Viscosity of Melanoidin from a Glycine–Xylose System. Support for the Equation Exhibiting a Relationship between Color Intensity and Molecular Weight

To obtain more intensive support for the validity of an equation, $E = kM^{\alpha}$ (E, $E_{450}{}^{1\%,1cm}$, absorbance of 1% solution in 1-cm path at 450 nm; M, molecular weight; k and α, constants), the intrinsic viscosity of the color components of melanoidin from a glycine–xylose system has been investigated in connection with their color intensity. Adherence to Staudinger's equation, $[\eta] = k'M^{\alpha'}$ ($[\eta]$, intrinsic viscosity), was observed in the relationship between the intrinsic viscosity and molecular weight of the color component, and the equation, $[\eta] = 0.096M^{0.48}$, has been derived. On the other hand, another equation was calculated to be $E = 2.90M^{0.29}$. Moreover, a linear relationship between log $[\eta]$ and log E led to $E = 11.8[\eta]^{0.61}$. The last equation can also be derived from the first two equations. Therefore, the validity of the equation, $E = k''M^{\alpha''}$, was judged by this experiment. Moreover, the color components of melanoidin from the glycine–xylose system were suggested to be a homologous series of symmetrically flexible chain polymers since Staudinger's equation held true for a melanoidin solution.

Melanoidin pigments prepared from a model system were fractionated into eight color components (designated as P1, P2, P3, P4, P5, P6, P7, and P8 in the order of elution position on DEAE-cellulose) on DEAE-cellulose column chromatography with stepwise elution by varying the concentration of sodium chloride (Motai, 1973). Fast eluting color components of melanoidin from a glycine–xylose system were found to change to slow eluting components with a concomitant increase of their molecular weight, probably as the result of polymerization during nonenzymatic oxidative browning (Motai and Inoue, 1974a). By utilizing the change of elution position on the DEAE-cellulose column during nonenzymatic oxidative browning, the color components formed by polymerization of the original color components were selectively separated. Molecular weight (M) and color intensity, $E_{450}{}^{1\%,1cm}$ or E (absorbance of a 1% solution in a 1-cm path at 450 nm), values of the color components increased in the order of their elution position on the DEAE-cellulose column and a linear relationship between log E and log M was unified as eq 1. This equation was also applicable to most of melanoidin from the model system (Motai, 1974). We concluded that nonenzymatic oxidative browning of melanoidin was attributable to an increase of its molecular weight and that the amount of color in the melanoidin solution increased according to eq 1. Nonenzymatic oxidative browning of shoyu (soy sauce), which was accompanied by deterioration of flavor and taste (Yokotsuka, 1960), could also be explained in terms of eq 1 (Motai and Inoue, 1974b).

$$E = kM^{\alpha} \quad (1)$$

To provide more intensive support for eq 1, this communication will describe a relationship among the intrinsic viscosity, molecular weight, and color intensity of the melanoidin from a glycine–xylose system.

EXPERIMENTAL SECTION

Preparation of Melanoidin. Melanoidin was prepared by heating a mixture of 0.2 mol of xylose and 0.2 mol of glycine in 150 ml of 0.2 M acetate buffer (pH 5.0) at 100° for 2 hr.

Separation of Color Components. Selective separation of color components using the change of elution position of color component on the DEAE-cellulose column during nonenzymatic oxidative browning was performed as previously described (Motai and Inoue, 1974a). After removing unreacted glycine and xylose from the color materials by passage through Sephadex G-15, color components P2 to P7 were fractionated from the color materials with stepwise elution by varying the concentration of sodium chloride. Purified color components P3 to P8 were obtained by a rechromatography through a DEAE-cellulose column after shaking these fractions in the presence of air. Then each color component was desalted by Sephadex G-15 and lyophilized.

Estimation of Molecular Weight. The molecular weights of color components were estimated by the gel filtration method on Sephadex G-25, G-50, G-75, and G-150 columns (2 × 100 cm) using dextran T-10 (mol wt 10,300), T-20 (mol wt 22,300), and T-40 (mol wt 44,400, Pharmacia Fine Chemicals) as a standard under the same conditions as previously described (Motai, 1974).

Determination of Intrinsic Viscosity. Viscosity was measured by using an Ostwald viscometer requiring 2.5 ml of solution with a water flow time of 192.5 sec at 20 ± 0.01°. Each sample was dissolved in distilled water.

RESULTS AND DISCUSSION

By studying the viscosity of a solution, valuable information can be obtained about the molecular size and shape of the dissolved molecule. In the present study, the viscosity of a melanoidin solution was measured in order to get information on the structure of the color components of melanoidin. The intrinsic viscosity of the color components is shown in Figure 1. Reduced viscosity was independent of the concentration of every color component. Intrinsic viscosity increased with an increase of the molecular weight of the color components. The authors previously reported that a series of color components formed during nonenzymatic oxidative browning appeared to be very similar in chemical structure based on spectral measurement, elemental analysis, and amino acid analysis and different in molecular weight and color intensity ($E_{450}{}^{1\%,1cm}$) (Motai and Inoue, 1974a). If melanoidin consists of a mixture of a homologous series of chain polymers with a different degree of polymerization of a similar structural unit, an empirical relationship between molecular weight and intrinsic viscosity of the color components of melanoidin can be found according to Staudinger's eq (2) (Staudinger, 1930; Hauwink, 1940; Flory, 1953):

$$[\eta] = k'M^{\alpha'} \quad (2)$$

Furthermore, eq 3 can be derived from eq 1 and 2. Conversely, the validity of eq 1 will be supported if eq 3 also applies to melanoidin molecules. Therefore, a relationship which fits eq 3 is expected to exist between intrinsic viscosity and color intensity of melanoidin.

$$E = k''[\eta]^{\alpha''} \quad (3)$$

As shown in Figure 2, the points in log $[\eta]$ vs. log M plots were fairly well arranged on a defined straight line. Apparently, Staudinger's equation was found to be applicable for the color components of melanoidin from the

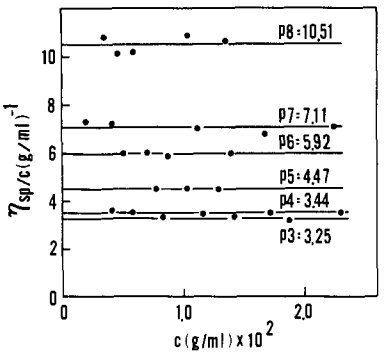

Figure 1. Intrinsic viscosity of color components of melanoidin from a glycine-xylose system.

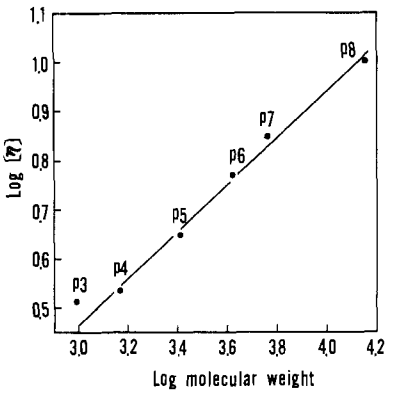

Figure 2. Log intrinsic viscosity ([η]) vs. log molecular weight (M) of color components of melanoidin from a glycine-xylose system; empirical equation: $[\eta] = 0.096M^{0.48}$.

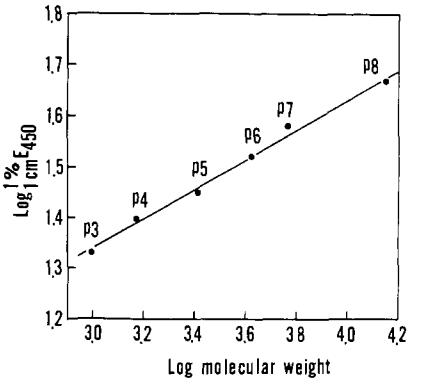

Figure 3. Log $E_{450}^{1\%,1cm}$ (E) vs. log molecular weight (M) of color components of melanoidin from a glycine-xylose system; empirical equation: $E = 2.90M^{0.29}$.

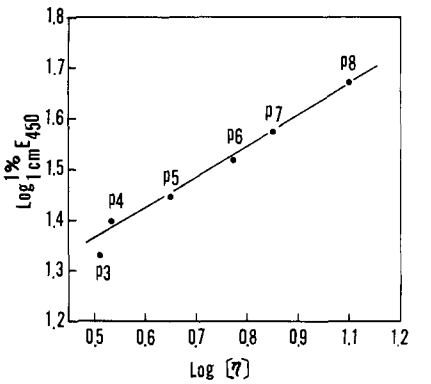

Figure 4. Log $E_{450}^{1\%,1cm}$ (E) vs. log intrinsic viscosity ([η]) of color components of melanoidin from a glycine-xylose system; empirical equation: $E = 11.5[\eta]^{0.61}$.

glycine-xylose system. The data in Figure 2 lead to eq 4.

$$[\eta] = 0.096M^{0.48} \quad (4)$$

On the other hand, from the data in Figure 3, the following equation is derived:

$$E = 2.90M^{0.29} \quad (5)$$

Equation 5 was fairly close to that previously reported (Motai, 1974), although values for color intensity and molecular weight were a little higher as compared to those of the previous experiment (Motai, 1974).

As expected from eq 3, the relationship between log E and log [η] was observed to be linear (Figure 4) and was expressed in the following empirical equation (6). Equation 6 was in good agreement with that calculated from eq 4 and 5.

$$E = 11.5[\eta]^{0.61} \quad (6)$$

From the above results, especially from eq 6, the validity of eq 1 has been demonstrated. Moreover, the color components of melanoidin from the glycine-xylose system are suggested to be a homologous series of flexible chain polymers since eq 2, which is uniquely applicable to only homologous chain polymers, was applicable for the melanoidin. It is also thought that the chemical structure of melanoidin is symmetrically flexible since these color components showed no optical rotation in the region of visible wavelength (Motai and Inoue, 1974a).

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