

31678

Immunochemical Studies of Dextran Coupled Ragweed Pollen Allergen, Antigen E¹

TE PIAO KING, LUCIA KOCHOUMIAN, KIMISHIGE ISHIZAKA, LAWRENCE M. LICHTENSTEIN, AND PHILIP S. NORMAN

The Rockefeller University, New York, New York 10021, and The Johns Hopkins University, Baltimore, Maryland 21239

Received January 21, 1975

The major allergen of ragweed pollen antigen E has been coupled to periodate oxidized dextrans, followed with sodium borohydride reduction to stabilize the linkages. Two products having molecular weights of about 100,000 and 140,000 were prepared, and the molar ratio of dextran to antigen E in both products was the same in the range of 2-5. The antigenic and the allergenic activities of the products were on a molar basis about seven to eight fold less than those of antigen E. On dextranase digestion of the products, their biological activities were restored. The products were immunogenic in rabbits to stimulate the formation of antibodies reacting with antigen E and with dextran.

Antigen E is a major allergen of ragweed pollen. It is an acidic protein containing two polypeptide chains which are held together by noncovalent forces, and the two polypeptides have molecular weights of about 16,000 and 22,000 (2-3). We have been studying chemical modifications of this protein with the purposes of (a) obtaining a better understanding of its allergenic properties and (b) obtaining suitable derivatives with reduced allergenic activities which may be useful as therapeutic agents in ragweed sensitive patients. Chemically modified derivatives of antigen E containing decreased number of antigenic determinants are likely to have reduced allergenic activities as allergic responses are generally believed to require the interaction of a multivalent antigen with its specific cell surface bound immunoglobulins of the IgE class (cf. Ref. 4).

Our previous studies have shown that sixteen out of eighteen lysine residues of antigen E are accessible for acetylation, succinylation, or butyrimidination (2, 5).

¹This work was supported in part by Grants AI-08445, AI-07290 and AI-0866 of the National Institutes of Health, and by Grant GB-41443 from the National Science Foundation.

The acetylated and the succinylated derivatives were unstable as they slowly polymerized on standing but the butyrimidinated derivative was stable. The allergenic and the antigenic properties of the butyrimidinated antigen E were the same as those of the native antigen as shown respectively by skin tests on sensitive patients and by precipitin analysis with sheep anti-antigen E sera. These findings suggest that the positive charges of ϵ -amino groups of lysyl residues of antigen E are necessary for the conformational stability of the molecule, and that the butyrimidinyl groups introduced are too small in size to interfere with the reactivity of the allergenic or antigenic determinants of antigen E with antibodies. We therefore sought to prepare other derivatives of antigen E having substituents of larger molecular size which may sterically hinder the reactivity of the determinants of antigen E.

In this paper will be reported our studies on the preparation and the immunochemical properties of derivatives of antigen E coupled to dextrans of different molecular weights. Antigen E was coupled via its ϵ -amino groups of lysyl residues to the aldehyde groups of periodate treated dex-

trans and the resulting by sodium borohydride. These reactions are they have been previously for the coupling of polysaccharides (6). The procedure of proteinogen bromide activation (8, 9) was first explored practical because of of antigen E with dextran the competing self-dextran molecules.

EXPERIMENTAL

Materials

Antigen E was isolated according to the published method (1) obtained in several isolectin forms B and C (1). In the present study and the

Dextrans of different molecular weights obtained from Pharmacia Chemical Corporation. Bovine serum albumin obtained from Armour Chemical Company. Sheep anti-antigen E serum from the Laboratory.

Chemical Analysis

Aldehyde content of dextran was analyzed by the method of Hodge (7). Dextran concentrations were determined by the phenanthroline-sulfuric acid method adapted for use in a Technicon Autoanalyzer. Flow rates of anthrone streams being, respectively, 0.5 ml/min, and the sample flow rate was 0.5 ml/min with a pickup time of 0.5 min (corresponding to about 10 sec heating period at 95°C). Absorbance was measured at 570 nm. Under these conditions of dextran gave an absorbance of 0.1. Absorbance was not maximum of the anthrone reaction not have available the acid analysis was carried out (2); the samples (about 100 μ l) at 110°C in test tubes.

Periodate oxidation under the conditions described (1) was terminated each 10 ml of the reaction mixture was dialyzed in Visking dialysis tubing against water overnight then

trans and the resulting linkages were stabilized by sodium borohydride reduction. These reactions are shown in Fig. 1 and they have been previously used by others for the coupling of proteins to other polysaccharides (6). The more commonly used procedure of protein coupling with cyanogen bromide activated polysaccharides (7, 8, 9) was first explored but it was found not practical because of the low coupling yield of antigen E with dextran and because of the competing self coupling reaction of dextran molecules.

EXPERIMENTAL PROCEDURES

Materials

Antigen E was isolated from ragweed pollen according to the published procedures (1, 10). It can be obtained in several isoelectric forms. The two predominant forms B and C (1) were used interchangeably in the present study and they gave identical results.

Dextrans of different molecular weights were obtained from Pharmacia Company. Dextranase and lysozyme were obtained from Worthington Biochemical Corporation. Bovine plasma albumin was obtained from Armour Company. *N*- α -Acetyl-L-lysine was obtained from Cyclo Chemical Corporation. Sheep anti-antigen E sera was a gift from Center Laboratory.

Chemical Methods

Aldehyde content of periodate oxidized dextrans was analyzed by the ferricyanide method (11). Dextran concentrations were determined by the anthrone-sulfuric acid method (12). The procedure was adapted for use in a Technicon autoanalyzer with the flow rates of anthrone reagent, air, and sample streams being, respectively, 1.44, 1.20, and 0.32 ml per min, and the sampler was set for 60 samples per h with a pickup time of about 10 s for each sample (corresponding to about 50 μ l). After about a 10-min heating period at 95°C, the mixture was analyzed at 570 nm. Under these conditions a 1 mg per ml solution of dextran gave an absorbance reading of 0.3–0.4. Absorbance was not measured at the absorption maximum of the anthrone-dextran product as we did not have available the appropriate color filter. Amino acid analysis was carried out as described previously (2); the samples (about 3 nmol) were hydrolyzed in 6 N HCl (200 μ l) at 110°C for 20 h in sealed and evacuated tubes.

Periodate oxidations of dextrans were carried out under the conditions described in Table I. The reaction was terminated by adding 0.25 ml of glycerol to each 10 ml of the reaction mixture. The mixture was dialyzed in Visking 18/32 tubing against distilled water overnight then lyophilized. The weight recover-

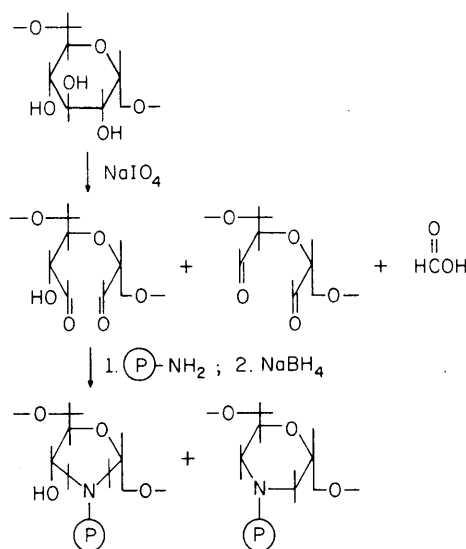


FIG. 1. Scheme for coupling of protein to periodate oxidized dextran. Dextran is a 1,6-linked polyglucose with periodic branching at the 3-position, and the branching is now shown.

ies were in the range of 90–95%. The number of aldehyde groups formed per 10⁶ g of oxidized dextran was measured by the ferricyanide method using dextrose as a standard. To determine if any glycosidic linkage cleavage occurred during oxidation the samples (10 mg per ml) after reduction with 0.04 M sodium borohydride in borate buffer (pH 9.05) as described below were examined by chromatography on a 105 × 0.9 cm column of Sephadex G-100 or Sepharose 6 B.

Coupling of proteins with periodate oxidized dextrans. A mixture of protein (7–10 mg) and oxidized dextran (25 or 50 mg) in 2.5 ml of buffer was allowed to stand at 25°C for the desired time period. A freshly prepared solution (100 μ l) of 1 M sodium borohydride in the same buffer was added. After 30 min, acetone (100 μ l) was added to decompose the excess borohydride. After another 30 min, the mixture was chromatographed on a 100 × 0.9 cm column of Sephadex G-100 or a 200 × 0.9 cm column of Sepharose 6 B. The column was eluted with 0.1 M ammonium bicarbonate at a flow rate of about 10 ml per h. Fractions of 2 ml volume were collected. They were analyzed for protein content by absorbance at 280 nm and for dextran content by the anthrone reaction. The desired product was concentrated by lyophilization or ultrafiltration through Visking 8/32 tubing (1). The compositions of buffers used for coupling were as follows: (a) pH 7.80, 0.183 M Na₂HPO₄ and 0.017 M NaH₂PO₄; (b) pH 8.55, 0.100 M H₃BO₃ and 0.025 M Na₂B₄O₇; (c) pH 9.05, 0.024 M H₃BO₃ and 0.025 M Na₂B₄O₇.

The molecular weights of the were estimated from their elution profile on Sepharose 6 B chromatography on the

different changes in their properties (Table II). A freshly prepared solution of oxidized dextran in solutions of pH greater than 7 showed negligible ultraviolet absorption, but on standing an absorption peak at 240 nm slowly developed. Accompanying this change is a decrease in the molecular weight as estimated by its elution position from a Sepharose 6 B column. These elution position changes are shown in Fig. 2 for the oxidized dextrans T-70, T-500 and T-2,000 with 56, 48, and 48 aldehyde groups per 10^5 g, respectively. These changes probably result from alkaline degradation of the glycosidic linkages via β -elimination which have been reported to occur for the dialdehyde cellulose (16).

Coupling of periodate oxidized dextrans with proteins. The coupling reactions were carried out in basic solutions at 25°C. After coupling the reaction mixtures were treated with sodium borohydride to stabilize the linkages of the coupled product (Fig. 1). The mixtures were then analyzed by chromatography on Sephadex or Sepharose gels to establish the extent of coupling as well as to estimate the molecular size of the product. Three different proteins were studied, and they are lysozyme, bovine

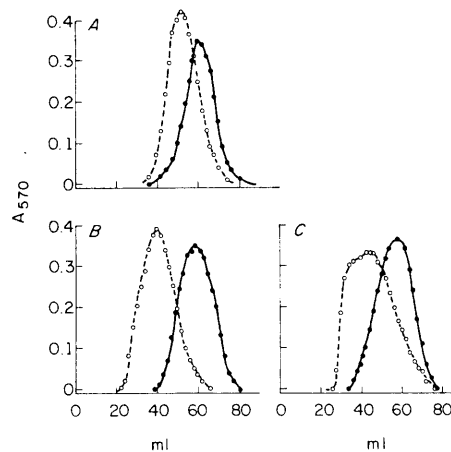


FIG. 2. Chromatography of dextrans and periodate treated dextrans on a column (105×0.9 cm) of Sepharose 6 B. Pattern A. Dextran T-70; B. Dextran T-500; and C. Dextran T-2000. The native dextrans and the periodate oxidized dextrans after sodium borohydride reduction gave indistinguishable elution curves; only those for the native dextrans are shown, designated by open circles. The curves with closed circles are for periodate oxidized dextrans after standing at pH 9.05 for 24 h followed with borohydride reduction. The void and the total volumes of the column were 27 and 47 ml, respectively.

plasma albumin and antigen E. These experiments are summarized in Table III.

Experiments 2, 3, and 4 show that the coupling between lysozyme and dextran T-70 with 56 aldehyde groups per 10^5 g required 24 h to give better than 95% yield at pH 8.55 and 25°C, and that the reaction was slower at pH 7.8 than that at pH 8.55. Under identical reaction conditions dextran T-70 with 22 aldehyde groups per 10^5 g (Experiment 1) had a lower capacity to couple with lysozyme than the same dextran with 56 aldehyde groups (Experiment 4). The optimal conditions established for the coupling of lysozyme with oxidized dextran T-70 were also applicable for bovine plasma albumin (Experiment 5).

The optimal conditions established for lysozyme and albumin coupling to dextran T-70 with 56 aldehyde groups were not directly applicable to antigen E. Chromatographic separation of the reaction mixture of Experiment 6 showed that only about 60% of the antigen E was coupled to the dextran (Fig. 3A); the dextran coupled antigen E was eluted at about 100 ml while

TABLE II
ULTRAVIOLET SPECTRAL AND MOLECULAR WEIGHT
CHANGES OF PERIODATE OXIDIZED DEXTRANS IN
ALKALINE SOLUTIONS^a

Dextran sample, aldehyde groups per 10^5 g	pH of solution	Absorbance at 240 nm		Average molecular weight after 24 h
		0 h	24 h	
T-70, 228 groups	7.80	0.67	3.72	
T-70, 56 groups	7.80	0.25	0.66	
T-70, 56 groups	8.55	0.18	1.55	30,000
T-70, 56 groups	9.05	0.18	2.74	18,000
T-70, 22 groups	9.05	0.23	1.24	35,000
T-500, 48 groups	9.05	0.20	2.35	25,000
T-2000, 48 groups	9.05	0.32	2.16	30,000

^a The concentration of dextran was 10 mg per ml. The following buffers were used: phosphate for pH 7.80 and borate for pH 8.55 and 9.05. Absorbance was measured in cuvettes of 1 cm light path. Molecular weight was estimated from the elution position of the sample by chromatography on Sepharose 6 B (see Fig. 2), and the reproducibility of the estimated values was about 20%.

TABLE III
COUPLING REACTION OF PERIODATE OXIDIZED DEXTRANS WITH PROTEINS^a

Experiment number	Dextran, aldehyde groups per 10 ⁵ g	Protein	pH	Reaction time in h	Coupling product		
					Yield (%)	Estimated <i>M_r</i>	Composition by wt % Dex-tran Pro-tein
1	T-70, 22 groups	Lysozyme	8.55	24	34		
2	T-70, 56	Lysozyme	7.80	6	11		
3	T-70, 56	Lysozyme	8.55	6	65		
4	T-70, 56	Lysozyme	8.55	24	100	70,000	80 20
5	T-70, 56	Bovine plasma albumin	8.55	24	95	140,000	60 40
6	T-70, 56	Antigen E	8.55	24	60	80,000	75 25
7	T-70, 56	Antigen E	9.05	22	90	80,000	68 32
8	T-500, 48	Antigen E	9.05	20	80	120,000	77 23
9	T-500, 48	Antigen E	9.05	20	99	120,000	78 22

^a In Experiments 1-8 the concentration of dextran was 10 mg per ml and in Experiment 9 it was 20 mg per ml. The protein concentrations were 4 mg per ml for lysozyme or albumin and 2.8 mg per ml for antigen E.

The reaction mixtures of Experiments 1-4 were analyzed by chromatography on a 105 × 0.9 cm column of Sephadex G-100; the coupled product and the excess dextran were both eluted at 33 ml and unreacted lysozyme was eluted at 69 ml. The molecular weight of the coupled product was measured by chromatography on a 200 × 0.9 cm column of Sepharose 6 B; the chromatographic results are not shown.

The reaction mixtures of Experiments 5-9 were analyzed by chromatography on a 200 × 0.9 cm column of Sepharose 6 B. The results of Experiments 6-9 are shown in Fig. 3 and that of Experiment 5 is not shown, the dextran coupled antigen E was eluted at 90-100 ml while the native antigen E was eluted at 122 ml.

the excess dextran and antigen E were both eluted at about 120 ml. The coupling yield was increased to about 90% by carrying out the reaction at pH 9.05 (Experiment 7 and Fig. 3B). The coupling yield of antigen E with oxidized dextran T-500 at pH 9.05 was about 80% in Experiment 8, and the larger size of the product is indicated by its elution position being at about 94 ml (Fig. 3C). By doubling the concentration of dextran used in Experiment 8 the coupling reaction was made essentially complete in Experiment 9; this was indicated by the virtual absence of the antigen E peak in Fig. 3D.

The dextran-antigen E coupled products all have similar compositions of about 25% protein and 75% dextran as indicated in Table III. The average molecular weights of the oxidized dextrans T-70 and T-500 are reduced to about 18,000 and 25,000, respectively, due to degradation under the coupling conditions (Table II). Using these values for the molecular weights of dextrans and the value of 38,000 for the

molecular weight of antigen E (1), we calculated the molar ratios of dextran to antigen E to be 6.3 and 4.5, respectively, for the products obtained with oxidized dextrans T-70 and T-500. Taking the average molar ratio value of 5, we calculated the minimal molecular weights of the coupled products with dextrans T-70 and T-500 to be about 130,000 and 160,000, respectively. The reliability of these calculated values depends on the assumption that the dextran in the coupled products was degraded to the same extent as that of the control experiment in the absence of antigen.

The molecular weights given in Table III were estimated from the elution positions of the products. They are 80,000 and 120,000, respectively, for the products with dextrans T-70 and T-500, and they are about 30% lower than the corresponding calculated minimal molecular weights. This difference is a result of two factors. One is the experimental error in selecting the true elution positions as the peaks in

FIG. 3. Chromatography on Sepharose 6 B. and 7, 7.2 mg of amounts were used. Closed circles refer to eluted at 122 ml decompose the rechromatography in the text.

Fig. 3 are relative the products are that of native as the dextrans size and they gain conditions. The molecular weight plot of the elution logarithm of the dextrans as standard weights of globular in this manner underestimates molecular weight estimated from its conditions give true value. The for the products 53% as the product dextran and methods used for lar weights both will use the average 140,000 obtained to refer to the order of the text

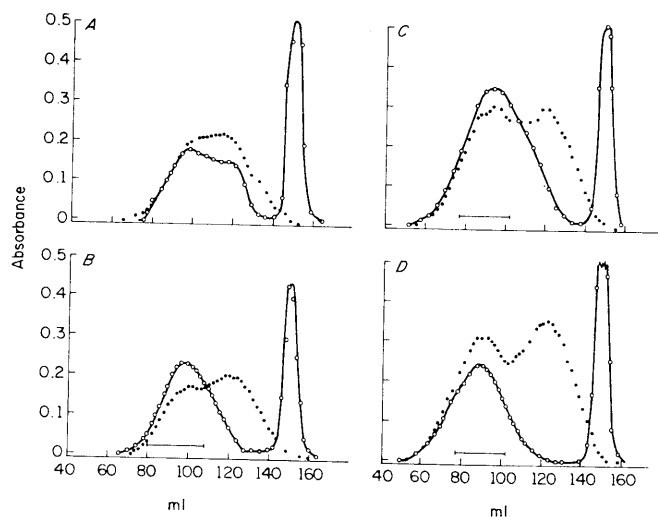


FIG. 3. Chromatography of antigen E-dextran coupled products on a column (200×0.9 cm) of Sepharose 6 B. Patterns A-D are for Experiments 6-9 of table III, respectively. In Experiments 6 and 7, 7.2 mg of antigen E and 25 mg of oxidized dextran were used; in Experiment 8 twice these amounts were used. In Experiment 9, 7.2 mg of antigen E and 50 mg of oxidized dextran were used. Closed circles refer to dextran concentration determined by the anthrone reaction. Open circles refer to protein concentration determined by absorbance at 280 nm. Native antigen E was eluted at 122 ml. The ultraviolet absorbing peak at 150 ml represents excess acetone used to decompose the borohydride. The indicated cuts were recovered on ultrafiltration. They were rechromatographed on the same column and then used for the immunochemical studies described in the text.

Fig. 3 are relatively broad. These peaks of the products are about twice as broad as that of native antigen E (result not shown) as the dextrans used are heterogenous in size and they gave broad peaks under these conditions. The second factor is that the molecular weights were estimated from a plot of the elution position versus the logarithm of the molecular weights with dextrans as standards. When the molecular weights of globular proteins are estimated in this manner, they are known to give underestimates (12). For example the molecular weight of native antigen E estimated from its elution position under the conditions given in Fig. 3 is only 53% of its true value. The extent of underestimation for the products is probably much less than 53% as the products contain about 75% of dextran and 25% of protein. The two methods used for the estimation of molecular weights both have their pitfalls, and we will use the averaged values of 100,000 and 140,000 obtained from these two methods to refer to the two products in the remainder of the text.

Amino acid analyses of acid hydrolysates of the dextran-antigen E coupled products with molecular weights of 100,000 and 140,000 showed that they each contained 16.0 ± 0.6 residues of lysine per mole of product as compared to the lysine content of 18 residues for native antigen E. The contents of the other amino acid residues of the product were within experimental error of $\pm 3\%$ the same as those of antigen E, with the single exception of histidine which was 6.9 residues instead of the expected value of 6.0 for antigen E. A coupling product of dextran (Sample I, Table I) and *N*- α -acetyl-lysine containing 11 mol of lysine per 10^5 g of dextran was acid hydrolyzed. On amino acid analysis, the only ninhydrin positive product found was lysine in a yield of about 14%. The finding suggests that the oxidized dextran-lysine product on acid hydrolysis does not regenerate lysine in high yield.

Immunochemical studies of the oxidized dextran-antigen E products. Precipitin analyses of the products and the native antigen were carried out with a sheep

produced anti-sera against antigen E and dextran with hemagglutination titres of about 1/5000 and 1/1000, respectively. Immunodiffusion analyses of the two rabbit anti-dextran coupled antigen E sera and the sheep anti-antigen E sera against antigen E showed complete identity of the precipitin lines.

The allergenic activity of the product with molecular weight of 140,000 was tested (Table IV). Skin tests on three ragweed sensitive patients showed that at

equal molar concentrations the product consistently gave a lower grade of skin reaction than antigen E did, suggesting that on a molar basis the product was less than ten-fold as reactive as antigen E. Histamine release assays with leukocytes of nine ragweed sensitive donors showed that the activity of the product varied from 2.4- to 16-fold less than that of antigen E, and the average value is 6.5 ± 2.7 .

DISCUSSION

Condensation of antigen E with periodate oxidized dextrans gave mainly products with the approximate molecular composition of one molecule of antigen and about five molecules of dextran. Two samples of oxidized dextrans with molecular weights of 70,000 and 500,000 were used for the reaction, and the two products formed with antigen E had average molecular weights of about 100,000 and 140,000, respectively. The molecular weights of the products are lower than those expected because the oxidized dextrans are unstable under the conditions used for the condensation. They undergo glycosidic cleavages to give dextrans in the molecular weight range of 18,000–25,000. Amino acid analysis of the products showed that about two out of the 18 lysine residues of antigen E may be involved in the coupling reaction. This value is much lower than that estimated from the composition of the products. The difference may result from the incorrectly assumed molecular weight of the degraded dextran in the product (see Results).

The oxidized dextrans are multifunctional with many reactive aldehyde groups per molecule just as antigen E is with many reactive amino groups. Therefore, in principle, the coupling between these two types of molecules could yield a complex mixture of products differing in their molecular sizes and compositions and in their sites of coupling. The chromatographic analyses of the products described in the results section indicated that the products are not grossly heterogeneous in their molecular sizes and their compositions. However, the immunological findings indicate that the products are heterogeneous in their sites of

TABLE IV
ALLERGENIC ACTIVITIES OF ANTIGEN E AND ITS
DEXTRAN COUPLED PRODUCT^a

A. Skin tests on ragweed sensitive patients				
Test antigen	Concentration $\mu\text{g/ml}$	Grade of skin reaction on patients		
		H. B.	J. G.	J. C.
Antigen E	10^{-6}	4+	4+	4+
Antigen E-dextran	10^{-6}	4+	4+	4+
Antigen E	10^{-7}	3+	2+	3+
Antigen E-dextran	10^{-7}	3+	1+	1+
Antigen E	10^{-8}	2+	±	±
Antigen E-dextran	10^{-8}	1+	±	±
Antigen E	10^{-9}	±		
Antigen E-dextran	10^{-9}	±		

B. Histamine release assays with leukocytes of ragweed sensitive patients			
Patient	Concentration required for 50% release		Concentration ratio
	Antigen E ng/ml	Antigen E-dextran ng/ml	
LML	0.24	1.10	4.6
FA	0.12	0.45	3.7
JA	0.095	0.65	6.8
HaCl	0.095	0.85	8.9
Atherton	0.13	0.80	6.2
Waker	0.09	0.45	5.0
Solter	0.025	0.40	16.0
Debner	0.65	3.2	4.9
Johnson	7.0	17.0	2.4
Average			6.5
			± 2.7

^a Only the dextran coupled product with molecular weight of 140,000 was studied. The concentrations given above for the product refer only to the protein portion of the molecule, and they do not include the dextran portion of the molecule.

coupling. That is, they are mixtures of derivatives, each with a different reduced number of antigenic determinants as compared to the determinants present in antigen E, and/or they are mixtures, each with a different number of determinants of reduced affinities for the antibodies. The findings in support of this conclusion are as follows: (a) On immunodiffusion against sheep anti-E sera there was complete fusion of the precipitin lines of the products with that of antigen E. (b) The products inhibited the hemagglutination of antigen E coated cells with sheep anti-E sera as antigen E did and their molar inhibitory activity was about one-eighth of that of antigen E. (c) There was complete fusion of the precipitin lines of antigen E with sheep anti-antigen E sera and with rabbit anti-dextran coupled antigen E sera.

The number as well as the size of dextran substituents in the products are apparently still not sufficient to reduce greatly by steric hindrance the reactivities of the antigenic or allergenic determinants of antigen E with antibodies.² This is shown by the following findings: The products reacted with sheep anti-E sera to give immune precipitates though in reduced amounts when compared to antigen E. The binding activity assay with antigen E-specific human IgG indicated that the product with molecular weight of 140,000 retained all the antigenic determinants with only small decrease in their binding affinities. The molar allergenic activity of the product with molecular weight of 140,000 was about one-fifth of that of antigen as shown by skin tests on patients and by histamine release assays with ragweed sensitive leukocytes. The modest reduction in allergenic activity suggests that the product is of limited use for the desen-

sitization of patients, even though it showed good immunogenicity in rabbits. Another drawback in the use of dextran coupled antigen E in man may be the enhanced immunogenicity of the dextran portion of the product, and this is evidenced by the formation of anti-dextran antibodies in rabbits immunized with the product. Dextrans with molecular weights of less than 70,000 are nonimmunogenic in man (17) and in rabbits, mice and guinea pigs (9). However, dextran-protein conjugates are immunogenic in rabbits, sheep and guinea pigs (9, 18).

The fact that products larger than that with two to five molecules of dextran and one molecule of antigen E are not formed, would suggest that the large size of the attached dextran molecules sterically hinders the approach of an additional molecule of dextran. One would expect that the reaction of the dextran-antigen E products with antibodies be subjected to the same steric hindrance since the molecular weights of IgG or IgE are several times larger than those of dextran. It is therefore surprising that the products showed only modest reductions in their antigenic and allergenic activities. Two possible explanations may be advanced. One is the shape difference of globular antibody molecules and relatively open chained dextran molecules (19). A dextran molecule in solution is much larger in size than a protein molecule of the same molecular weight (13). Therefore, the steric hindrance for the reaction of the dextran-antigen E product with dextran can be greater than that of the reaction of dextran-antigen E product with a protein. The other is that the high binding affinity of antibody for the antigenic determinant can overcome the steric hindrance by possibly displacing the flexible dextran side chain away from the surface of the product.

Other investigators have modified rye grass allergen by formaldehyde treatment (20) and ragweed antigen E by glutaraldehyde treatment (21) to yield cross linked derivatives of reduced allergenic activities. The formaldehyde treated rye allergen was a mixture of cross linked derivatives, mainly dimers and trimers in the molecu-

lar weight range. histamine release assays with sen-

² The reduced reactivities of the determinants may be a consequence of steric hindrance as suggested above or alternatively it may be a consequence of conformational changes of antigen E in the products. The alternative is considered less likely as the positive charges of the lysyl residues of antigen E are retained in the products and the positively charged lysyl residues were shown previously to be of importance in maintaining the conformational stability of antigen E (2).

1. KING, T. P., N. (1964) *Biochem*
2. KING, T. P., N. *Immunoch*
3. GRIFFITHS, B. 1275.
4. ISHIZAKA, K. (ed.), Vol. I.
5. KING, T. P., N. M. (1967) A

lar weight range of 40-80 thousand. On histamine release assays with sensitive leukocytes, its activity on a weight basis was in the range of 0.001-0.1% of that of native rye allergen. Glutaraldehyde treatment of antigen E gave a mixture of polymerized antigens, and two fractions having molecular weight ranges of 0.2-4 million and 4-20 million were isolated. The allergenic activities of these two fractions were both reported to be on a weight basis 1-10% of that of native antigen E as shown by passive transfer skin tests and by histamine release assays with sensitive leukocytes.

REFERENCES

1. KING, T. P., NORMAN, P. S., AND CONNELL, J. T. (1964) *Biochemistry* **3**, 458.
2. KING, T. P., NORMAN, P. S., AND TAO, N. (1974) *Immunochemistry* **11**, 83.
3. GRIFFITHS, B. W. (1973) *Can. J. Biochem.* **51**, 1275.
4. ISHIZAKA, K. (1973) *In The Antigens* (Sela, M., ed.), Vol. I, p. 479, Academic Press New York.
5. KING, T. P., NORMAN, P. S., AND LICHTENSTEIN, L. M. (1967) *Ann. Allergy* **25**, 541.
6. SANDERSON, C. J. (1971) *Immunology* **20**, 1061.
7. AXEN, R., AND ERNBACK, S. (1971) *Eur. J. Biochem.* **18**, 351.
8. KAGEDAL, L., AND AKERSTROM, S. (1971) *Acta Chem. Scand.* **25**, 22.
9. RICHTER, W., AND KAGEDAL, L. (1972) *Int. Arch. Allergy* **42**, 885.
10. KING, T. P. (1972) *Biochemistry* **11**, 367.
11. PARK, J. T., AND JOHNSON, M. J. (1949) *J. Biol. Chem.* **181**, 149.
12. SPIRO, R. C. (1966) *Methods Enzymol.* **8**, 3.
13. CHURMS, S. C. (1970) *Adv. Carbohydr. Chem.* **25**, 13.
14. ISHIZAKA, K., KISHIMOTO, T., DELESSESSE, G., AND KING, T. P. (1974) *J. Immunol.* **113**, 70.
15. LICHTENSTEIN, L. M., AND OSLER, A. G. (1964) *J. Exp. Med.* **120**, 507.
16. GUTHRIE, R. D. (1961) *Adv. Carbohydr. Chem.* **16**, 105.
17. KABAT, E. A., AND BEZER, A. E. (1958) *Arch. Biochem. Biophys.* **78**, 306.
18. HIMMELSPACH, K., AND WREDE, J. (1971) *Fed. Eur. Biochem. Soc. Lett.* **18**, 118.
19. WEELY, W. B. (1960) *Adv. Carbohydr. Chem.* **15**, 341.
20. MARSH, D. G. (1971) *Int. Arch. Allergy*, **41**, 199.
21. PATTERSON, R., SUSZKO, I. M., PRUZANSKY, J. J., AND ZEISS, C. R. (1973) *J. Immunol.* **110**, 1413.

s, even though it
ogenicity in rabbits.
the use of dextran
man may be the
icity of the dextran
ct, and this is evi-
tion of anti-dextran
immunized with the
h molecular weights
nonimmunogenic in
ts, mice and guinea
xtran-protein conju-
ic in rabbits, sheep
).

cts larger than that
ules of dextran and
n E are not formed,
ie large size of the
cules sterically hin-
an additional mole-
ould expect that the
-antigen E products
jected to the same
ce the molecular
are several times
xtran. It is therefore
oducts showed only
their antigenic and
o possible explana-
l. One is the shape
antibody molecules
ined dextran mole-
molecule in solution
ze than a protein
molecular weight
ic hindrance for the
-antigen E product
reater than that of
-antigen E product
er is that the high
ibody for the anti-
overcome the steric
displacing the flexi-
n away from the

have modified rye
ldehyde treatment
n E by glutaralde-
yield cross linked
llergenic activities.
ed rye allergen was
inked derivatives,
ers in the molecu-