Thermal Behavior of Annealed Acetic Acid-Soluble Wheat Gluten

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ABSTRACT

The effect of annealing acetic acid-soluble wheat gluten at temperatures below its glass transition temperature (T_g) was investigated using differential scanning calorimetry (DSC). Acetic acid-soluble wheat gluten was annealed at 10°C below its T_g for various lengths of time. Subsequent DSC of the annealed gluten revealed an endothermic peak near its T_g. The area of this peak, which depended both on the time annealed and the DSC heating rate, appears to be associated with the relaxation of glassy gluten toward a lower energy state and can be explained by retardation kinetics. Thus, there is no need to invoke first-order thermodynamic processes to explain the energy absorption of amorphous gluten observed in the DSC after annealing. The calorimetric data obtained with isolated gluten fit a theory of enthalpy relaxation used to explain endothermic peaks in amorphous synthetic polymers. Energy absorption and peak temperature from the thermogram can be used to estimate the relaxation rate and activation energy for enthalpy relaxation.

An endothermic peak near or below the glass transition temperature (T_g) of annealed glassy synthetic polymers has been observed using differential scanning calorimetry (DSC) (Foltz and McKinney 1969, Petrie 1972, Berens and Hodge 1982, Hutchinson and Ruddy 1988). This peak, known as the endothermic overshoot, was suggested by Petrie (1972) to provide a quantitative measure of the enthalpy relaxation that occurs in a polymer during sub-T_g annealing. Glasses always exist in a nonequilibrium state and relax toward a lower energy, metastable state upon annealing (aging) (McKenna 1988). This relaxation brings about the endothermic peak in DSC heating scans (Petrie 1972, Berens and Hodge 1982). The peak's position and size is controlled by cooling and subsequent heating rates, as well as annealing temperature and time (Petrie 1972, Hutchinson and Kovacs 1976, Berens and Hodge 1982). The peak can be explained by enthalpy retardation kinetics (Hutchinson and Kovacs 1976).

Slade (1984) showed, and Hoseney et al (1986) later confirmed, that wheat gluten is a glassy polymer at room temperature and low-moisture content. Wheat gluten can undergo a glass transition either by elevating its temperature or by adding a plasticizer (water) (Slade 1984, Hoseney et al 1986, Doescher et al 1987, Levine and Slade 1988, Fujio and Lim 1989).

An endothermic peak, apparently similar to that we described, has been reported for gluten and glutenin by others (Hoseney et al 1986, Cocero and Kokini 1991), but no attempt has been made to characterize it. The purpose of this study was to determine whether the endothermic peak observed by Hoseney et al (1986) and Cocer and Kokini (1991) in DSC thermograms of isolated gluten and glutenin represents the same phenomena as the endothermic overshoot described for other synthetic amorphous polymers.

MATERIALS AND METHODS

Protein Extraction

The acetic acid-soluble (ACS) gluten was isolated from a defatted Scout 66 flour with a protein content of 11.7%, db (%N × 5.7). The gluten-solubilization procedure is outlined in Figure 1. Two acetic acid supernatants were combined and lyophilized, and the dried ACS gluten was stored at 4°C. All of the fractions from the isolation procedure were analyzed for nitrogen content in triplicate by AACC method 46-13 (1983). Moisture determination for the flour, flour residue, and ACS gluten was performed by drying a 200-mg sample in a preweighed drying bottle and placing the sample in an air oven at 100°C until the sample was a constant weight. Starch was determined by an enzymatic method patterned after the method of Solomonsson et al (1984). Glucose was determined using a diagnostics kit (Sigma Chemicals, St. Louis, MO).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the Scout 66 flour, ACS gluten, and hand-washed gluten, according to a modified procedure of Laemmli (1970), on 12% (w/v) acrylamide resolving gel. All three samples were reduced in SDS reducing buffer before electrophoresis. Samples were extracted in a 0.5*M* Tris-HCl (pH 6.8) buffer containing 10% (w/v) SDS and 5% (v/v) β-mercaptoethanol. Samples were diluted 1:20 with buffer and heated for 10 min at 95°C. After centrifugation, supernatants were analyzed on a vertical gel apparatus (Protean II xi slab cell, Bio-Rad, Richmond, CA) with 1.5-mm gels. The samples were subjected to electrophoresis for about 4 hr at 35 mA per gel until the tracking dye migrated off the gel. The gel was stained overnight with 0.1% (w/v) Coomassie Brilliant Blue R250 in 12.5% (w/v) trichloroacetic acid. The gel was destained with the same solution without Coomassie Blue until the gel background was clear for photography. Molecular weight standards (Bio-Rad) used were phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

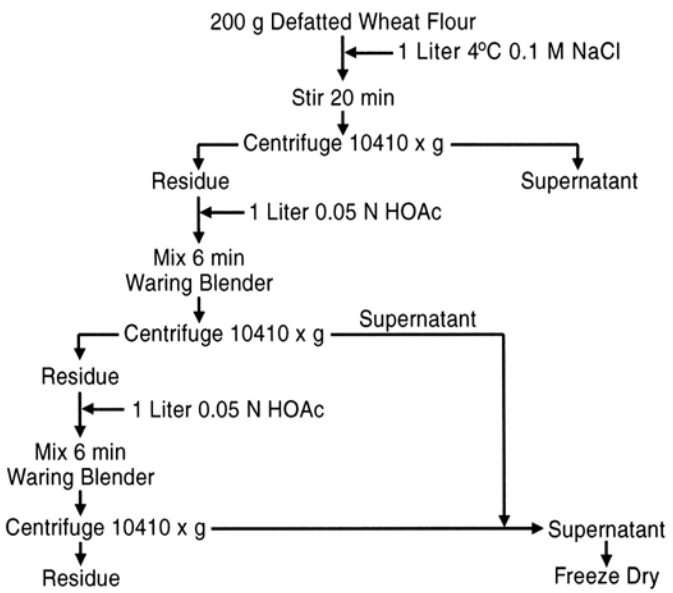

Fig. 1. Procedure for the laboratory isolation of acetic acid-soluble gluten from flour.

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DSC

A Perkin-Elmer (Norwalk, CT) differential scanning calorimeter (model DSC-7) was used to examine the isolated wheat gluten. The calorimeter was calibrated with water and indium. Approximately 3 mg (as is) of gluten was scaled into a tared aluminum calorimeter sample pan and sealed. To erase prior thermal histories, samples were placed in the calorimeter, heated from 0 to 100°C, and cooled to 30°C at 20°C/min. After initial heating and cooling, the sealed samples were placed in an air oven to anneal at 47°C, about 10°C below their T_g. This T_g of the isolated ACS gluten was obtained from the thermogram of the sample at annealing time 0; it was measured as the midpoint of the transition. Annealing times were 0, 0.5, 1, 2, 4, 8, 16, 24, 48, 72, 144, 240, 960, and 4,080 hours (170 days). After annealing, the samples were placed back into the calorimeter and rescanned from 0 to 100°C at 20°C/min. Peak temperature and enthalpy (ΔH) of the peak was calculated using thermal analysis software (TAS-7, Perkin-Elmer).

The effect of heating rate on annealed ACS gluten was investigated using this procedure with the following exceptions. All samples were annealed at 47°C for a single time (144 hr). After annealing, samples were rescanned at one of the following heating rates: 1, 2.5, 5, 7.5, 12.5, 15, or 20°C/min.

RESULTS AND DISCUSSION

Preliminary work, using the conditions of Hoseney et al (1986), revealed an endothermic peak in the initial DSC thermogram of vital wheat gluten (Fig. 2). Because no evidence exists for a gluten-denaturation peak (Eliasson and Hegg 1980, Hoseney et al 1986), another explanation is needed to explain this peak. This peak and the peaks shown in the thermograms of Hoseney et al (1986) and Cocero and Kokini (1991) are quite similar to endothermic peaks observed in the thermograms of annealed glassy synthetic polymers. Vital wheat gluten contains starch, another polymer with a glass transition (Zeleznak and Hoseney 1986, Slade and Levine 1988), which also could be responsible for the endothermic peak (Shogren et al 1992). Therefore, we decided to use an ACS gluten to eliminate the possibility of starch contributing to the endothermic peak.

The extracted ACS gluten had a Kjeldahl nitrogen value of 17.7% (db) (100% protein, 5.7 × N) and a moisture content of 8.3%. The yield of the ACS gluten was 9.43% of the flour (80.9% of the total flour protein). The flour residue contained 0.2% unextracted nitrogen (1.1% protein, 5.7 × N), 11.8% of the flour protein was extracted as salt-solubles. If the salt-solubles are excluded from the protein yield, then 90% of the gluten protein was extracted by acetic acid. The gluten that was not extracted was probably high molecular weight glutenin (Bietz and Huebner 1980). Figure 3 shows that glutenin was extracted by acetic acid, as evidenced by the high molecular weight glutenin subunits.

Starch content of the ACS gluten was 0.82% (db). Shogren showed that the area (ΔH) for the endothermic peak of starch annealed below its T_g; it decreased as moisture content of the starch decreased. He also showed that starch at a moisture content of 11.1% and annealed for 28 days had an endothermic peak with a ΔH of ~0.75 J/g. The ΔH for pure starch at 8.3% moisture is assumed to be lower than 0.75 J/g. In our ACS gluten sample, the starch contribution to the endothermic peak would be over 100 times lower than that seen by Shogren. We believe that the starch content of the ACS gluten is too low to contribute significantly to the endothermic peak.

The effect of annealing time on peak formation during heating in the calorimeter is illustrated in Figure 4. As gluten is annealed for longer periods of time, its peak temperature (T_{max}) is increased. The measured area of the peak (ΔH) also increases with increased annealing time. DSC and differential thermal analysis thermograms of synthetic polymer glasses that show a similar dependence on sub-T_g annealing times have been observed by Petrie (1972) and others (Foltz and McKinney 1969, Wolpert et al 1971, Berens and Hodge 1982, Hutchinson and Ruddy 1988).

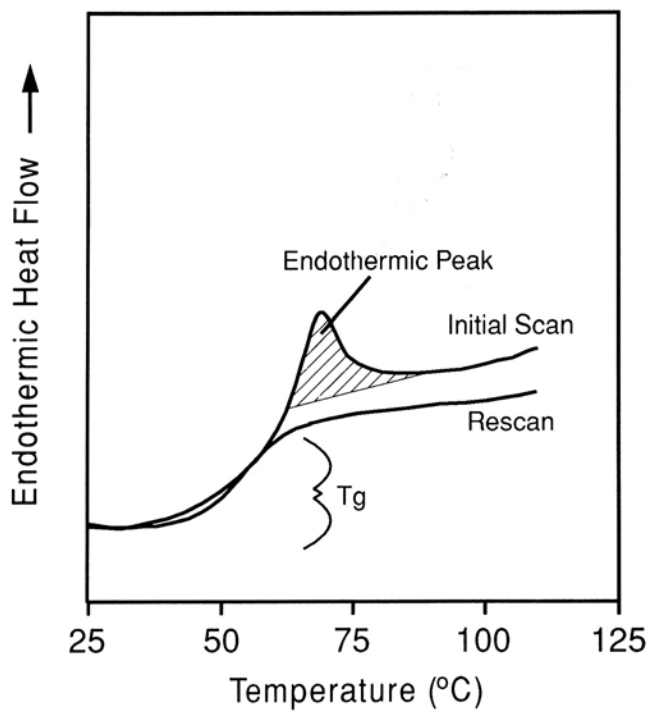

Fig. 2. Differential scanning calorimetry thermogram of laboratory-isolated vital wheat gluten heated at 20°/min from 0–110°C. Initial scan presented the endothermic peak (shaded) just before glass transition temperature (T_g). Immediate rescan shows only the step change in the heat capacity characteristic of (T_g).

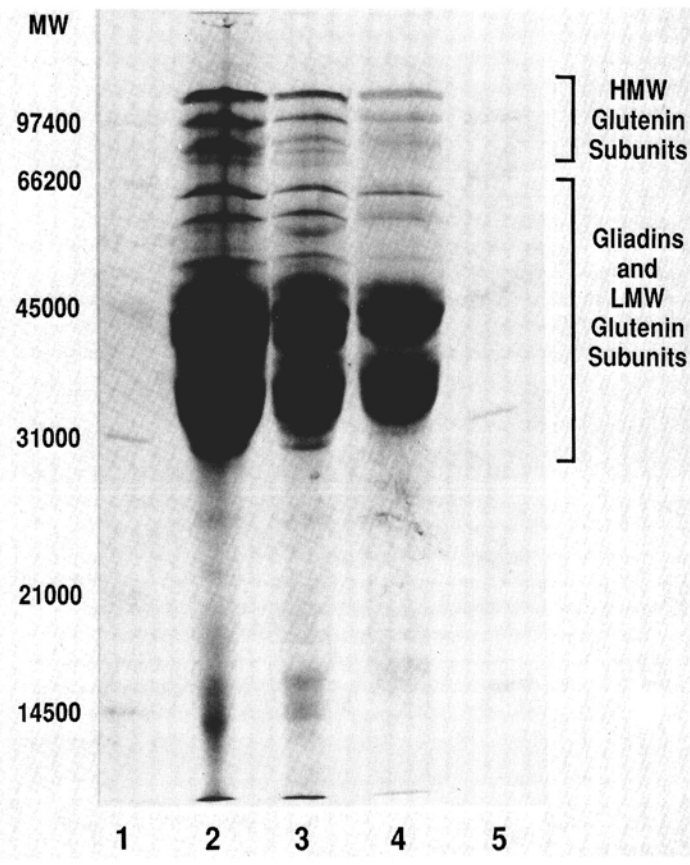

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lane 1, standard proteins; Lane 2, hand-washed gluten from Scout 66 flour; Lane 3, Scout 66 flour; Lane 4, acetic acid-soluble gluten from Scout 66 flour; Lane 5, standard proteins. MW = molecular weight; HMW = high molecular weight; LMW = low molecular weight.

The existence and behavior of this peak can be explained by retardation kinetics (Hutchinson and Kovacs 1976). Gluten, when rapidly cooled from above its *Tg* to the glassy state, does not have enough time to reorient physically and approach equilibrium conditions. When this gluten is maintained at temperatures below its T_g, molecular relaxation (through short-range, rotational reorientations) takes place. This results in a loss in enthalpy and changes in other physical properties (Foltz and McKinney 1969). When the annealed sample is heated in the calorimeter, the loss in enthalpy must be made up at T_g. This results in the endothermic overshoot peak.

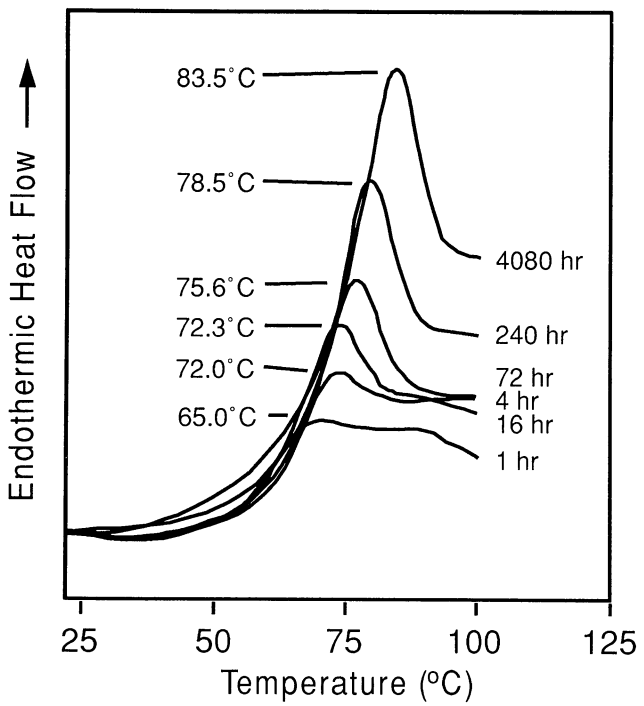

Fig. 4. DSC thermograms of annealed acetic acid-soluble gluten. Samples annealed at 47°C then heated at 20°C/min from 0–100°C. Annealing times and peak temperatures as indicated.

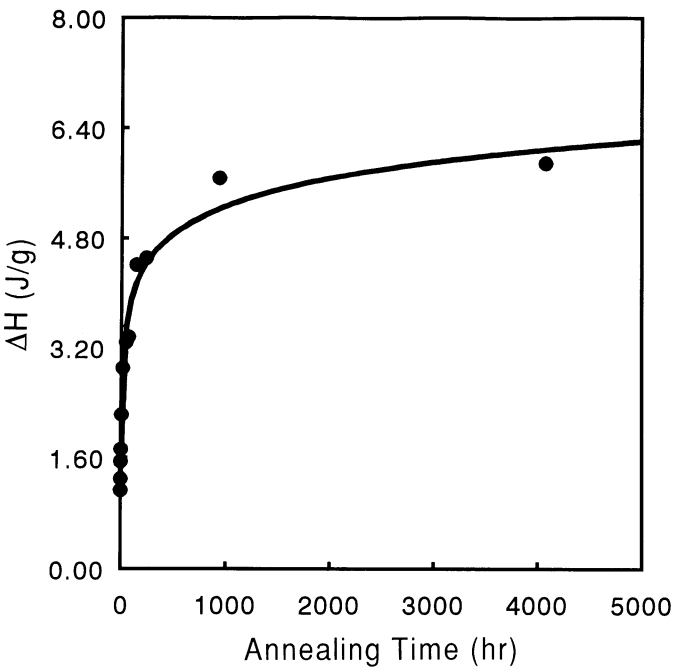

Fig. 5. Thermal energy absorbed at glass transition temperature as a function of annealing time. Samples annealed at 47°C. Heating rate 20°C/min.

The peak area of annealed ACS gluten samples, plotted as a function of their annealing time, is shown in Figure 5. The data suggest that the peak areas are approaching a limiting value. This would be expected in the case of an endothermic overshoot. In fact, Petrie (1972) reported that this limiting value is based on the equilibrium state (actually a metastable steady state of lowest energy) of the system under investigation. This limiting value is the total enthalpy loss caused by the material relaxing to its lowest energy amorphous state at that specific temperature. Consequently, the amount of heat absorbed at the glass transition cannot exceed this limiting value, regardless of the length of the annealing period. The rate at which a glass approaches this limiting value is a function of the temperature difference between the annealing temperature and T_g (Petrie 1972). The farther the annealing temperature is below T_g, the slower the material anneals to a steady state. This relationship probably holds down to approximately 50°C below its T_g, where all appreciable motion should have disappeared (Gordon 1965).

A rough estimate of the time required for a polymer to reach such a low-energy steady state (Struik 1978) is:

$$t_\infty = 100 \cdot e^{0.77(T_g - T)} \quad (1)$$

where t_∞ is the time required to reach the fully relaxed state (in seconds) and T is the annealing temperature. According to equation 1, the ACS gluten used in this study would take about 61 hr to reach steady state. The actual data (Fig. 5) show that the equation's estimate was quite low. There are at least two possible reasons for the underestimate. First, the equation is very sensitive to temperature. Changing the $T_g - T$ term in the equation from 10 to 11°C changes the t_∞ estimate from 61 to 132 hr. Any error in the determination of T_g or the measurement of T greatly changes the calculated t_∞. Second, t_∞ in equation 1 is based solely on how far the annealing temperature is below the material's T_g. The equation completely ignores structural aspects of the macromolecules that would affect the rate at which they approach a low-energy metastable state. Marshall and Petrie (1975) showed that molecular weight of polystyrene affects its relaxation time (longer chains have longer relaxation times). This presents some interesting possibilities. If the annealing process is affected greatly by molecular structure, then it may be possible to use this procedure to differentiate between strong and weak glutans. The areas (ΔH) of the peaks also can be plotted as a function of log annealing time, as shown in Figure 6 (Petrie 1972).

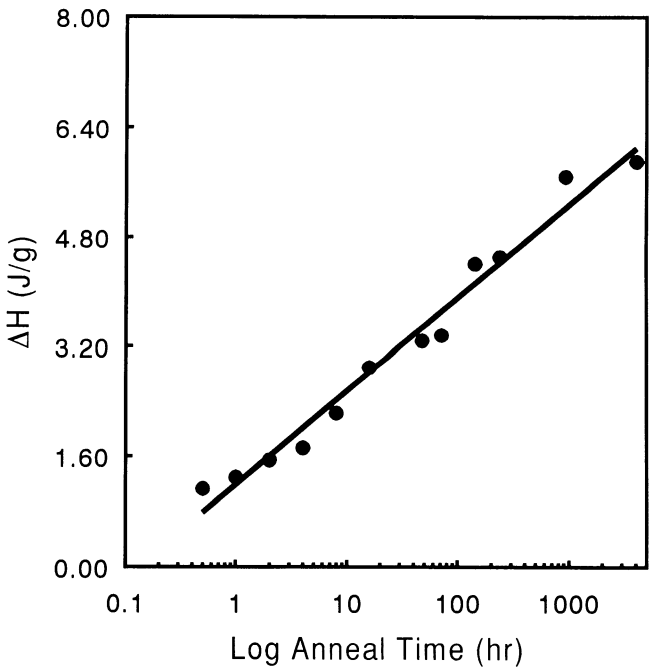

Fig. 6. Enthalpy relaxation of acetic acid-soluble gluten annealed at 47°C. $R^2 = 0.97$.

This plot is linear, and its slope reflects the rate of enthalpy relaxation of the glassy ACS gluten.

Heating rate in the calorimeter also affects the endothermic peak, as shown in Figure 7. The peak increases in size and is shifted to higher temperatures as heating rate increases. This phenomenon also exists for organic and inorganic glasses (Wunderlich et al 1964, Moynihan et al 1974, Hutchinson and Ruddy 1988). Figure 8 shows the data in Figure 7 plotted as inverse peak temperature versus the natural log of the heating rates (Moynihan et al 1974). This produces a linear plot with slope b equal to:

$$d \ln|q| d/ T_{max} = b \approx \Delta h^* / R \quad (2)$$

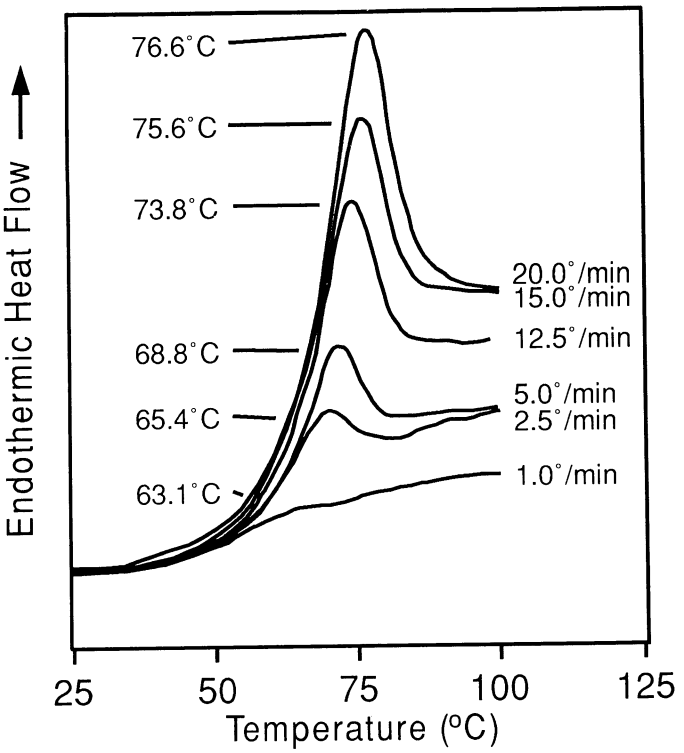

Fig. 7. Differential scanning calorimetry thermograms of acetic acid-soluble gluten annealed for 144 hr. Heating rates as indicated. Samples annealed at 47°C.

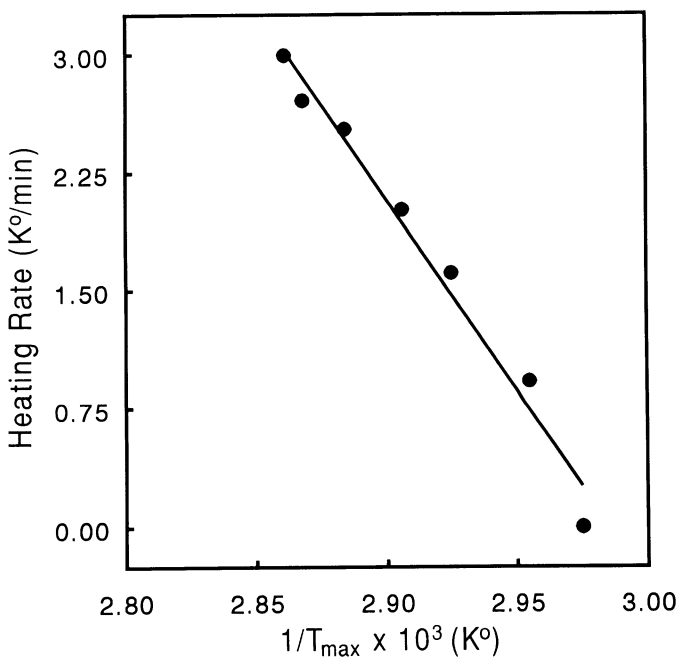

Fig. 8. Natural log of the heating rate vs. 1/peak temperature. Slope = −24.38. $R^2 = 0.98$. Samples annealed at 47°C for 144 hr.

where q is the heating rate, T_{max} is the peak temperature, Δh^* is the activation energy, and R is the gas constant.

Previous studies on the isothermal enthalpy or volume relaxation of glasses in the glass transition region have shown that Δh^* for enthalpy relaxation is generally the same as Δh^* for shear viscosity (Moynihan et al 1974, 1976). The Δh^* calculated from the slope of Figure 8 is 48.6 kcal/mol. This Δh^* for enthalpy relaxation of ACS gluten is lower than that predicted for synthetic amorphous polymers (Ferry 1980). However, the Δh^* calculated from Figure 7 was acquired with gluten that was plasticized with about 8% water. To get a true Δh^* for gluten, either this procedure would have to done with bone-dry samples or results would have to be extrapolated back to the dry state for samples of various moisture contents.

CONCLUSIONS

The endothermic peak seen in DSC thermograms of glassy ACS gluten, annealed (aged) at temperatures below its Tg, is a kinetic event associated with the relaxation of amorphous gluten to its lowest energy metastable glassy state. This appears to be the same phenomenon that occurs in synthetic amorphous polymers and can be explained without invoking first-order thermodynamic transitions such as denaturation or melting (of either gluten or starches). These data agree with and extend those of Slade (1984) and Hoseney et al (1986) and strengthen the conclusion that wheat gluten has essentially no long-range order, and it acts as an amorphous random polymer (Levine and Slade 1988, 1989; Slade and Levine 1989).

The relaxation process takes time to occur, and the gluten takes time to reach steady state, which, consequently, affects the storage of gluten. Gluten stored near or above its Tg could have different physical properties than those of gluten stored at a temperature significantly below its Tg. For example, gluten containing 10% moisture would have a Tg at approximately 50°C (Slade 1984, Hoseney et al 1986, Levine and Slade 1988, Fujio and Lim 1989, Cocero and Kokini 1991). Equation 1 estimates it should take this glassy gluten 726 years to reach low-energy steady state at 25°C. However, it would take only about 12 days for that same gluten to reach the same steady state if stored at 38°C.

Heating rate also affects the size and position of the endothermic peak seen in the DSC thermograms. Increasing heating rates increases the peak size and shifts it to higher temperatures. The slope of the inverse of the peak temperature versus the natural log of the heating rate can be used to calculate an activation energy for the enthalpy relaxation of glassy gluten. The activation energy for the gluten used in this study was calculated to 48.6 kcal/mol.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods for the AACC, 8th ed. Method 46-13, approved October 1976, reviewed October 1982. The Association: St. Paul, MN.

BERENS, A. R., and HODGE, I. M. 1982. Effects of annealing and prior history on enthalpy relaxation in glassy polymers. I. Experimental study on poly(vinyl chloride). Macromolecules 15:756.

BIETZ, J. A., and HUEBNER, F. R. 1980. Structure of glutenin: Achievements at the Northern Regional Research Center. Ann. Technol. Agric. 29:249.

COCERO, A. M., and KOKINI, J. L. 1991. The study of the glass transition of glutenin using small amplitude oscillatory rheological measurements and differential scanning calorimetry. J. Rheol. 35:257.

DOESCHER, L. C., HOSENEY, R. C., and MILLIKEN, G. A. 1987. A mechanism for cookie dough setting. Cereal Chem. 64:158.

ELIASSON, A. C., and HEGG, P. O. 1980. Thermal stability of wheat gluten. Cereal Chem. 57:436.

FERRY, J. D. 1980. Dependance of viscoelastic behavior on temperature and pressure. Pages 289-320 in: Viscoelastic Properties of Polymers. John Wiley and Sons: New York.

FOLTZ, C. R., and McKINNEY, P. V. 1969. Quantitative study of the annealing of poly(vinyl chloride) near the glass transition. J. Appl. Polym. Sci. 13:2235.

FUJIO, Y., and LIM, J. 1989. Correlation between the glass-transition point and color change of heat-treated gluten. Cereal Chem. 66:268.
GORDON, M. 1965. Thermal properties of high polymers. Pages 209-249 in: Physics of Plastics. P. D. Ritchie, ed. D. Van Nostrand: Princeton.
HOSENEY, R. C., ZELEZNAK, K., and LAI, C. S. 1986. Wheat gluten: A glassy polymer. Cereal Chem. 63:285.
HUTCHINSON, J. M., and KOVACS, A. J. 1976. A simple phenomenological approach to the thermal behavior of glasses during uniform heating and cooling. J. Polym. Sci., Pt. B. 14:1575.
HUTCHINSON, J. M., and RUDDY, M. 1988. Thermal cycling of glasses. II. Experimental evaluation of the structural (or nonlinearity) parameter χ. J. Polym. Sci., Pt. B. 26:2341.
LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680.
LEVINE, H., and SLADE, L. 1988. Water as a plasticizer: Physicochemical aspects of low-moisture polymeric systems. Water Sci. Rev. 3:79.
MARSHALL, A. S., and PETRIE, S. E. B. 1975. Rate-determining factors for enthalpy relaxation of glassy polymers. Molecular weight. J. Appl. Phys. 46:4223.
McKENNA, G. B. 1988. Glass formation and glassy behavior. Pages 311-363 in: Comprehensive Polymer Science, Vol. 2. Polymer Properties. G. Allen and J. C. Bevington, eds. Pergamon Press: Oxford.
MOYNIHAN, C. T., EASTEAL, A. J., DeBOLT, M. A., and TUCKER, J. 1976. Dependence of the fictive temperature of glass on cooling rate. J. Am. Ceram. Soc. 59:12
MOYNIHAN, C. T., EASTEAL, A. J., WILDER, J., and TUCKER, J. Dependence of the glass transition temperature on the heating and cooling rate. J. Phys. Chem. 78:2673.
PETRIE, S. E. B. 1972. Thermal behavior of annealed organic glasses. J. Polym. Sci., Pt. A-2 10:1255.
SHOGREN, R. L., SWANSON, C. L., and THOMPSON, A. R. 1992. Extrudates of cornstarch with urea and glycols: Structure/mechanical property relations. Starch/Staerke 44:335.
SLADE, L. 1984. Staling of starch-based products. Cereal Foods World 29:508.
SLADE, L., and LEVINE, H. 1988. Non-equilibrium melting of native granular starch. I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. Carbohydr. Polym. 8:183.
SLADE, L., and LEVINE, H. 1989. Influences of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs and baked products. Pages 157-330 in: Dough Rheology and Baked Product Texture. H. Faridi and J. M. Faubion, eds. Nostrand/AVI: New York.
SOLOMONSSON A.-C., THEADER, O., and WESTERLUND, E. 1984. Chemical characterization of some Swedish cereal whole meal and bran fractions. Swed. J. Agric. Res. 14:111.
STRUIK, L. C. E. 1978. Basic aspects of aging: Effects on the mechanical properties at small strains. Pages 13-47 in: Physical Aging in Amorphous Polymers and Other Materials. Elsevier Scientific: New York.
WOLPERT, S. M., WEITZ, A., and WUNDERLICH, B. 1971. Time-dependent heat capacity in the glass transition region. J. Polym. Sci., Pt. A-2. 9:1887.
WUNDERLICH, B., BODILY, D. M., and KAPLAN, M. H. 1964. Theory and measurements of the glass-transformation interval of polystyrene. J. Appl. Phys. 35:95.
ZELEZNAK, K. J., and HOSENEY, R. C. 1986. Annealing and glass transition of starch. Starch/Staerke 38:289.

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