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α_1-Antitrypsin: Apparent Molecular Weight Heterogeneity Shown by Two-Dimensional Electrophoresis

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SUMMARY

Two-dimensional (2-D) gel electrophoresis was used to examine charge and molecular weight variability of α_1-antitrypsin. Two-D electrophoresis resolved distinctive differences among individual phenotypes. Microheterogeneity of charge was seen for the different alleles that corresponded to the charge variability observed on isoelectric focusing gels. The molecular weights of the major components of each allele appeared to differ from each other by approximately 1,000, suggesting, that in addition to sialic acid, there may be differences in neutral sugar composition between the individual components. In comparison to the *M* allele components, the corresponding *S* and *Z* components had higher molecular weights. The *MZ* and *MS* phenotypes showed characteristic patterns of protein spot doublets. Computerized quantitation was used to separate and estimate the contribution of each component to the overall allele composition. The *Z* allele components contained about 15% of the total *MZ* quantity, while the *S* allele components contained about 40% of the total *MS* quantity. The 2-D electrophoresis technique may offer a new approach for molecular structural studies of α_1-antitrypsin variants and similar glycoproteins.

INTRODUCTION

The principal protease inhibitor of human serum α_1-antitrypsin (alternately called α_1-protease inhibitor) has a high degree of inherited polymorphism and structural microheterogeneity with more than 40 recognized electrophoretic and quantitative

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variants. These variants are determined genetically by a set of codominant alleles at the protease inhibitor (PI) locus [1]. Currently, identified alleles are designated by the letters of the alphabet: *B*, *E*, *F*, *G*, *I*, *L*, *M* (M_1, M_2, M_3), *N*, *P*, *R*, *S*, *V*, *W*, *X*, and *Z*, and by "null," indicating the approximate electrophoretic mobility of each allele. The *M* phenotype frequency in the United States has been estimated to be about 90%; the *MS* to be 6.4%; and the *MZ*, 2.2% [2]. The *Z* phenotype with an estimated frequency of about 1/5,000 is associated with hereditary adult emphysema and childhood cirrhosis.

Starch gel electrophoresis followed by immunoelectrophoresis has been used to identify the genetic variants of α_1-antitrypsin. Isoelectric focusing is an alternate method for demonstrating variation. With either method, α_1-antitrypsin has been found to form a characteristic pattern of five to eight protein bands of differing intensities [3–7]. The individual bands appear to differ by approximately single charges. Variable quantity of sialic acid is likely to account for much of the charge variability since neuraminidase treatment causes a stepwise migration of the bands toward a single band [7, 8]. These methods for allele identification are sensitive to charge but do not reveal molecular weights. Current estimates of α_1-antitrypsin molecular weight (MW) by SDS gel electrophoresis range from 50,000 [9, 10] to 56,000 [8]. The MW of the individual components has not yet been investigated.

The 2-D gel electrophoresis technique of O'Farrell [11] separates molecules by the two independent parameters of charge and MW, and it potentially provides more information than is obtained by a single-dimensional separation. For this reason, I have studied various α_1-antitrypsin phenotypes by 2-D gel electrophoresis in order to examine genetic variability of α_1-antitrypsin and the MW of the individual components. I found that 2-D electrophoresis can be used to resolve α_1-antitrypsin phenotypes. In addition, the MWs of the individual components of α_1-antitrypsin were determined for the first time. In comparing the components of one allele with another, it appeared that greater variability of MW is present than has been previously recognized.

MATERIALS AND METHODS

Sample Preparation

Serum samples were obtained from normal volunteers and patients suspected of having pulmonary disease or neonatal hepatitis for whom PI typing had been requested. Samples were kept frozen or on ice prior to use to prevent deterioration. Comparisons by electrophoresis of stored frozen samples to fresh samples showed no apparent differences. Samples of various other PI phenotypes and standards for PI typing including *M* subtypes were kindly supplied by Drs. F. Kueppers, R. Norum, and R. Erickson. PI typings were confirmed by starch gel electrophoresis followed by crossed immunoelectrophoresis [3].

For 2-D electrophoretic analysis of serum samples, 10 μl of serum was added to 20 μl of sample buffer, consisting of 9.4 M urea (Schwartz-Mann, Spring Valley, N.Y.), 2% NP-40 (Bethesda Research, Bethesda, Md.), 5% beta-mercaptoethanol (Eastman, Rochester, N.Y.), and 1.6%, pH 5–7, and 0.4%, pH 3.5–10, ampholines (LKB, Hicksville, N.Y.). The sample buffer was made fresh prior to use, mixed with the sample, and applied directly to isoelectric focusing (IEF) gels. To determine the 2-D position of α_1-antitrypsin, serum of type M_1 was immunoprecipitated with rabbit antiserum (Cappell, Cochranville, N.Y.), and washed three times with saline. The immunoprecipitate formed was mixed with sample

buffer and applied to IEF gels. To determine the effect of removal of sialic acid from α_1-antitrypsin, neuraminidase (*V. cholerae*, 125 U/ml of saline, Calbiochem, San Diego, Calif.) was added 1:1 to type M1 serum at 37°C for 1 hr, and the resulting sample was analyzed by electrophoresis. In a second set of experiments, the effect of another type of neuraminidase (*C. perfringens*, 12 U/ml, Bethesda Research) that was found to produce a more complete desialidation was examined after 0, 5, and 60 min and 24 hrs of reaction.

Two-D gel electrophoresis was carried out using the technique of O'Farrell [11] with minor modification [12–15]. Pyrex tubes with internal diameter of 2.5 mm and length of 14 cm were filled to 11 cm with a 10% polyacrylamide gel mixture consisting of 9.46% acrylamide (Sigma, St. Louis, Mo.), 0.54% bis-acrylamide (Bio-Rad, Rockville Center, N.Y.), 2% NP-40, 55% (w/v) urea (Schwartz-Mann, "Ultrapure"), and 2% ampholines (4 parts, pH 5–7, and 1 part, pH 3.5–10). The polymerization was initiated by adding 5 μl of Temed and 10 μl of freshly made ammonium persulfate to 10 ml of the mixture. The gels were overlayed with 0.5 M urea and allowed to polymerize for 2 hrs. After polymerization, the urea overlay was removed, the samples applied, and overlayed with 0.5 M urea in 1% agarose. The tubes were placed in a tube electrophoresis apparatus with the lower chamber filled with 10 μM H_3PO_4, and the upper chamber, with a mixture of 40 mM NaOH plus 20 mM $CaOH_2$.

The gels were subjected to an initial voltage gradient of 200 V. The voltage increased to a 400-V maximum using a constant power source. Four hundred V were applied for 16 hrs followed by a 1-hr final hyperfocus of 800 V. The gels were removed from the tubes by gentle water pressure and equilibrated for 2 hrs in sodium dodecyl sulfate (SDS) sample buffer: 23% SDS (Gallard-Schlesinger, Carle Place, N.Y.), 5% beta-mercaptoethanol, 10% glycerol, and 62.5 mM Tris-HCl, pH 6.8.

To form the 10% separating gel, 33 ml of stock acrylamide solution (acrylamide 29.2% and bis-acrylamide 0.8%) was added to 25 ml of separating gel buffer (0.4% SDS, 1.5 M Tris-HCl, pH 8.8) plus 41 ml of water. One ml of 10% ammonium persulfate, and 50 μl of Temed was added to initiate polymerization. The solution was degassed under a vacuum for 10 min and poured into a slab gel apparatus with dimensions of 12 cm by 10 cm by 1.5 mm. It was overlayed with 0.1% SDS and allowed to polymerize overnight. To make the 1-cm stacking gel, 4 ml of the stock acrylamide solution was added to 6.25 ml of stacking gel buffer (0.4% SDS), 0.5 M Tris-HCl, pH 6.8, and 15 ml of water. After degassing, 125 μl of ammonium persulfate and 25 μl of Temed were added to initiate polymerization. The solution was applied to the upper 1 cm above the separating gel and allowed to polymerize for several hours.

The equilibrated first-dimensional gel was placed on top of the stacking gel and attached with melted 1% agarose in SDS sample buffer containing 0.0015% bromphenol blue as a marker dye. The upper and lower chambers were filled with SDS running buffer consisting of 0.192 M glycine, 25 mM Tris, and 0.1% SDS. Electrophoresis was carried out at 20 mA per gel until the dye front had come to within 1 cm of the bottom. The gels were fixed and stained in 35% methanol, 10% acetic acid, and 0.1% Coomassie Brilliant Blue for 1 hr. They were destained in successive changes of 35% methanol and 10% acetic acid. The gels were then equilibrated in 10% methanol, 10% acetic acid, and 1% glycerol, and dried on 3-MM (Whatman) filter paper using a gel drying apparatus (Hoeffer). A larger-sized apparatus was used in some experiments to increase the separation and resolution as suggested by Garrels [14] and Comings and Peters [15]. The apparatus produces gels of size 20 cm by 20 cm. A separating gel of 13% polyacrylamide was employed. These modifications appeared to increase the separations of heterozygous phenotypes.

Direct pH measurements are difficult to reproduce on narrow tube gels. To overcome this problem, Anderson and Hickman [16] have proposed a technique for standardizing comparisons of pH measurements between laboratories. Their method is to incorporate in the gel a carbamylated protein such as CPK with single-charge additions that can be clearly seen. This allows accurate and reproducible comparisons of the internal standard CPK isoelectric coordinates from laboratory to laboratory. Therefore, in addition to direct

surface pH electrode measurements, carbamylated CPK was prepared as described [16] and employed in these experiments to determine the pH of α_1-antitrypsin. MW markers (Pharmacia, Piscataway, N.J.) included phosphorylase B (94,000), albumin (67,000), ovalbumin (43,000), and carbonic anhydrase (30,000).

Quantification of α_1-Antitrypsin Components

Computer analysis was used to determine the relative quantity of protein in each subcomponent spot of α_1-antitrypsin. A transparent photograph was made of the region of the gel containing α_1-antitrypsin. This transparency was scanned using a high-speed raster-scanning microdensitometer (Optronix). The resulting 2-D density matrix was entered into a PDP 11/70 computer. A program to analyze 2-D gels [17] and modified to run on a PDP 11/70 computer was employed in these experiments. The program quantitated spots by fitting each protein peak to a 2-D Gaussian function. Peaks with non-Gaussian shapes were approximated as the sum of a set of Gaussians by combining overlapping Gaussians. Using this program, overlapping protein spots were separated from one another and an estimate of their relative volumes obtained. The volume fit was accurate to within approximately ±2% for isolated spots [17]. A series of stained protein standards of increasing quantity was examined, and the quantitation was found to be linear over the protein quantity range employed here. Following the fitting of the protein patterns by 2-D Gaussians, the regenerated Gaussian functions were plotted as a density contour map for comparison to the original density pattern.

RESULTS

α_1-Antitrypsin showed microheterogeneity of charge in the isofocusing dimension and size in the SDS dimension on 2-D gels. The position of α_1-antitrypsin on 2-D gels was identified by comparing the position of immunoprecipitated α_1-antitrypsin with the pattern of whole serum proteins (fig. 1*a* and *c*). This agreed with the position previously identified by Anderson et al. [12]. Six distinct spots were seen for the phenotype M_1. They had isoelectric points within a range of −19 to −12 relative to carbamylated CPK isomeres corresponding to a pH range of 4.3 to 4.9 in 9 M urea. Each spot corresponded in approximate size and position to each of the individual bands seen on IEF [7] and to the individual peaks seen on crossed immunoelectrophoresis [4].

Two-D electrophoresis of α_1-antitrypsin revealed heterogeneity of MW in the second dimension. The six individual spots had an estimated apparent MW of approximately 58,000, 57,000, 56,000, 55,000, 54,700, and 54,000 (±1,000), respectively, as shown in figure 1*d* and diagrammatically in figure 2. It appeared that the distance between spots 5 and 6 is greater than the distance between the other spots. This may reflect some secondary heterogeneity in underlying structure leading to an increased charge separation. Alternatively, a slightly increased separation of the pH gradient may be occurring in this region of the gel. Treatment of serum with *V. cholerae* neuraminidase for 1 hr caused a shift of the spots toward the basic end of the gel (fig. 1*c*), indicating that sialic acid partly accounted for the charge heterogeneity seen. As shown in figure 3, when *C. perfringens* neuraminidase was employed, a more complete desialidation occurred. The spots moved toward a predominate major single spot and a secondary minor spot. The major spot was 10 charge U more basic than spot 1 with an apparent MW of about 53,000. The minor spot was about 12 charge U more basic than spot 1. It had an MW of about 52,000.

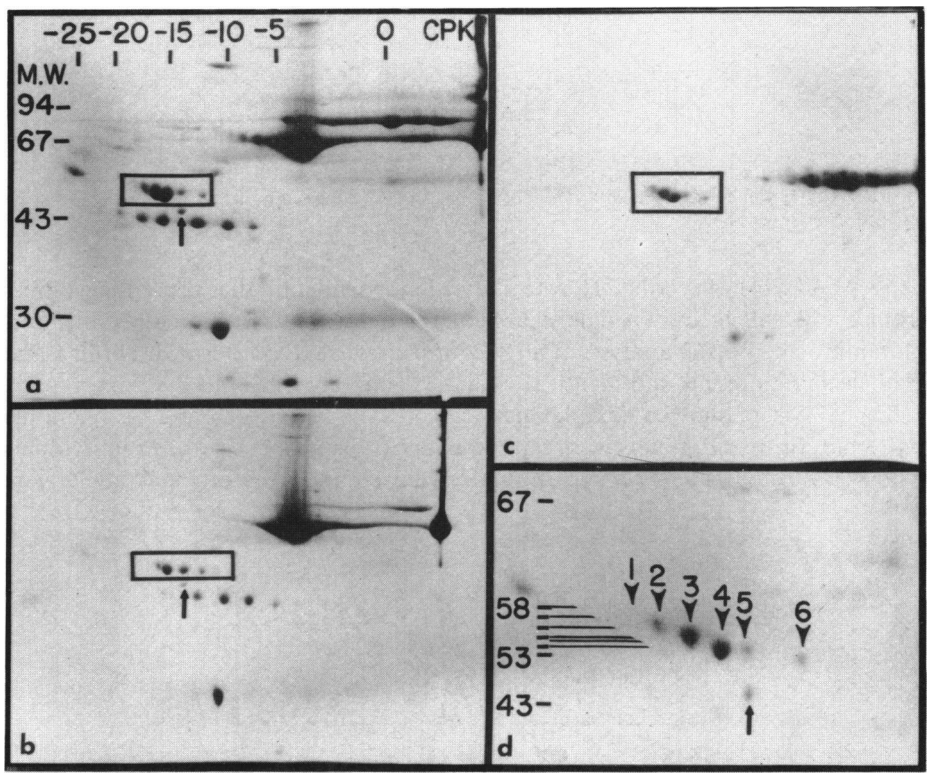

FIG. 1.—2-D gel electrophoresis of α_1-antitrypsin showing position, charge, MW, immunoprecipitation, and effect of neuraminidase treatment. *a*, 2-D electrophoresis of serum from a person of M_1 α_1-antitrypsin phenotype. The α_1-antitrypsin is enclosed in *rectangle*. An unidentified marker protein whose position was found to be a constant reference spot is indicated by *arrow*. *b*, Effect of neuraminidase treatment on α_1-antitrypsin pattern. Incubation with neuraminidase (*V. cholerae*) resulted in a partial stepwise shift of the α_1-antitrypsin spots toward the basic end of gel. *c*, Immunoprecipitation of α_1-antitrypsin from serum using α_1-antitrypsin antiserum (Kappell-rabbit). The immunoprecipitated α_1-antitrypsin region is *enclosed*. The immunoglobulin heavy chains are *above to the right*, and the light chains, *below*. *d*, Enlargement of α_1-antitrypsin spots *numbered 1–6* showing MW; 1 μl was applied to reduce overloading of the prominent spots.

Different PI phenotypes showed distinctive patterns of spots on 2-D gels as shown in figure 4. The *MZ* phenotype, figure 4*b*, showed a characteristic doubling of the 4th, 5th, and 6th *M* spots. This doubling was due to overlap of the *Z* allele spots with the *M* allele spots. There appeared to be a heavier relative MW for the *Z* allele of approximately 1,000. Yoshida el al. [18] and Jeppson [19] have shown that the *Z* allele differs from the *M* allele by a single amino acid substitution of lysine for glutamic acid. This would result in a double charge shift toward the basic end of the gel. The *Z* allele (figure 4*c*) shows three main spots (2, 3, and 4) with faint 1st, 5th, and 6th spots. The main *Z* component, 4, appears to differ from the main *M* component by slightly less than 2 charge U. This might be due to configurational differences in the molecule caused by the amino acid substitution.

The *S* allele, figure 4*e*, shows a similar pattern to the *M* pattern but with a basic shift of approximately 1 charge U. This is consistent with observations by Owen et

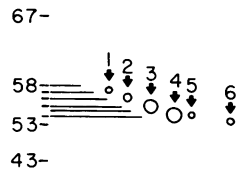

FIG. 2.—Diagrammatic representation of α_1-antitrypsin as seen in figure 1*d*, showing relative charge separation of spots and MWs.

al. [20] and Yoshida et al. [21], who showed independently that the *S* allele differs from the *M* allele by a single charged amino acid substitution of valine for glutamic acid. Two-D analysis of an SZ sample revealed a pattern with both *S* and *Z* allele spots present and identical with a 1:1 mixture of S and Z serum.

Type *MS* sera showed complex patterns on 2-D gels (fig. 4*f* and *g*). A unique pattern of protein doublets was seen for α_1-antitrypsin from seven *MS* individuals who were tested. The MWs of the individual components of the *S* allele were

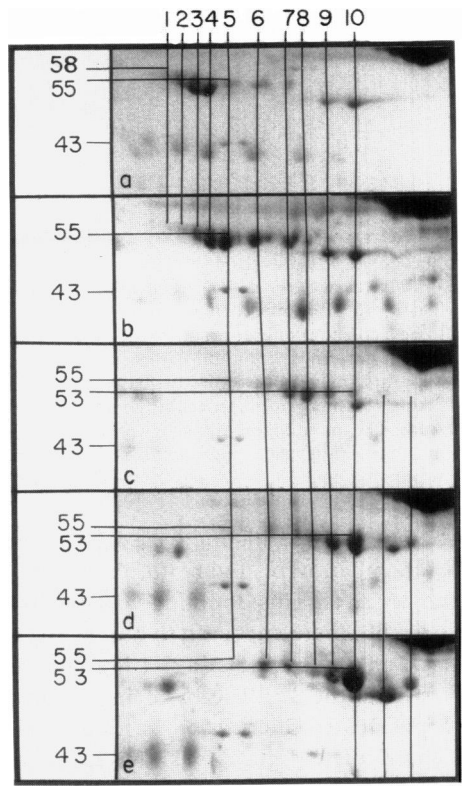

FIG. 3.—Incubation series showing sequential removal of sialic acid from α_1-antitrypsin using neuraminidase (*C. perfringens*). Incubation times at 37°C: *a*, 0 time; *b*, 5 sec; *c*, 5 min; *d*, 1 hr; *e*, 24 hrs. *Vertical bars* indicate charge U relative to spot 1. They pass through the center of each spot and are not always parallel because of irregular stretching of the gels during drying.

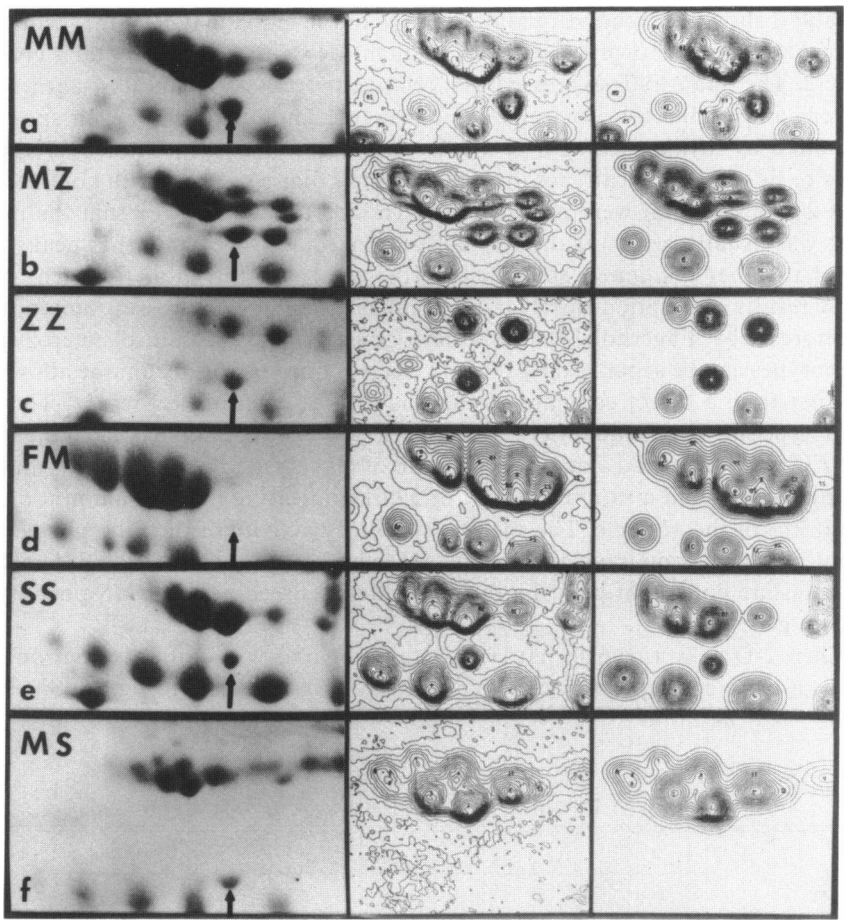

FIG. 4.—2-D gel electrophoresis and computer analysis of α_1-antitrypsin phenotypes. *a*, M_1 type. The major spot (*4*) *lies above and to left of the marker protein spot* with constant position (*indicated by arrows*). *b*, M_1Z type. A characteristic set of spot doublets is seen. The pattern appears as a combination of *M* and *Z* type patterns. *c*, *Z* type. Compared with type *M*, there is a shift of the spots *toward the basic end of the gel* and a reduced relative quantity of protein present. *d*, M_3F type. An overlap of the *M* type pattern with the *F* type pattern results from an apparent acidic shift of the *F* type by about 2 charge U. *e*, *S* type. A basic shift of about 2 charge U relative to the *M* type pattern is seen. *f*, M_1S type. A set of characteristic spot doublets is present. The *S* spots have a heavier MW relative to the *M* spots. Gels *a–e* are 10% and *f*, 13%, polyacrylamide.

approximately 1,000 heavier than the corresponding *M* components. This was confirmed with a 1:1 mixture of *M* and *S* sera that produced an identical pattern to the *MS* pattern as shown in figure 6*b*. Two samples of *FM* sera showed a blurring of the 2nd, 3rd, and 4th spots, suggesting incomplete resolution of the spots of the two alleles due to similar MWs (fig. 4*d*). A sample of *MP* sera, figure 6*d*, showed overlap of the 2nd, 3rd, and 4th components, indicating similar MW of these two alleles.

Table 1 shows the relative quantity of each of the individual α_1-antitrypsin spots of the phenotypes shown in figure 4. The quantities of three MS samples is given in table 2. The quantity is expressed as a relative percent of the total α_1-antitrypsin present and of the total of each allele present. The quantity of each spot was estimated by fitting each spot to a 2-D Gaussian function to allow overlapping spots to be mathematically resolved as shown in figure 4. Spots with volumes less than 2% of the total were not included. These estimates showed that the major peak was usually peak 3 with 30%–40% of the total protein and that peaks 2, 3, and 4 together contained 80%–90% of the total α_1-antitrypsin for each allele. These results correspond closely to the results of quantitative crossed immunoelectrophoresis by Fagerhol [4] for homozygous phenotypes. Since heterozygous phenotypes can be separated by the 2-D electrophoresis, this technique allows for the first time a direct estimate of the quantity of each allele present. The *MZ* phenotype showed the quantity of the *Z* allele protein to be about 15% of the total. The *FM* phenotype had a quantitative ratio of *F* allele to *M* allele of 53:47, and the *MS* phenotype had a ratio of *S* to *M* of 39:61, indicating that the amount of *S* is about 2/3 of the *M* allele. The specific inhibitory activity of the *S* homozygote is about 65% of normal and that of the *MS* heterozygote, about 80% [21]. These activities are consistent with the relative quantities seen here for *MS* phenotypes on 2-D gels.

When 2-D α_1-antitrypsin patterns were compared with patterns seen on IEF alone, a close correspondence is seen (compare figs. 4 and 6 to fig. 5). Since the

TABLE 1

RELATIVE PERCENTAGES OF COMPONENTS OF α_1-ANTITRYPSIN

	COMPONENT SPOT						
PI TYPE	1	2	3	4	5	6	TOTAL
MM	6.7	15.7	32.1	30.2	8.7	6.6	...
MZ:							
M	2.2	15.0	42.7	25.6	12.3	4.1	...
M/MZ	1.9	12.7	36.3	21.7	10.4	3.5	86.5%
Z	...	*	47.3	52.7	*	...	...
Z/MZ	...	*	7.1	7.9	*	...	15.0%
ZZ	...	22.6	37.7	39.7	*	...	...
FM:							
F	1.4	20.7	30.7	34.2	12.9	0.1	...
F/FM	0.7	10.9	16.2	18.0	6.8	0.1	52.8%
M	1.2	28.1	37.7	31.7	1.3	*	...
M/FM	0.6	13.3	17.8	15.0	0.6	*	47.2%
SS	*	15.7	40.9	32.6	6.4	4.4	...
MS:							
M	3.4	13.7	46.0	34.0	2.8	*	...
M/MS	2.1	8.4	28.2	20.9	1.7	*	61.4%
S	*	6.5	26.5	50.7	16.3	*	...
S/MS	*	2.5	10.2	19.5	6.3	*	38.6%

* Not estimated (less than 2%).

TABLE 2

RELATIVE % OF MS COMPONENTS

	1		2		3		4		5		6	
	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
M.....	3.47	0.6	18.9	4.6	40.3	5.5	29.7	4.15	3.4	2.76	5.75	0.63
M/MS	2.17	0.49	11.75	3.08	24.97	2.85	19.38	2.15	2.13	1.8	3.6	0.56
S.....	*	*	6.97	0.99	34.7	7.13	48.45	2.4	7.47	7.7	2.8	0.85
S/MS	*	*	2.67	0.57	13.2	2.8	18.4	1.16	3.1	2.77	1.06	0.42

NOTE: Table is based on the analysis of three *MS* samples (fig. 6*e*, *g*, and *h*). Total *M*/total *M* + *S* = 61.8% (2.4 SD). Total *S*/total *M* + *S* = 38.2% (2.5 SD).

* Not estimated (less than 2%).

allelic components were resolved with respect to MW as well as charge on 2-D gels, the *MS* and *MZ* patterns are distinctive and well delineated. *M* subtypes (M_1, M_2, and M_3) that can be resolved by IEF alone [22] were examined and did not appear to be well resolved by 2-D electrophoresis. This could be due to the lateral diffusion of protein in the IEF gel that may occur during the 2-hr equilibration step prior to the SDS gel electrophoresis, or during electrophoresis into the second dimension. This could obscure the slight pH separation of *M* subtype components. Comparison of type M_1S (fig. 6*f*) with type M_3S (fig. 6*g*) showed a slightly greater separation of M_3 and *S* than of M_1 and *S*, indicating that *M* subtype differences may be perceptible on 2-D analysis of heterozygous types.

DISCUSSION

As the results have shown, 2-D electrophoresis can be used to resolve the multiple components of α_1-antitrypsin by charge and MW separation. The 2-D patterns of different PI alleles showed charge separation patterns similar to α_1-antitrypsin patterns that are produced by IEF alone or by crossed immunoelectrophoresis. In addition, underlying differences of component MWs were simultaneously revealed.

Various workers have reported differing values for the MW of α_1-antitrypsin when estimated by SDS gel electrophoresis: Crawford [9] and Roll et al. [10] reported a value of 50,000, Yoshida et al. [21] observed an MW of 53,000, Berninger and Talamo [23] reported that isolated α_1-antitrypsin had an MW of 53,500, and Jeppson et al. [8] found a value of 56,000. The results here allowed the MWs of the individual components to be estimated for the first time and showed them to vary from a value of 58,000 for spot 1 to 54,000 for spot 6. Estimation of α_1-antitrypsin MW by ultracentrifugation analysis has produced values of 49,000 $\pm$ 3,000 [9] and 47,500 [10], with no differences noted between the MW of M, S, and Z phenotypes. The differences in MW estimation of α_1-antitrypsin by SDS electrophoresis and ultracentrifugation are difficult to assess, and the true MW of α_1-antitrypsin may not be established with certainty until the complete amino acid sequence is known.

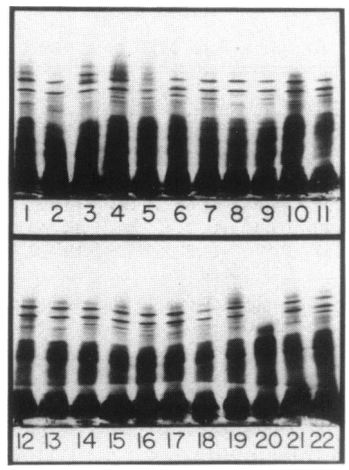

FIG. 5.—IEF of α_1-antitrypsin phenotypes. The phenotypes are: 1, M_1; 2, M_1Z; 3, M_3F; 4, M_1S; 5, S; 6, M_1P; 7, M_1S; 8, M_1; 9, M_1S; 10, M_2; 11, M_3S; 12, M_1; 13, M_1; 14, M_1S; 15, M_1S; 16, M_1S; 17, M_1; 18, M_3S; 19, M_1Z; 20, Z; 21, M_3S; 22, M_1P. (M subtyping confirmed by courtesy of Dr. F. Kueppers.)

The apparent heterogeneity of MW revealed by 2-D electrophoresis may relate to the variable oligosaccharide composition of the molecule. The multiple charge components of α_1-antitrypsin for a given allele appear to be partly due to differing amounts of sialic acid. The major bands numbered 2, 3, and 4 in figure 1 were reported to have 8, 7, and 6 sialic acid residues, respectively [9, 10]. With an increase in the number of sialic acid residues, an increase in apparent MW on SDS gels is seen. Adding one sialic acid residue should increase the overall MW by about 309. But components 1 through 4 appear to differ progressively by about 1,000, component 5 by 300, and component 6 by about 700 (figs. 1*d* and 2).

Increasing the oligosaccharide content of proteins causes a greater increase in MW on SDS gels than calculated [24]. This effect of anomalously increased apparent MW of sialoglycoproteins on SDS gels is due to decreased binding of SDS as compared with standard protein, and the degree of MW anomaly for a given sialoglycoprotein appears to be a direct linear function of its percent carbohydrate composition. Segrest el al. [24] showed that the estimated MW for a given sialoglycoprotein is about 1,000 above its real MW for every 10% of the molecule represented by carbohydrate. This analysis of the anomalous effects of sialoglycoproteins on SDS gels suggests that for α_1-antitrypsin with 12% carbohydrate composition an overestimate of about 1,200 for each component could be expected. Subtracting 1,200 from the apparent SDS MWs yields a corrected estimate for the M allele components of: 1, 56,800; 2, 55,800; 3, 54,800; 4, 53,800; 5, 53,500; and 6, 52,800. The addition of one sialic residue would increase the carbohydrate concentration by about 0.5%, causing a relative overestimate of about 50 between the individual components. Based on this, the expected difference between the individual components would be 50 plus the 309 weight of sialic acid or 359. However, a difference of about 1,000 between the major components was observed

(fig. 2). The differences in the MW of the individual components appear to be greater than can be accounted for by single additions of sialic acid alone. Hodges et al. [25] have proposed a structure for the oligosaccharide chains consisting of one type A triantennery carbohydrate chain and three type B biantennery carbohydrate chains per molecule of α_1-antitrypsin (fig. 7). Mega et al. [26, 27] independently proposed a similar structure for the carbohydrate side chains with a variable ratio of B to A type chains. (Their type B chain was identical with the type A, and their type A was identical with the type B of Hodges et al.) However, Mega et al. reported finding not four but three oligosaccharide chains per molecule of α_1-antitrypsin. These models of oligosaccharide composition were based on an analysis of *M* type α_1-antitrypsin, 90% of which would be expected to be a mixture of components 2, 3, and 4 (table 1). Altering a type A chain to a type B chain could occur by the loss of the neuNAc-Gal-GlcnAc type A side chain (fig. 7). This would result in an MW change of about 1,000. If component 1 has three type A chains with no B chains, while component 4 has three type B chains with no A chains, such a difference in ratio of side chains might account for the observed differences of approximately 1,000 between the major components observed on 2-D gels. The smaller differences in MW between components 4, 5, and 6 may represent some

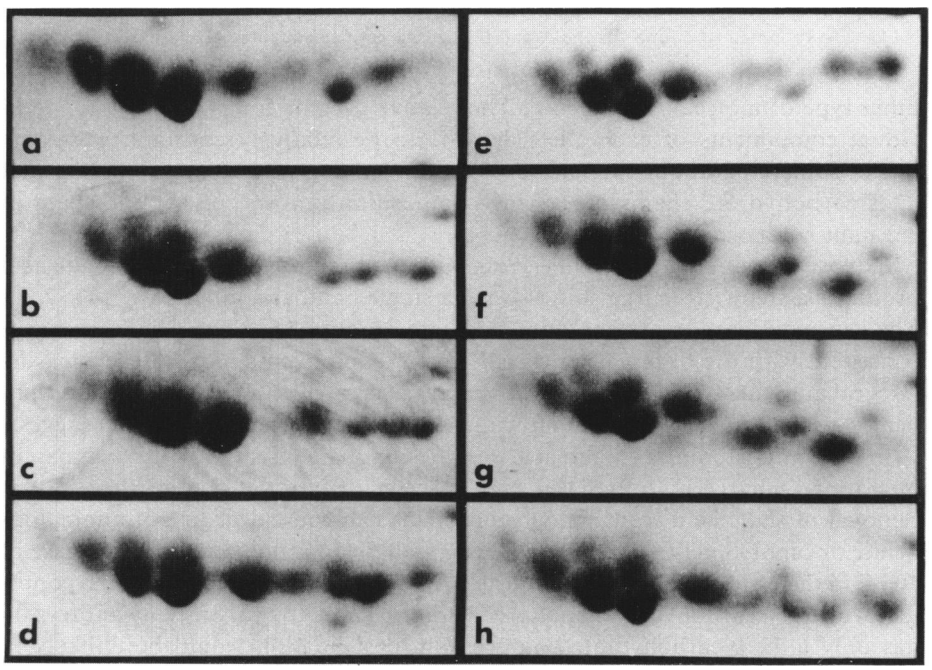

FIG. 6.—2-D gels indicating MW heterogeneity of *MS* and *MP* types *a*, M_1 type. *b*, A 1:1 mixture of M_1 and *S* types (*a* and *c*) showing MW heterogeneity of the *MS* pattern. *c*, *S* type. *d*, M_1 *P* type showing incomplete separation of the M_1 and *P* components. *e–h*, Four different MS samples illustrating the characteristic doublet of the pattern. The *S* allele has a basic shift and heavier apparent MW relative to the *M* allele. Figure 6 samples that correspond to figure 5 samples are: *c* to *5*, *d* to *6* and to *22*, *e* to *18*, *f* to *4*, and *g* to *11* (*h* not typed by IEF).

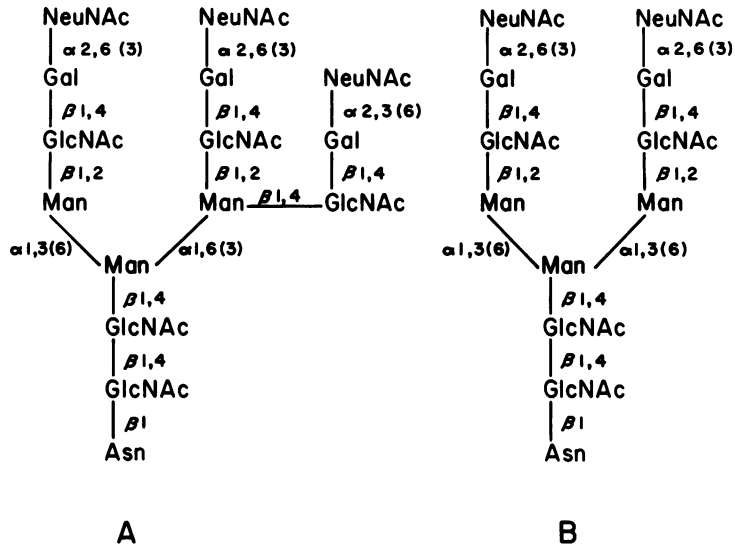

FIG. 7.—Proposed structures for the A and B oligosaccharide sidechains of α_1-antitrypsin [25]

other type of molecular difference. The relative percent composition of the individual components of each allele appears to be relatively constant. This may reflect something about the nature of the underlying molecular processing that adds carbohydrate chains of varying composition onto a polypeptide chain of constant composition.

Extensive treatment of α_1-antitrypsin with neuraminidase to remove sialic acid produces a stepwise charge shift toward a single band at a more basic pH [7, 8]. The neuraminidase treatment employed here produced a stepwise shift in charge consistent with the observation that the main components may have decreasing integral amounts of sialic acid. The apparent decrease in MW of the main component, 4, from 55,000 to 53,000 following neuraminidase treatment (fig. 3) suggests the removal of about six sialic acid residues and is consistent with its apparent migration 6 charge U to the basic end of the gel. Segrest et al. [24] showed that removal of sialic acid from sialoglycoproteins can cause a degree of anomalous behavior on SDS gels. For a glycoprotein with 50% carbohydrate, an increase of 100% in the estimated MW was observed following desialidation. For a glycoprotein with 25% carbohydrate, only a 20% increase was found. Since α_1-antitrypsin has only a 12% carbohydrate composition, desialidation would be unlikely to cause as much of an anomaly in behavior on SDS gels.

This report has indicated that the individual components of α_1-antitrypsin appear to differ in MW by more than can be accounted for by simple additions of sialic acid and may suggest differences in underlying oligosaccharide residue composition. Similar MW differences of about 1,000 between individual components

of serum transferrins, haptoglobins, and α_2-HS glycoprotein have been reported [28]. These reports may suggest that a general type of posttranslational modification occurs to liver specific proteins that leads to a variable ratio of A to B type chains and causes MW differences of about 1,000 between individual components. Further, the individual *S* and *Z* allele components appeared to have approximately 1,000 greater MW compared with individual *M* allele components, possibly suggesting differences in the underlying oligosaccharide residue compositions of the corresponding components of *S* and *Z* compared with *M* alleles of α_1-antitrypsin. This raises the possibility that there is a greater complexity of underlying structure among the different alleles than has been suspected.

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