting turbidity was measured spectrophotometrically at 420 nm with a Jasco UVIDEC-2 spectrophotometer.

Cross-Linking of Gum Arabic. Gum arabic was insolubilized by cross-linking with epichlorohydrin according to the preparation of Sephadex (Flodin, 1962). One hundred grams of gum arabic purchased from Kanto Chemical Co. Inc. (Tokyo, Japan) was immersed in 80 ml of water and 120 ml of 5 *N* NaOH was added. After gums were completely dissolved, 27 g of epichlorohydrin was mixed and vigorously stirred at 40° until the mixture solidified into a block of gel, which occurred after 15 min. The reaction was allowed to continue for 24 hr, and then the reaction temperature was raised to 70°. After standing at this temperature overnight, the brittle gel was broken into portions, swollen in water, and homogenized in a Waring Blendor. It was thoroughly washed with water, and fine particles were removed by decantation.

Electrophoresis. Disc electrophoresis on 15 or 7.5% polyacrylamide gels at pH 4.3 was conducted as described by Reisfeld et al. (1962). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol was performed by the method of Weber and Osborn (1969). To determine the molecular weight, 5% polyacrylamide gel in the presence of sodium dodecyl sulfate was used, since Dunker and Rueckert (1969) pointed out that a molecular weight–mobility plot was linear below 20,000 daltons on the 5% polyacrylamide gels. Gels were stained with 0.1% Coomassie Brilliant Blue and destained in 7% acetic acid. Migration distances were expressed as relative mobilities, i.e., the ratio of each migration distance to that of α-chymotrypsinogen. The following proteins were used as internal standards: bovine serum albumin, 69,000; ovalbumin, 46,000; α-chymotrypsinogen, 26,000; myoglobin, 17,600; lysozyme, 14,300; ribonuclease A, 13,600; cytochrome *c*, 12,400; mixture of A and B chains of insulin, 2900; bacitracin, 1450. Isoelectric focusing was conducted in 7.5% polyacrylamide gels with a pH gradient of 3 to 10 (Wrigley, 1971).

Gel Chromatography in 6 M Gdn·HCl.[1] The column and samples were prepared by the method of Fish et al. (1969) with slight modifications. Hemagglutinin and standard proteins used in the gel filtration were reduced with 0.05 *M* dithiothreitol in 6 *M* Gdn·HCl at pH 8.6 for 2 hr, and then carboxymethylated by the addition of a twofold molar excess (with respect to SH groups of dithiothreitol) of solid, recrystallized iodoacetic acid at the same pH. Seven proteins (mol wt 69,000–1450) were used to calibrate a column of Sepharose 6B (1.5 × 86 cm) that had been equilibrated previously with 6 *M* Gdn·HCl. Samples were applied to the column together with blue dextran and Dnp-alanine. Elution positions were normalized in terms of a distribution coefficient, K_d.

Gel Chromatography. A column of Sephadex G-75 calibrated with native proteins was used to estimate the molecular weight of hemagglutinin by the method of Andrews (1964).

Ultracentrifuge Analyses. Sedimentation velocity experiments were conducted with a Hitachi UCA-1A ultracentrifuge equipped with Schlieren optics. Sedimentation coefficients of the protein in 8 *M* urea or in 1 *M* NaCl containing 1 *M* D-galactose were determined using a synthetic boundary cell at 56,200 rpm. All values were corrected to water at 20°. Sedimentation equilibrium experiments were performed with a Beckman Spinco Model E ultracentrifuge using interference optics. The protein was dissolved in 1 *M* NaCl containing 1 *M* D-galactose and was dialyzed extensively against the same solution. The protein concentration was adjusted to 3.5 mg/ml and the resultant solution was used for centrifugation experiments. Partial specific volume was assumed to be 0.72 ml/g, which was calculated from the amino acid composition (Cohn and Edsall, 1943).

Amino Acid Analyses. Samples were hydrolyzed in 6 *N* HCl at 110° for 24, 48, and 72 hr in sealed evacuated tubes. Quantitative analyses were performed by the method of Spackman et al. (1958) with a JEOL automatic amino acid analyzer Model JLC-6AH.

Carbohydrate Analysis. The phenol–sulfuric acid method (Dubois et al., 1956) was used for carbohydrate analysis with D-galactose as the standard.

Determination of Protein. Protein in crude extract was estimated by absorbance at 280 nm. The amount of purified hemagglutinin was quantitated by its absorbance at 280 nm using $E_{1cm}^{1\%} = 23.4$.

Absorption Spectra. Absorbance measurements were performed at room temperature with a Shimazu MPS-50L multipurpose recording spectrophotometer. Circular dichroism measurements were carried out at 25° with a Jasco J-20 automatic recording spectropolarimeter. A mean residue weight of 102 daltons was used in the calculations of molar ellipticities.

Results

Purification of the Streptomyces Hemagglutinin. To 25 l. of culture filtrate was added 110 ml of swollen cross-linked gum arabic gel, and this suspension was stirred at 4° overnight. The gel was collected by sedimentation and packed into a column. The column was washed successively with 0.15 *M* NaCl, 1 *M* NaCl, and then with 0.2 *M* D-galactose in 1 *M* NaCl to remove contaminating proteins. The hemagglutinin was eluted by washing the column with 1 *M* D-galactose in 1 *M* NaCl. A typical elution profile is illustrated in Figure 1. The active fractions were pooled and dialyzed against water or applied to a column of Sephadex G-15 to remove salts and sugars. The resulting hemagglutinin solution was freeze-dried and stored at −20°. The yield was about 60 mg, corresponding to 64% of the total activity found in the original culture filtrate (25 l.).

Purity. A single protein band was obtained on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol (Figure 2A,B), and also by isoelectric focusing (Figure 2C). Disc gel electrophoresis at pH 4.3 usually gave one band (Figure 2D); however, in some instances two bands (Figure 2E) could be detected. Nevertheless, no significant differences could be found in the amino acid compositions of the two bands. The extra

[1] Abbreviations used are: Gdn·HCl, guanidine hydrochloride; Et, ethyl; F_3EtOH, trifluoroethanol; Dnp, dinitrophenyl.

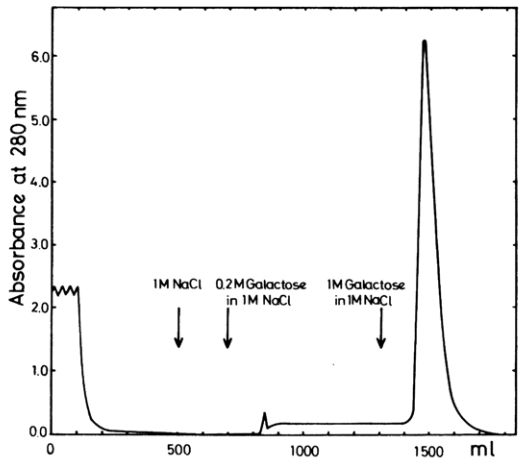

FIGURE 1: Purification of the *Streptomyces* hemagglutinin by affinity chromatography on insolubilized gum arabic. Insolubilized gum arabic (110 ml) was added to the culture filtrate (25 l.) and the mixture was stirred overnight at 4° to adsorb the hemagglutinin. Then the gel was packed into a column (2.8 × 18 cm) and the column was successively washed with 0.15 *M* NaCl, 1 *M* NaCl, and 1 *M* NaCl containing 0.2 *M* D-galactose. The hemagglutinin was finally eluted from the column with 1 *M* NaCl containing 1 *M* D-galactose.

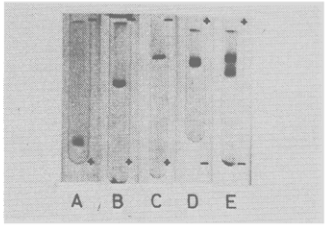

FIGURE 2: Electrophoresis of the *Streptomyces* hemagglutinin: (A) sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% gel at pH 7.2; (B) sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 15% gel at pH 7.2; (C) isoelectric focusing on polyacrylamide gel (gradient of pH, 3–10); (D) and (E) disc gel electrophoresis on 7.5% gel at pH 4.3.

protein band is probably derived from aggregation of the hemagglutinin. Investigations are now in progress to clarify this problem further in detail. Purity of the samples was also examined by ultracentrifugal analyses under the conditions where aggregation of the hemagglutinin would not occur. A single symmetrical peak was observed in 8 *M* urea and in 1 *M* NaCl containing 1 *M* D-galactose. Furthermore, Sephadex gel filtration gave a single symmetrical peak. Thus, the hemagglutinin purified in the present study is thought to be homogeneous judging from these results.

Molecular Weight Determination. The molecular weight was calculated to be 10,400 ± 500 from sedimentation equilibrium analysis. A similar value (10,300 ± 2000) was also estimated from the mobility–molecular weight relationship on 5% polyacrylamide gel in the presence of sodium dodecyl sulfate and 2-mercaptoethanol. The values agree fairly well with that obtained by Sepharose 6B gel filtration in 6 *M* Gdn·HCl (11,800 ± 2000). From these results, the molecular weight of the hemagglutinin is assumed to be 11,000.

Amino Acid and Carbohydrate Analyses. Amino acid analyses of the hemagglutinin revealed that the contents of alanine, glycine, and valine residues are very high, but no phenylalanine residue is present (Table I). A minimum molecular weight of 10,700 was calculated from the amounts of lysine and proline residues. This value is in good agreement with molecular weights determined by other methods. The content of neutral sugar in a purified desalted sample measured by the phenol–sulfuric acid method was 1.8%, which corresponds to 1 mol of hexose per 11,000 daltons. The result might suggest that the hemagglutinin contains a few moles of hexose per mole of the protein. However, although the hemagglutinin actually contains some sugars, it is not certain at the moment whether they are bound covalently to the protein or not.

Table I: Amino Acid Composition of the *Streptomyces* Hemagglutinin.

Amino Acid	nmol/47.5 μg of Hemagglutinin	No. of Amino Acid Residues per 11,000 g[a]
Lys	4.42	1
His	11.23	2
Arg	29.54	6
Asp	30.08	6
Thr	37.2[b]	8
Ser	23.9[b]	5
Glu	41.04	9
Pro	3.90	1
Gly	78.83	17
Ala	89.48	19
Cys		3[c]
Val	59.99	13
Met	11.01	2
Ile	13.96	3
Leu	15.23	3
Tyr	12.0[b]	3
Trp		4[d]

[a] Calculated assuming that 4.67 nmol equals 1 residue/mol. The value (4.67 nmol) was calculated from the experimental values for Lys, Pro, Ile, and Leu assuming that 1 mol of Lys and Pro and 3 mol of Ile and Leu were contained in 1 mol of protein. [b] Extrapolated value to zero hydrolysis time. [c] Determined as cysteic acid. [d] Estimated by the method of Edelhoch (1967).

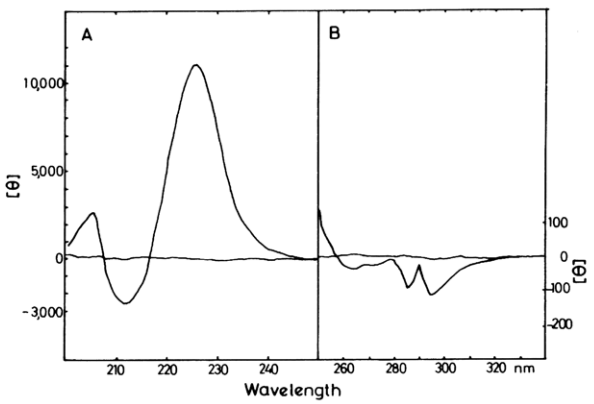

FIGURE 3: CD spectra of the *Streptomyces* hemagglutinin. The spectra were measured in 50 m*M* phosphate buffer (pH 6) at a protein concentration of 0.71 mg/ml in a (A) 1 mm or (B) 10 mm cuvette.

Spectral Studies. The ultraviolet absorption spectrum of the protein has an absorption peak at 279 nm with shoulders at 286 and 272 nm. The circular dichroic (CD) spectrum of the *Streptomyces* hemagglutinin is quite different from those of usual proteins in that it has a prominent positive band at 226 nm (θ = 10,000) and a negative band at 212 nm (θ = 2600) as shown in Figure 3. The spectrum did not change significantly when it was measured in 0.1 *N* NaOH (pH 12.7) or in 8 *M* urea. The positive peak, however, decreased somewhat in 1 *N* NaOH (pH 13.6), and

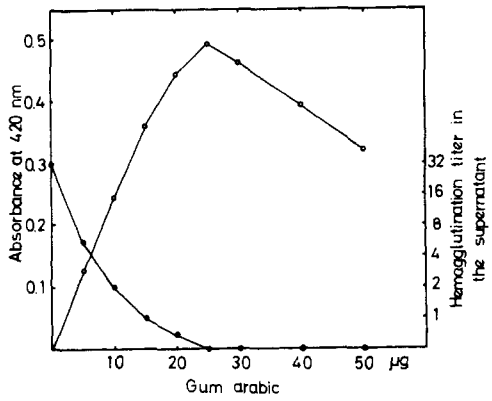

FIGURE 4: Quantitative precipitation curve of the *Streptomyces* hemagglutinin with gum arabic (○). Turbidity is plotted against polysaccharide concentration. The reaction mixture (total volume, 200 μl) contained 20 μg of the hemagglutinin and the final concentration of NaCl was 0.15 *M*. Hemagglutinating activity in the supernatant is also plotted against the polysaccharide concentration (●).

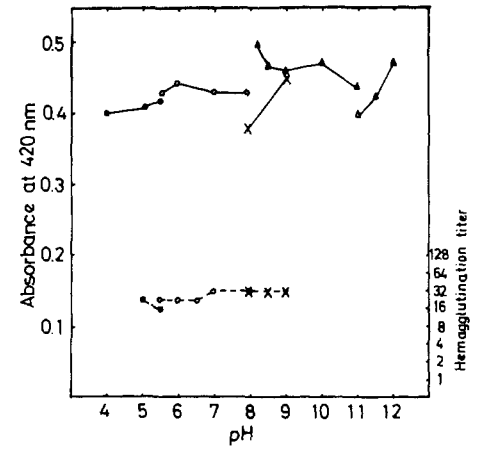

FIGURE 5: Effects of pH on the interaction of the hemagglutinin with gum arabic (—) and on the hemagglutinating activity for human blood group B erythrocyte (- - -). The reaction mixture (total volume, 200 μl) contained 20 μg of hemagglutinin and 25 μg of gum arabic, and the final concentrations of NaCl and buffer were 0.15 *M* and 20 m*M*, respectively. Reaction was carried out at 37° for 15 min: (●) citrate buffer; (○) phosphate buffer; (×) Tris-HCl buffer; (▲) NH_3–NH_4Cl buffer; (△) Na_2HPO_4–NaOH buffer.

Table II: Hemagglutination of Human Erythrocytes by the *Streptomyces* Hemagglutinin.[a]

	Blood Group			
	A	B	O	AB
Hemagglutination titer	4	1024	4	256

[a] Protein concentration, 0.96 mg/ml.

Table III: Hemagglutination–Inhibition Assay of the *Streptomyces* Hemagglutinin with Human Saliva.

	Dilution of Saliva							
Saliva	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
BSe[a] (YM)	0	0	0	0	2+	2+	2+	2+[c]
Bse[b] (TI)	0	1+	2+	2+	2+	2+	2+	2+
Bse[b] (ST)	0	2+	2+	2+	2+	2+	2+	2+
Without saliva	2+	2+	2+	2+	2+	2+	2+	2+

[a] Secretor individual of blood group B. [b] Nonsecretor individual of blood group B. [c] 1+, 2+: degree of agglutination.

Table IV: Inhibition of the *Streptomyces* Hemagglutinin by Various Saccharides.

Saccharide	Minimum Amount (m*M*) for Complete Inhibition
D-Galactose	2.1
D-Galactono-1,4-lactone	>167
D-Galactitol	>57
2-Amino-2-deoxy-α-D-galactopyranoside	>167
2-Acetamido-2-deoxy-α-D-galactopyranoside	>167
Methyl α-D-galactopyranoside	2.1
Methyl β-D-galactopyranoside	1.3
Phenyl α-D-galactopyranoside	0.52
Phenyl β-D-galactopyranoside	0.52
L-Rhamnose	0.21
L-Arabinose	2.6
D-Fucose	5.2

was rapidly diminished by heating, or in acidic media, 6 *M* Gdn-HCl, or 0.1% sodium dodecyl sulfate. Since the hemagglutinating activity was lost under the latter conditions, disappearance of the activity might correlate with the diminution of the positive peak at 226 nm.

Quantitative Precipitation. Typical precipitation curves between purified hemagglutinin and gum arabic are shown in Figure 4. When the turbidity is plotted against increments of gum arabic, a sharp maximum is obtained at the equivalence zone. If the complex was removed by centrifugation, the hemagglutinating activity in the supernatant fraction decreased with increased precipitation and disappeared at the point of maximum complexation. Guar gum or locust bean gum, which seem to react with the hemagglutinin, cannot be examined in this system because the solution is turbid in itself.

Effect of pH. Precipitation reaction with gum arabic and hemagglutination of the agglutinin were not affected when pH values of the reaction mixture were changed from 4 to 12 as shown in Figure 5. Below pH 4, the protein was irreversibly denatured, and the activity was rapidly lost.

Blood Group Specificity. The hemagglutinin strongly agglutinated all the samples of human B and AB erythrocytes tested, the anti-B activity being stronger than those for A and O erythrocytes (Table II). The minimum concentrations of the purified agglutinin required to agglutinate human B, AB, A, and O cells were 0.9, 3.8, 240, and 240 μg/ml, respectively. The anti-B activity was confirmed by the facts that the purified hemagglutinin was inhibited by salivas from secretor individuals of blood group B, but not inhibited appreciably by those from nonsecretors (Table III).

Hapten Inhibition Studies. The sugar specificity of purified hemagglutinin was examined by hemagglutination-inhibition tests. Results are summarized in Table IV. The hemagglutination was inhibited by D-galactose with the pyranose form, but not by that with the lactone ring. α-D-Galactosides were not necessarily better inhibitors than β-D-galactosides. L-Rhamnose was the best inhibitor among the monosaccharides tested, and its inhibitory effect was 10 times as strong as that of D-galactose. These results are the

same as previous results which were obtained with partially purified samples.

Discussion

In a previous paper, it was reported that the *Streptomyces* hemagglutinin was purified by conventional column chromatographic methods. The procedure was time consuming and the recovery (0.8%) was not satisfactory. In the present paper, one-step purification of the hemagglutinin was achieved using affinity chromatography on insolubilized gum arabic prepared by cross-linking with epichlorohydrin. With this improved method, a larger scale preparation can easily be carried out and the whole purification procedure can be finished within 2 days. Furthermore, the overall yield was 64% which is about 80 times higher than that with the previous conventional method.

The hemagglutinin strongly bound to a gum arabic column, and was not released from the column by washing with 1 *M* NaCl, 8 *M* urea, or 0.1 *M* ammonia solution. The hemagglutinin was eluted only slightly from the column by washing with 1 *M* NaCl containing 0.2 or 0.4 *M* galactose. However, by raising the sugar concentration in the eluent to 1 *M*, it could be eluted as a sharp peak. Compared with other hemagglutinins which were released from their affinity adsorbents by 0.1–0.4 *M* inhibitory sugars (Lis and Sharon, 1973), the interaction between the *Streptomyces* hemagglutinin and the adsorbent appears to be stronger. Due to this interaction, it might be possible to use a batchwise method to adsorb the hemagglutinin onto the adsorbent directly from the culture filtrate.

Although the hemagglutinin seems to bind to L-rhamnose and/or L-arabinose at a nonreducing terminal of gum arabic, the actual mode of interaction between the hemagglutinin and the adsorbent is not certain at the moment. Since the hemagglutinin can be purified to homogeneity with the cross-linked gum arabic, analyses of the mode of binding to the adsorbent are not so important for practical purposes.

The hemagglutinin purified by affinity chromatography was homogeneous on sodium dodecyl sulfate–polyacrylamide gel electrophoresis at pH 7.2, disc gel electrophoresis at pH 4.3, isoelectric focusing, and by ultracentrifugation.

Sedimentation coefficients ($s_{20,w}$) of the hemagglutinin were measured at various protein concentrations (from 2.7 to 6.2 mg). Average $s_{20,w}$ values were 1.35 S in 8 *M* urea and 1.49 S in 1 *M* NaCl containing 1 *M* D-galactose. From these results together with data described in the Results section, the molecular weight of this hemagglutinin was concluded to be 11,000.

In a previous paper (Fujita et al., 1973), the molecular weight of the hemagglutinin was reported as 5000 ± 1000 as determined by gel column chromatography on Sephadex G-75. It was later found that the hemagglutinin interacted with dextran and was therefore retarded in eluting from a Sephadex G-75 column. Thus, the molecular weight estimated by this method was smaller than the present value. The interaction was partially abolished by inhibitory sugars such as D-galactose, and the hemagglutinin was eluted earlier from a Sephadex G-75 column in the presence of D-galactose than in its absence. Sephadex interaction was also affected by the degree of cross-linking in dextran. The hemagglutinin interacts with Sephadex G-25, G-50, and G-75, but not with Sephadex G-15. A similar phenomenon has been found with concanavalin A, which binds to Sephadex G-50 but not to Sephadex G-25 (Agrawal and Goldstein, 1965).

As mentioned above, the molecular weight of the present protein is one of the smallest hemagglutinins so far reported (Lis and Sharon, 1973). However, it is open to question whether the hemagglutinin acts upon erythrocytes as it is or whether it forms aggregates to express the hemagglutinating activity.

Amino acid composition of the hemagglutinin is peculiar in that the content of alanine, glycine, and valine is very high, 47% of the total amount of amino acid residues. Since the amount of carbohydrates detected in the protein is very low, we consider at present that the *Streptomyces* hemagglutinin may not be a glycoprotein.

The ultraviolet absorption spectrum of the protein closely resembles that of free tryptophan. The CD spectrum of the *Streptomyces* hemagglutinin has a positive band at 226 nm. Most proteins, of which secondary structures are mixtures of α helix, β structure, and unordered conformations, show negative circular dichroism at this region. The CD spectrum of the hemagglutinin is quite different from ordinary proteins in this respect. However, similar peculiar CD patterns have been reported for poly(L-tryptophan) (Cosani et al., 1968), cobrotoxin (Yang et al., 1968), and the gene 5 protein of fd bacteriophage (Day, 1973), with positive peaks at 226 nm ($\Delta_{abs} = 14.1 \times 10^4$), 228 nm ($\theta = 4800$), and 228 nm ($\theta = 3362 \pm 150$), respectively. In the case of the gene 5 protein, Day has assigned the positive 228-nm peak to the phenolic transition of tyrosine residues judging from its pH dependence and the data obtained with model compounds. In contrast, the positive band at 226 nm of the hemagglutinin did not change by raising the pH to 13. Consequently the band may not be attributed to phenolic group transitions.

The CD pattern of poly(L-tryptophan) ([γ-Et-DL-Glu]$_{160}$[L-Trp]$_{32}$) in trifluoroethanol in the 200–250-nm region has a strong resemblance to that of the *Streptomyces* agglutinin. Cosani et al. (1968) have concluded that poly(L-tryptophan) is in some helical conformation in F_3EtOH, and that strong overlapping of CD bands from side-chain chromophores and peptide chromophores in the wavelength range 185–240 nm does not allow definite conclusions to be drawn about the type of helical conformation which exists in poly(L-tryptophan) in F_3EtOH solution. In the present case of the *Streptomyces* agglutinin, it is highly likely that the tryptophan side chains contribute to the positive CD band at 226 nm.

The infrared spectrum of the hemagglutinin suggested the presence of the β structure. However, the negative CD band around 217 nm cannot be seen due to the large positive contribution of the side-chain chromophores. Further details on this problem will be published elsewhere.

Numerous hemagglutinins have been purified to homogeneity, but typical anti-B hemagglutinins have not been obtained from plants. In 1974, however, Hayes and Goldstein purified and characterized from *Bandeiraea simplicifolia* seeds a so-called anti-B lectin, the existence of which had been known through its anti-B activity (Mäkelä and Mäkelä, 1956). The lectin is specific for α-galactopyranosyl residues, but agglutinates both human blood group A_1 and B erythrocytes. The hemagglutinating activity for A_1 cells is one-quarter of that for B cells.

In contrast, the purified *Streptomyces* agglutinin agglutinated human B cells 256 times as strongly as A or O cells. Thus, as far as serological activity is concerned, the *Streptomyces* agglutinin is more suitable to be called an anti-B hemagglutinin than the *B. simplicifolia* lectin.

As regards sugar specificity, the *Streptomyces* hemagglutinin is different from anti-B serum in binding only to D-galactose residues at nonreducing terminals of oligosaccharides as described in a previous paper (Fujita et al., 1973). In addition, hemagglutination-inhibition tests with methyl α- and β-galactopyranosides and phenyl α- and β-galactopyranosides reveal that α-galactosides are not necessarily better inhibitors than β-galactosides. These results are inconsistent with the fact that anti-B determinant requires α-D-galactose at the nonreducing terminal. However, the *Streptomyces* agglutinin agglutinates human blood group B erythrocytes, and its activity is inhibited by human B substances. In contrast, hemagglutinins which bind to D-galactose are not always specific for blood group B erythrocytes (Gilboa-Garber, 1972; Van Wauwe et al., 1973; Olsnes et al., 1974). Anti-B activity of the *Streptomyces* hemagglutinin may relate to other structures on the erythrocyte membrane besides D-galactosyl residues. We will further examine this problem using enzyme treatments of blood group B substances and quantitative precipitation analyses.

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