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Studies on the molecular weight distribution of cosmetic protein hydrolysates

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Synopsis

MOLECULAR WEIGHT has been thought to be an important feature of COSMETIC GRADE PROTEINS and to be a critical factor affecting PROTEIN SUBSTANTIVITY to HAIR. The study reported in this paper was undertaken to show the relationship of molecular weight to protein substantivity. Using gel filtration and ultracentrifugation data were obtained that indicate peptides in the range of molecular weight 1000 are more substantive than the very high molecular weight molecules.

INTRODUCTION

Cosmetic grade protein hydrolysates are complex mixtures of various molecular weight polypeptides. Only approximate number average molecular weights have been previously reported. This study was undertaken to provide additional information about the distribution of molecular weights of collagen hydrolyzed by several methods, and to show if there is a relationship between molecular size and hair substantivity.

Two techniques were used for investigating the molecular weight distribution of cosmetic grade protein hydrolysates, gel filtration and ultracentrifugation.

Gel filtration is an established chromatographic method for the separation of molecules according to their size. The use of Sephadex®*, a bead-formed dextran gel, for gel filtration was introduced in 1959, and since has become a well-established method for fractionation and separation of molecules according to their size. Sephadex is cross-

*Sephadex®, Pharmacia Fine Chemical Inc., 800 Centennial Avenue, Piscataway, NJ.

linked dextran; the degree of crosslinking determines the molecular weight fractionation range.

Sephadex Type	Fractionation Range (Molecular Weight)	
	Peptides	Dextrans
G-10	– 700	– 700
G-15	– 1,500	– 1,500
G-25	1,000– 5,000	100– 5,000
G-75	3,000– 70,000	1,000– 50,000
G-200	5,000–800,000	1,000–200,000

Molecules of molecular weight above the upper limit of the ranges shown in the above chart are totally excluded from the gel. Molecules of molecular weight below these ranges are eluted from the Sephadex column in the above chart at a volume about equal to the total bed volume. Molecules between the upper and lower limits are eluted from the Sephadex column in a specific relationship to the molecular weight. Over a considerable range, the elution volume is approximately a linear function of the logarithm of the molecular weight.

The other method of determining molecular weight is by ultracentrifugation. The ultracentrifuge produces high centrifugal forces in order to measure the movement or redistribution of sedimenting particles. The distribution of the particles is observed by an interference pattern. From this interference pattern, a molecular weight or molecular weight range can be calculated.

In work with the ultracentrifuge, the material under investigation is placed in the centerpiece of the cell assembly. This cell assembly is constructed in a manner that permits light rays to pass through its entire length. After the cell is assembled and placed in a rotor hole, the rotor is then installed in the rotor chamber; the chamber is evacuated; and the rotor accelerates. The sample material is subjected to high centrifugal forces that causes the molecular particles to sediment. As the particles are redistributed, the light from the optical system light source can be transmitted through the rotating cell. By means of this light, the optical elements translate particle movement into an optical pattern, from which molecular weight can be calculated.

METHODS AND MATERIAL

MATERIALS

Protein Hydrolysates

1. Collagen hydrolyzed with papain to a formol nitrogen of 10.0*
2. Collagen hydrolyzed with steam to a formol nitrogen of 6.0*
3. Collagen hydrolyzed with acid to a formol nitrogen of 10.0*

*Inolex Corporation, Chicago, IL.

Sephadex Gels

Sephadex G-10
G-15
G-25
G-75
G-200

METHODS

Gel Filtration

Cosmetic grade protein hydrolysates were thought to have average molecular weights of between 1,000 and 10,000. With this range, Sephadex G-15 or G-25 would be the gel of choice for the separation of these peptide molecules. Chromatographic columns were prepared by packing 1.5 × 100 cm columns with gel previously swollen in 0.25 *M* NaCl. These columns were equilibrated with the saline. Protein hydrolysate samples were dissolved in the equilibration solution at a concentration of 180 mg/ml, and 3 ml of this solution was applied to the top of the column. The material was eluted at the rate of 0.25 ml/min. The presence of polypeptides was detected by measuring the absorbance at 280 nm using a continuous flow spectrophotometer. The curves generated showed that all of the sample was eluted in the void volume (above in the upper exclusion limit) from the Sephadex G-15 column, and some of the sample was eluted in the void volume from the Sephadex G-25 column. These gels, therefore, were not the appropriate ones for the separation of the cosmetic grade protein hydrolysates being investigated.

After the initial chromatographic runs were completed, Sephadex G-75 and G-200 columns were packed in the same manner as the G-15 and G-25 columns. From the absorbance graphs of these runs, it was determined that G-75 was the most useful in determining the molecular weight distribution of the hydrolysates being studied.

The 3 commercially available cosmetic grade proteins described above were studied using the Sephadex G-75 column. The peptides were eluted with 0.25 *M* NaCl at a rate of 0.15 ml/min and detected with absorbance measurements at 280 nm.

To determine the molecular weight relationship to elution volume, the Sephadex G-75 column was calibrated. First, the void volume was determined by eluting Blue Dextran; the elution volume is equal to the void volume. The total volume was calculated from the geometry of the column. A calibration curve was generated by eluting proteins of known molecular weights from the same Sephadex G-75 column and under the same conditions used in the experimental run.

Protein	Molecular Weight
Aldolase	158,000
Ovalbumin	45,000
Chymotrypsinogen	25,000
Ribonuclease A	13,700

The elution volumes of the proteins shown in the above chart were plotted against the logarithm of the molecular weight.

PROTEIN SUBSTANTIVITY OF MOLECULAR WEIGHT FRACTIONS

Evaluation of the 3 unfractionated cosmetic grade proteins on hair showed that the enzyme digested hydrolysate was the most substantive. For this reason, it was selected as the protein for study of the substantivity properties of its various fractions. A large G-75 Sephadex column (5.0 × 100 cm) was used, since much greater quantities of material than could be obtained from the small column were required to conduct the substantivity measurements on the various fractions.

The sample of the enzyme hydrolyzed material, 50 ml of an 18 per cent solution in 0.25 *M* NaCl, was applied to the bottom of the column and eluted at a rate of 0.5 ml/min. After 4 h, the column was inverted and the flow allowed to proceed as a descending chromatograph. This procedure insures a straight horizontal front.

Fractions were collected every 15 min. These fractions were then pooled into 4 main fractions: (1) greater than molecular weight 30,000; (2) 30,000–5,000; (3) 5,000–1,000; and (4) less than 1,000. It was necessary to desalt each of the fractions, because each was dissolved in 0.25 *M* NaCl. Desalting was accomplished on a Sephadex G-10 column. Each of the fractions was first freeze-dried, redissolved in 10 ml of water, applied to the column, and eluted with water. The solid material was used to prepare 5 per cent solutions of each fraction.

The relationship of molecular weight to substantivity to bleached and bleached-waved hair was then investigated. Hair swatches of each type of hair were prepared and each was then treated with one of the 5 per cent solutions. A water control and an hydroxyproline treated swatch were also included in the analysis. The treatment consisted of soaking for 10 min, blotting off the excess, and rinsing for 30 sec in warm running tap water.

To determine the amount of protein sorbed on the hair, the swatches were hydrolyzed with barium hydroxide, and the hair hydrolysate analyzed for hydroxyproline, the amino acid found in collagen protein but not in hair. In addition, the amount of hydroxyproline in each fraction was determined in order to correlate per cent hydroxyproline to per cent protein (6).

Ultracentrifugation

Two of the fractions separated by the Sephadex G-75 column were prepared for ultracentrifugation on a Beckman Ultracentrifuge* by diluting to 2 mg/ml in 0.25 *M* NaCl. This solution was placed in 1 side of a filled-Epon double sector synthetic boundary center piece,* and 0.25 *M* NaCl was placed in the other side. The cell with sapphire windows was placed in an AN-D rotor, and the run started. The conventional sedimentary equilibrium method of determining molecular weight was used. The experimental set-up for the 2 runs was as follows.

*Beckman Instruments Inc., Palo Alto, CA.

	Run One	Run Two
Fraction	1,000–5,000	5,000–30,000
Left sector	0.15 ml 0.25 *M* NaCl	0.15 ml 0.25 *M* NaCl
Right sector	0.15 ml 2 mg/ml protein in 0.25 *M* NaCl	0.12 ml 2 mg/ml protein in 0.25 *M* NaCl
Temperature	13.00°C	13.00°C
Speed		
Overspeed	52,000 rpm–4 h	44,000 rpm–3 h
Equilibrium speed	44,000 rpm–18 h	30,000 rpm–18 h

The molecular weights determined by ultracentrifugation were compared to the results obtained by gel filtration as will be shown later in this paper.

RESULTS

GEL FILTRATION

From the elution diagrams (absorbance versus elution volume) of each of the three hydrolysates studied, graphs of molecular weight versus per cent by weight were drawn. These graphs represent the molecular weight distribution curves for these proteins.

The elution diagrams, Fig. 1, are curves generated by the continuous flow spectrophotometer. These diagrams are used in preparing molecular weight distribution curves.

The enzyme hydrolysate was preserved with methylparabens and propylparabens, as well as benzalkonium chloride. These materials absorb at 280 nm and were eluted from

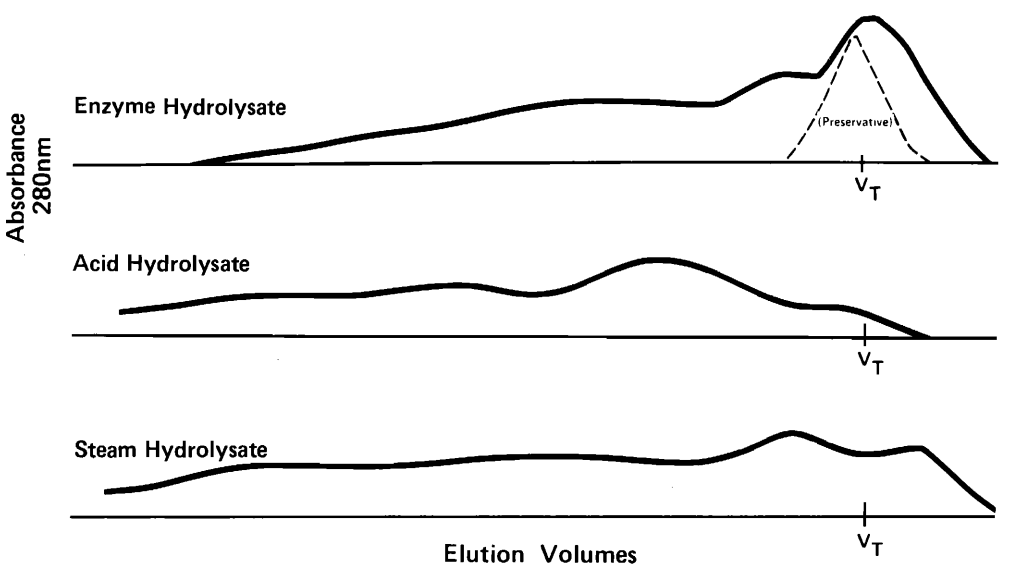

Figure 1. Elution diagrams, Sephadex G-75

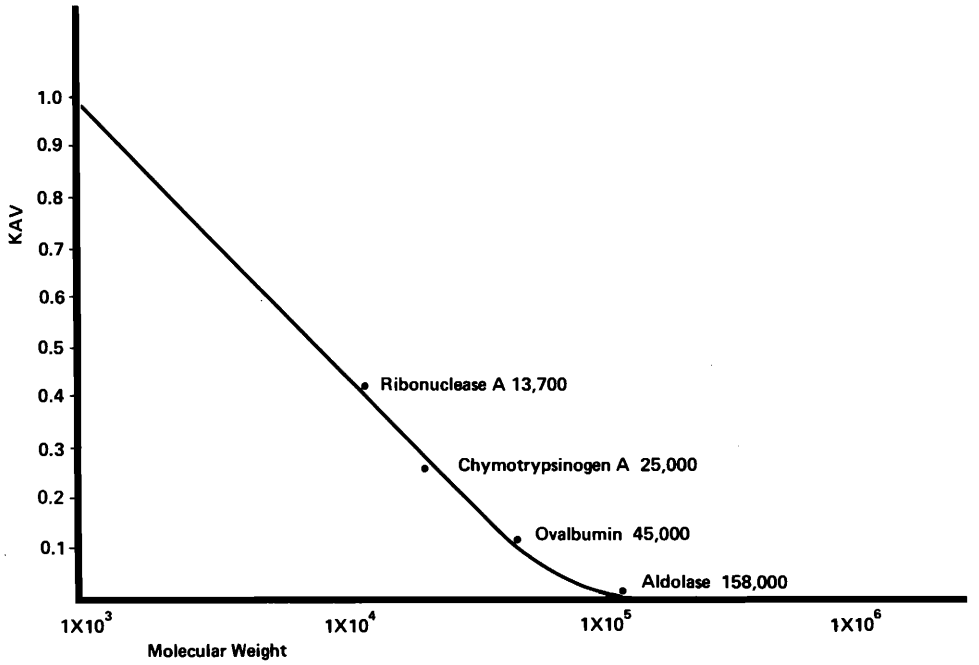

Figure 2. Calibration curve, G-75

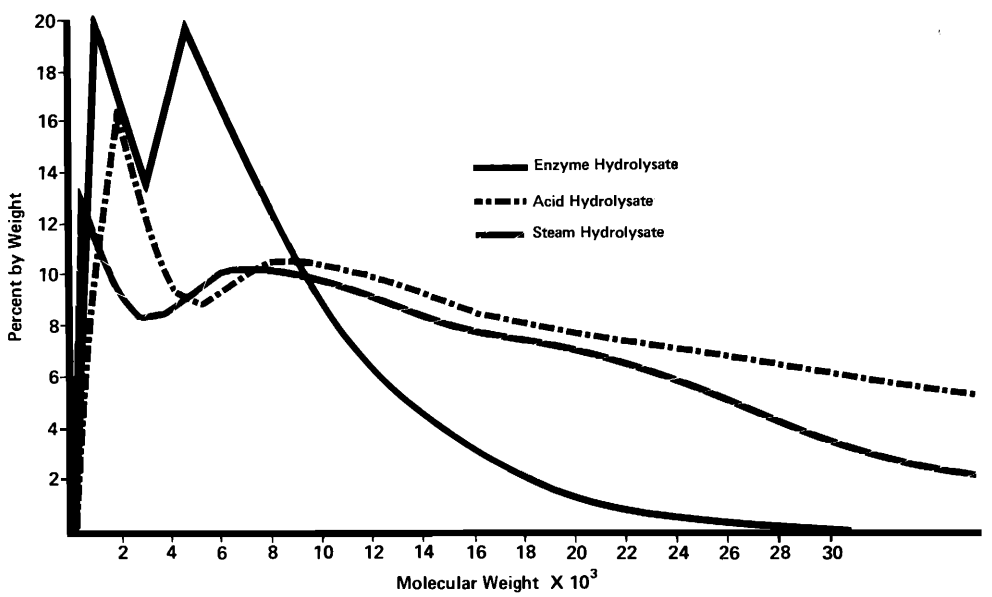

Figure 3. Molecular weight distribution curves

the column with the low molecular weight material. When preparing the molecular weight distribution curve of the enzyme hydrolysate, the absorbance due to the preservative had to be subtracted.

Figure 2 is the calibration curve, and shows the relationship of molecular weight to elution volume. The elution volume is expressed as Kav, which is the relationship of the elution volume to the void volume and the total bed volume.

$$Kav = \frac{Ve - Vo}{Ve - Vo}$$

As can be seen from the calibration curve, the elution volume has a straight line relationship to the logarithm of the molecular weight over most of the range between the void volume and the total bed volume.

From the elution diagrams and the calibration curve, molecular weight distribution curves were drawn. These graphs are shown in Fig. 3. These distribution curves are used to calculate both the weight average and the number average molecular weights.

The weight average and the number average molecular weights were calculated as follows.

$$M_n = \frac{P_i}{(P_i/M_i)}$$

where

M_w = weight average molecular weight
P = per cent of material at a constant elution volume interval
M_i = molecular weight at this interval $M_n = P_i/(P_i/M_i)$
M_n = number average molecular weight
P_i = percentage of material at a constant elution volume interval
M_i = molecular weight at this interval

In Table I the molecular weight averages calculated from the molecular weight distribution curves are shown.

ULTRACENTRIFUGATION

In Table II, the results of the ultracentrifuge run are compared to the gel filtration results.

Table I

Protein Hydrolysate	Weight Average	Number Average
Enzyme	4,000 ± 10 per cent	2,100 ± 10 per cent
Acid	9,300 ± 10 per cent	2,500 ± 10 per cent
Steam	7,800 ± 10 per cent	1,800 ± 10 per cent

Table II
Molecular Weight Ultracentrifugation versus Gel Filtration[a]

Gel Filtration	Ultracentrifugation
Over 5,000	8,700 – 3,600
5,000 – 1,000	3,000 – 1,500

[a]The molecular weights measured by these 2 methods correlate closely.

Table III

Fraction	Hair Type	Protein Substantivity mg Protein/100 g Hair
Greater than 30,000	Bleached	140
	Bleached-waved	300
30,000–5,000	Bleached	50
	Bleached-waved	290
5,000–1,000	Bleached	80
	Bleached-waved	370
Less than 1,000	Bleached	200
	Bleached-waved	510
Hydroxyproline	Bleached	40
	Bleached-waved	100

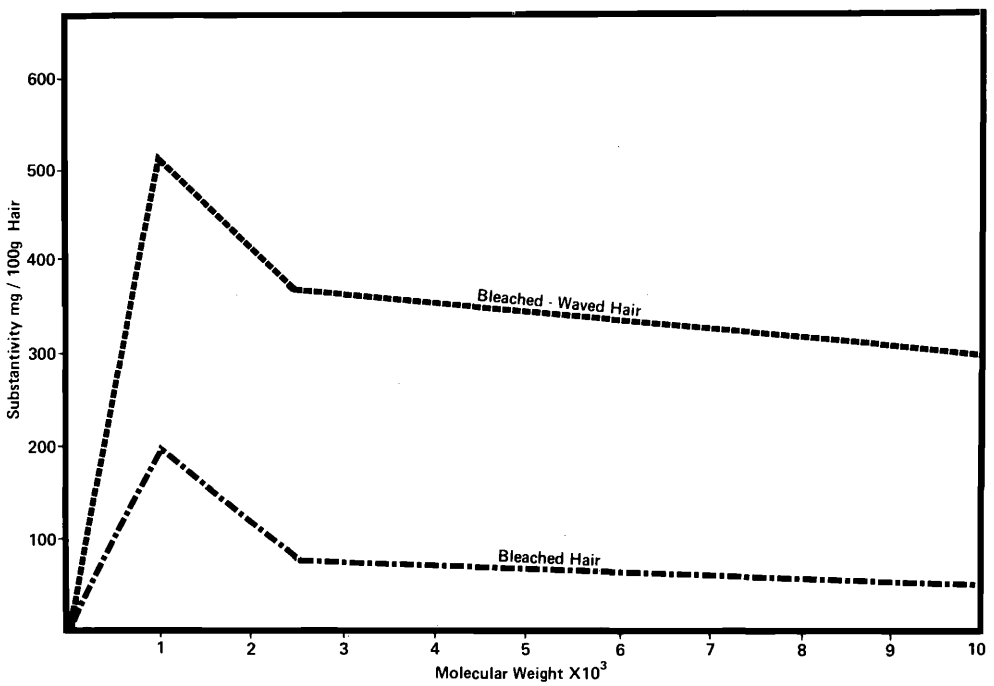

Figure 4. Substantivity of various molecular weight fractions

PROTEIN SUBSTANTIVITY

The relationship of molecular weight to protein substantivity is shown in Table III. The data in Table III show that peptides of molecular weight of about 1,000, but greater than the molecular weight of amino acids, gives the highest substantivity. Figure 4 shows the results in Table III graphically.

CONCLUSION

A study of the molecular weight distributions of 3 protein hydrolysates used in cosmetics was conducted. The effect of molecular weight on protein substantivity to damaged hair was also investigated in an attempt to find a relatively narrow weight range that gives optimum substantivity. Two conclusions can be drawn. (1) papain hydrolysis results in a narrow distribution of molecular weights. About 75 per cent of the molecules have weights between 500 and 10,000. As opposed to enzymatic hydrolysis, the acid and steam hydrolysates have much broader distributions. Seventy-five per cent of the molecules range in weights between 500 and 30,000. (2) The data have shown that polypeptides of molecular weight of about 1,000 but greater than the molecular weight of amino acids give the highest substantivity to damaged hair.

These conclusions are based on a preliminary study and a number of points still remain unresolved. Answering these questions will involve a more detailed separation of the molecules below molecular weight 1,000 and an in-depth study of their effect on substantivity. Separating polypeptides of molecular weight 0 to 1,000 is difficult. Gel filtration chromatography can be used, however, calibrating the column can pose a problem. Until these lower molecular weights are investigated, the conclusions that have been stated are rather broad. It will be the purpose of subsequent studies to narrow down the molecular weight range necessary for optimum substantivity.

Molecular weight has been thought to be an important feature of cosmetic protein hydrolysates. It was generally assumed that lower molecular weight polypeptides were more substantive to damaged hair. The study reported here has shown that peptides in the range of molecular weight 1,000 are more substantive than the very high molecular weight polypeptides.

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