MOLECULAR WEIGHT OF THE GAMMA-GLIADIN COMPONENT OF WHEAT GLUTEN[1]

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ABSTRACT

The molecular weight of electrophoretically pure gamma-gliadin from Ponca flour was determined in an ultracentrifuge by the sedimentation equilibrium method. In 6*M* guanidine hydrochloride plus 0.1*M* acetic acid (pH 3.3), both weight average and Z-average molecular weight are 31,000. In aluminum lactate-lactic acid ($\mu = 0.1$, pH = 3.1), gamma-gliadin is aggregated. Its apparent weight average is 33,000 and Z-average molecular weight, 42,000.

Wheat gluten contains a number of electrophoretic components (1,2). Some of these have been isolated by fractionation on a carboxymethylcellulose column (3) and their molecular weights determined in an ultracentrifuge by the approach-to-equilibrium method (4). The gamma-gliadin[2] fraction appeared homogeneous by both moving-boundary electrophoresis and the ultracentrifuge study. Molecular weight of gamma-gliadin in aluminum lactate-lactic acid ($\mu = 0.1$, pH = 3.1) was reported to be 37,000.[3]

Starch gel electrophoresis now demonstrates that the gamma-gliadin used previously contained a trace (less than 5%) of omega-gliadin. Although there is evidence (1,6) that omega-gliadin has a much higher molecular weight than gamma-gliadin, the trace of impurity was not detected by the approach-to-sedimentation-equilibrium technique. The inability of this method to detect a small degree of heterogeneity has been observed before.[4] Evidence has also been obtained that 6*M* guanidine hydrochloride is a better solvent for gluten fractions than is aluminum lactate buffer.

Because of these difficulties, the study of molecular weight of gamma-gliadin was repeated by using purified gamma-gliadin and the sedimentation-equilibrium centrifuge method. This technique is extremely sensitive to heterogeneity. Both guanidine hydrochloride and aluminum lactate were used as the solvent.

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[2] For discussion of the nomenclature of gluten fractions see references 2 and 4.

[3] This molecular weight was erroneously reported to be 47,000 (4). The correct value is 37,000. See erratum, reference 5.

[4] Nielson, H. C. Unpublished results.

Materials and Methods

The gamma-gliadin obtained from a carboxymethylcellulose column was purified either by continuous-flow, paper curtain electrophoresis or by preparative electrophoresis in a Tiselius cell. It was pure as determined by starch gel electrophoresis. Gluten from defatted Ponca hard red winter wheat was used. Jones and Dimler (7) have given a description of the lipid extraction. Complete details of the wheat used and of gluten preparation are given by Jones, Taylor, and Senti (1). Separation on carboxymethylcellulose is described by Woychik, Dimler, and Senti (3). The paper curtain electrophoresis was done by Huebner and Woychik.[5]

Impure gamma-gliadin was purified in a Tiselius cell, in Spinco Model H apparatus equipped with a macro cell. Protein solution was placed in the descending limb and alumimum lactate buffer in the ascending limb. The bottom cell section contained buffer plus enough sucrose to give a density greater than the density of the protein solution. Voltage was applied until gamma-gliadin appeared in the ascending side; then the cell was closed, and the pure component was removed. Only a few mg. can be obtained at a time.

The sedimentation-equilibrium procedure used is that described by Van Holde and Baldwin (8) and by Erlander (9). Time required to reach equilibrium was shortened by changing rotor speed as suggested by Hexner, Boyle, and Beams (10). This method gives M_W and M_Z for the entire sample, as well as M_W and M_Z at any point in the centrifuge cell.

Sapphire centrifuge cell windows replaced the usual quartz because distortion caused difficulty at low protein concentrations.

Results

Molecular weight of gamma-gliadin was determined in two solvents: aluminum lactate-lactic acid ($\mu = 0.1$, pH = 3.1) and 6*M* guanidine hydrochloride in 0.1*M* acetic acid (pH = 3.3). The log of protein concentration at various points in the cell was plotted against the square of the distance of the point from the center of rotation. Such plotting gives a straight line for a homogeneous protein and an upward curve if the material is heterogeneous (11). For gamma-gliadin in guanidine the curve is perfectly straight (Fig. 1), indicating a completely homogeneous protein. In aluminum lactate the plot is curved upward, showing in this case that the protein is aggregated. The approach-to-equilbrium method used previously (4) did not detect this aggregation.

[5] Huebner, F. R., and Woychik, J. H. In preparation.

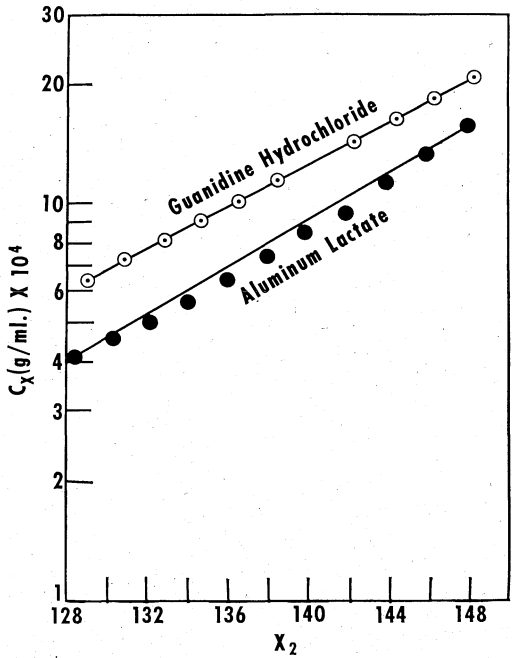

Fig. 1. Sedimentation equilibrium of gamma-gliadin.

In guanidine, purified gamma-gliadin has a weight-average and a Z-average molecular weight of 31,000. The molecular weight is uniform throughout the cell. Identical results were obtained at protein concentrations from 0.07 to 0.31%. For impure gamma-gliadin containing a trace of omega-gliadin, M_W is 33,000 and M_Z is 37,000. This difference again illustrates the greater sensitivity of the equilibrium method as compared with the approach-to-equilibrium method.

In aluminum lactate pure gamma-gliadin has an apparent weight-average molecular weight of 33,000 and an apparent Z-average of 42,000. Calculated weight average at the meniscus is about 24,000 and at the bottom of the cell 36,000. However, these values are not true molecular weights. In a system as heterogeneous as this one, some large particles are deposited on the cell bottom. The calculated values of molecular weight are therefore too low.

Apparent molecular weight was independent of protein concentration. Identical values of M_W were obtained in aluminum lactate buffer at initial protein concentrations of 0.09 to 0.17%. Effect of concentration on molecular size was studied by the procedure of Erlander, Senti, and Foster (12). Weight-average molecular weight was plotted against concentration at a constant weight fraction. The same results

were obtained regardless of whether the protein was dissolved directly in aluminum lactate or dissolved in guanidine and dialyzed against aluminum lactate.

Discussion

The type of aggregation is unusual in aluminum lactate, that is, independent of concentration. It might result from the low concentration and the narrow range of concentration. Using higher protein concentrations and acetate-chloride-dimethylformamide buffer, Winzor (13) found an increase in sedimentation coefficient with increasing concentration of wheat protein. The observations by Winzor, of course, could reflect properties of components other than gamma-gliadin in the gluten complex.

A value of 0.724 was calculated for the partial specific volume ($\overline{V}$) from the amino acid composition of gamma-gliadin (14,15). This procedure is valid and gives the molecular weight of the unsolvated polymer in a two-component system, such as water plus protein (16). A dilute salt solution can be treated as pure water. In a three-component system such as guanidine, water, and protein, however, the calculated or experimentally determined value of $\overline{V}$ cannot be used unless it is shown that the solute does not interact preferentially with either component of the solvent. To test for preferential binding of guanidine hydrochloride by gliadin, the apparent concentrations of gliadin in a synthetic boundary and from a velocity run in an ultracentrifuge were compared. The concentrations were the same. This similarity shows that there is no selective binding of solvent by gliadin in 6*M* guanidine hydrochloride; therefore, $\overline{V}$ of the dry polymer can be used in determining molecular weight.

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