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A Dynamic Light Scattering Study of β-Galactosidase: Environmental Effects on Protein Conformation and Enzyme Activity

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Dynamic light scattering (DLS) is a useful technique for analyzing the size, shape, and other structural characteristics of protein molecules in solution. The effects of various environmental conditions on the structure and activity of *Aspergillus oryzae* β-galactosidase were studied. DLS was used to determine protein particle size under various salt, pH, and temperature conditions. Changes in the activity and stability of this enzyme caused by different environmental conditions were found to correlate well with the size changes of the protein particles. This change in protein size can be attributed to protein unfolding and aggregation under extreme conditions. The presence of the enzyme substrate, lactose, in the protein solution greatly enhanced enzyme stability by inhibiting aggregation.

Introduction

Many enzymes are used in large-scale industrial processes. The hydrolysis of lactose in milk and cheese whey by using β-galactosidase is an example (Gekas and Lopez-Leiva, 1985). The design and operation of a large-scale enzymatic process should consider both the activity and long-term stability of the enzyme, which are affected by many process parameters such as the temperature, pH, ionic concentration, and composition of the reaction medium. The enzyme activity is determined in large part by the specific three-dimensional structure or conformation of the protein molecule. The effects of various process parameters on enzyme activity and changes in protein conformation thus are of interest, but they have not been well studied for many industrially important enzymes. The environmental effects on enzyme structure may include changes in size and shape, association or dissociation of subunits, denaturation or unfolding, degradation, and aggregation. These changes contribute to the inactivation of enzymes (Mozhaev & Martinek, 1982).

Dynamic light scattering (DLS), also known as quasi-elastic light scattering and photon correlation spectroscopy, is an established method for particle size analysis (Schurr, 1977). It has been applied in size and shape analyses of macromolecules and used to study various inter- and intramolecular interactions, including association, aggregation, gelation, micellization, and molecular conformation, and the effects of many different factors on molecular structure (Harding et al., 1992). DLS has been widely used in the study of polymers, aerosols, emulsions, and biopolymers (Berne and Pecora, 1976; Pecora, 1985; Schmitz, 1990). The dynamic light scattering technique has also been used extensively in studying solutions of many globular proteins, including bovine serum albumin, hemoglobin, enzymes, insulin, bovine growth hormone, and blood proteins, to determine their diffusion coefficients, size and shape characteristics, and aggregation and binding kinetics. In general, geometrical information (size, shape, etc.) can be extracted from the diffusion coefficient data gathered by DLS. More recently, light scattering techniques have also been applied to engineering studies of insulin aggregation (Sluzky et al., 1992), protein precipitation (Przybycien & Bailey, 1989), antigen–antibody complexes (Yarmush et al., 1988), aqueous two-phase (King et al., 1988) and reverse-micellar (Sheu et al., 1986) extraction systems, casein coagulation (Horne and Dalgleish, 1985), and molecular weight determination (Yu and Rollings, 1987).

In this work, we studied the effects of several environmental parameters, including pH, temperature, and salt concentrations, on the protein conformation and enzyme activity of β-galactosidase from *Aspergillus oryzae*. This enzyme has a pH optimum of 4.5, a temperature optimum of 55 °C, and is relatively small, with a molecular weight of ~100 000 (Tanaka et al., 1975). Several groups have investigated the effects of various process parameters on the activity and stability of this enzyme (Ohmiya et al., 1975; Tanaka et al., 1975; Mega and Matsushima, 1979; Park et al., 1979; Ogushi et al., 1980; Friend and Shahani, 1982; van Griethuysen et al., 1985, 1988; Illanes et al., 1990). However, no prior studies have attempted to explain the activity and stability of this enzyme in terms of changes in its protein structure. In this study, DLS was used to determine protein conformation (primarily size) under various processing conditions.

Theory of Dynamic Light Scattering

Dynamic light scattering is a technique of particle size analysis based on the random or Brownian movement of particles as a result of small thermal fluctuations in the bulk solution. The magnitude of this movement is dependent on the size and shape of the particle. For example, a large or rod-shaped particle moves slower than small or spherical particles, since the drag force on the former is larger. Thus, light scattered from the surface of particles varies with the size and shape of the particles and their movement in solution. The intensity of the scattered light that is detected is also dependent upon the scattering angle and time. In general, large particles scatter more light than smaller particles. Consequently, the presence of only a few percent (by number) of large particles in a polydisperse sample can significantly impact the average particle size measured with classical light scattering, which measures the time-averaged light

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intensity as a function of scattering angle. However, there is important particle size information available from the time dependence of scattered light. Since this time dependence is due to the random thermal fluctuations, the time-dependent light intensity fluctuates randomly.

In order to analyze these fluctuations and obtain worthwhile particle size information, a correlation technique that was originally developed to analyze electrical signal noise is used. This so-called autocorrelation function, $G(\tau)$ is

$$G(\tau) = [I(0)I(\tau)] = \lim_{t'\to\infty} \frac{1}{t'} \int_0^{t'} I(t)I(t+\tau)\, dt \qquad (1)$$

where t' represents the experimental duration, τ is the sample decay time, I is the scattered light intensity, and the brackets indicate the time-dependent nature of the intensity values. By using the autocorrelation function, the randomly fluctuating scattered light intensity is transformed into an exponentially decaying function.

In order to obtain particle size information, it is necessary to select a theoretical form of the correlation function and numerically fit this equation with the decaying experimental data. There are a variety of different theoretical functions reported in the literature, each dependent upon the type of phenomenon one is observing (i.e., simple translational diffusion, molecular flexibility, and directed motion). In addition, there are several numerical techniques commonly used to fit the experimental data to the selected theoretical correlation function. These techniques fall into two basic categories: (i) a cumulant method and (ii) histogram methods (Stock and Ray, 1985). In the cumulant method, an average particle diffusion coefficient is determined that best fits the experimental data. For histogram methods, a particle size distribution is calculated.

In this work, the cumulant method was used to monitor the effects of various process conditions on the apparent particle size. The cumulant equation is given by

$$0.5 \ln[G(\tau) - G(\infty)] = C_0 - \frac{K_1}{1!}\tau + \frac{K_2}{2!}\tau^2 - \frac{K_3}{3!}\tau^3 + \cdots \qquad (2)$$

where C_0 is a constant and the K values are called cumulants (Koppel, 1972; Phillies, 1988). The particle size information is obtained in the first cumulant, given by

$$K_1 = D_T q^2 \qquad (3)$$

where D_T is the particle translational diffusion coefficient and q represents the magnitude of the scattering wave vector:

$$q = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2} \qquad (4)$$

where n is the refractive index of the medium, λ is the wavelength of the incident light, and θ is the scattering angle. The particle size is obtained from an appropriate expression for the translational diffusion coefficient. Assuming the particle can be modeled as a hard sphere, the diffusion coefficient is described by the Stokes–Einstein equation:

$$D_T = \frac{k_B T}{3\pi\eta d} \qquad (5)$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the solution viscosity, and d is the particle diameter. For particles of different shapes, other equations for the diffusion coefficient are available.

It is noted here that the cumulant method does not determine a size distribution in a polydisperse sample, but it can be used to obtain the effective diameter of protein particles in solution. Since the effective diameter is heavily weighted toward the larger particles, the actual weight-average or number-average particle size should be smaller than the measured effective diameter using this method. However, increases in effective diameter would point to the onset of denaturation and aggregation. On the other hand, a lack of changes in the effective diameter would be indicative of stable conditions for the native protein (Sluzky et al., 1992).

Materials and Methods

β-Galactosidase Solution. *A. oryzae* β-galactosidase (EC 3.2.1.23, lot no. 97F0830) was obtained from Sigma Chemical Company. All enzyme solutions were prepared in a similar fashion. The salt, lactose, and urea solutions were prepared from stock solutions. Then, the enzyme was added directly to give a concentration of 2.00 mg/mL, unless otherwise noted. Next, the pH of the samples was adjusted with dilute hydrochloric acid or sodium hydroxide. All samples were filtered through a 0.2 μm syringe filter (Millipore, Millex GV) prior to DLS measurement.

Experiments. The effects of NaCl and KCl on protein particle size were studied for a wide concentration range from 0.01 to 4 M. The enzyme in solution was allowed to reach a steady-state size at 4 °C, before microfiltration and DLS analysis were performed. The effects of urea concentration on particle size and enzyme activity were studied in the range from 0 to 8 M. The protein concentration in this study was 6 mg/mL. The effects of pH on protein particle size and enzyme activity were studied for a pH range of 2–10, in the presence of 1 M NaCl. The effects of temperature were studied by first exposing the enzyme solutions to various temperatures, between 20 and 70 °C, for 10 min. The enzyme solutions were then immediately cooled down and assayed for their activities and particle sizes.

For thermal aggregation studies, the enzyme solutions were sealed with Paraffin wax and incubated in a hot-air oven. The change in particle size with incubation time was studied at 50 and 70 °C. The sample was taken to the DLS apparatus for size determination and immediately returned to the oven after each DLS measurement. The sample holder assembly was maintained at the same temperature as the incubation temperature. For thermal deactivation studies, the enzyme solutions were incubated in a constant-temperature water bath. Samples were taken at various time intervals and immediately placed in an ice bath until the enzyme activity assay was performed.

In order to examine any stabilizing effect of lactose on this enzyme, the pH and thermal aggregation studies were repeated with 0.135 M (4.6%) lactose in the enzyme solution. These results were compared with those containing no lactose.

Enzyme Activity Assay. The determination of β-galactosidase activity followed the Miller method (1972). The enzyme solution (0.05 mL), 1.5 mL of Z buffer, and 0.2 mL of the substrate, *o*-nitrophenyl β-galactoside (ONPG, 4 mg/mL), were mixed. The mixture was incubated at 30 °C for a predetermined time (between 5 and 20 min), and then the reaction was stopped by adding 0.5 mL of 1 M Na_2CO_3 solution. The colorless ONPG was converted to yellow *o*-nitrophenol, which was measured

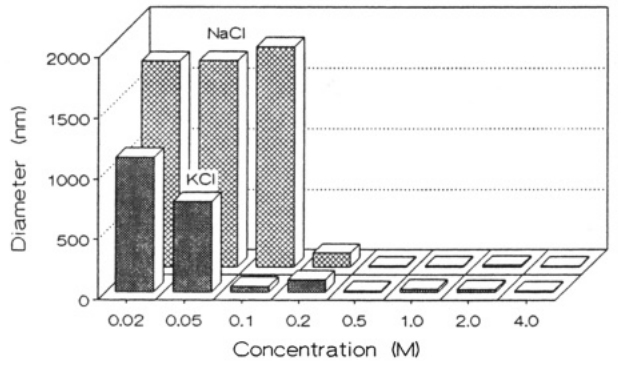

Figure 1. Effects of salt concentration on protein particle size in solution (pH 6.0, 25 °C).

as absorbance at 420 nm (OD_{420}) using a spectrophotometer. The enzyme activity and OD_{420} were linearly related.

DLS Measurements. The light scattering apparatus used in particle size analysis consisted of an argon ion laser (model XL-3000−3, Lexel Laser, Inc.) for illumination, a computer-controlled goniometer (Model BI-2005M, Brookhaven), and a 136-channel digital correlator and signal processor (Model BI-2030AT, Brookhaven), which incorporated an IBM-AT computer for measurement control, data collection, and particle size analysis. The entire sample holder assembly in the goniometer was fit with a temperature controller (Neslab, Model RTE-100B) and a submicrometer filter for dust removal (Brookhaven, Model BI-FC).

The glass sample cuvettes (Brookhaven, Model BI-RC12) had a refractive index of 0.514. Prior to preparation of the individual sample solutions, the cuvettes were rinsed thoroughly with deionized water and flushed with deionized water that had been filtered through a submicrometer filter. Next, the samples were filtered through the submicrometer filter directly into the cleaned cuvettes. After each experiment, the cuvettes were stored in a detergent solution (MICRO, International Products Corp.) designed to solubilize any materials that had adsorbed onto the glass surface.

All DLS measurements were made with a laser light wavelength $\lambda = 488$ nm at a scattering angle $\theta = 90°$, and the measurement duration for each sample was 2 min to reduce random noise and to ensure a stable baseline. With the exception of the study of the effect of temperature, all samples were maintained at 25 °C. The viscosity of salt solutions was obtained from the literature (Lange, 1961). The effect of the addition of protein to the solution viscosity η was assumed to be negligible due to the relatively small concentration of protein. The refractive index n of all samples was measured using an Abbe refractometer.

Data analysis was performed using canned programs available from Brookhaven Instrument Company. The available programs consisted of the cumulant (Koppel, 1972), a nonnegative regularized least-squares (NNLS) algorithm, and the CONTIN (Provencher, 1982a,b) methods. The cumulant method, as described previously, was used in this analysis.

Results and Discussion

Effects of Salt Concentration and Composition. The protein size as a function of NaCl and KCl concentrations at pH 6.0 is shown in Figure 1. For both salts, no aggregation was observed at salt concentrations higher than 0.2 M, and the protein size was ~12 nm in diameter. However, strong aggregation with particle sizes of 1−2 μm was found when KCl was below 0.1 M and NaCl was below 0.2 M, respectively. When the aggregated samples were left overnight, the solutions were observed to be completely turbid with some precipitate in the bottom of the container. This salting-in effect is due to the inability of the salt to effectively screen electrostatic interactions between protein molecules at very low salt concentrations. In addition, the difference in electrostatic screening of the two salts is in agreement with that predicted by the Hofmeister, or lyotropic, series. This series lists salts in order of their ability to disrupt hydrogen bonding in aqueous solutions (Belter et al., 1988). Experiments were performed up to salt concentrations of 4.0 M; however, no aggregation was observed. Although high salt concentrations have been used to precipitate proteins, the conditions of this experiment apparently were not conducive to aggregation.

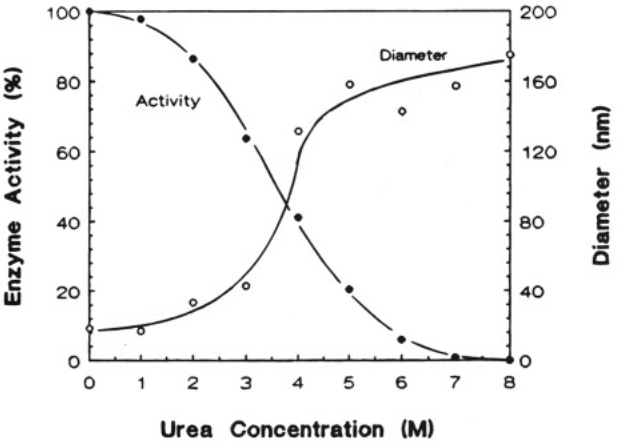

Figure 2. Effects of urea concentration on protein particle size and enzyme activity.

These results are in agreement with previously reported results for the effect of NaCl and KCl on the kinetics of lactose hydrolysis by *A. oryzae* lactase. At a pH of 4.0, the ion content was found to have little influence on protein activity. However, at a pH of 6.5, a significant reduction in enzyme activity was observed in the presence of 0.1 M of both KCl and NaCl. Since the isoelectric pH of this protein is 4.6, the protein would have a larger overall charge at pH 6.5 than at 4.0. Consequently, the amount of salt necessary to screen electrostatic interactions would be higher at pH 6.5. At the low pH value, the net charge on the protein is smaller, and the presence of 0.1 M salt apparently is sufficient to reduce aggregation (van Griethuysen et al., 1985).

It is noted that even though all of the protein solution samples were filtered before DLS measurement, some large aggregates (greater than the 200 nm pore size of the membrane) were still observed. This may be explained as follows: (1) Large aggregates might be broken up during filtration but reaggregated quickly after they passed the membrane. This was consistent with the finding that aggregation was fast under low-salt conditions. (2) The aggregates were not rigid spheres; they might be forced to penetrate through the membrane pore during filtration. (3) The size calculation was based on a spherical shape assumption. A nonspherical particle (e.g., a rod) would give a larger apparent size due to its smaller diffusivity in solution in the DLS analysis.

Effects of Urea. Urea is a chaotropic agent that disrupts the bonding that holds the protein in its characteristic three-dimensional structure. The folding behavior of *A. oryzae* lactase has not been previously reported. In order to shed some light on the folding behavior of this protein, DLS was used to monitor the effect of urea on protein size.

The graph of particle size versus urea concentration shown in Figure 2 has a common shape. The measured

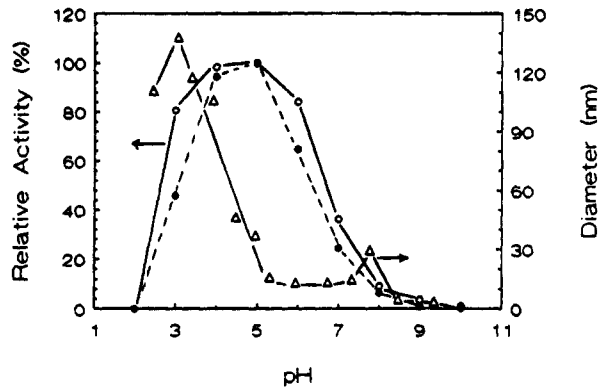

Figure 3. Effects of pH on protein particle size and enzyme activity (all sample solutions contained 1 mg/mL protein and 1 M NaCl).

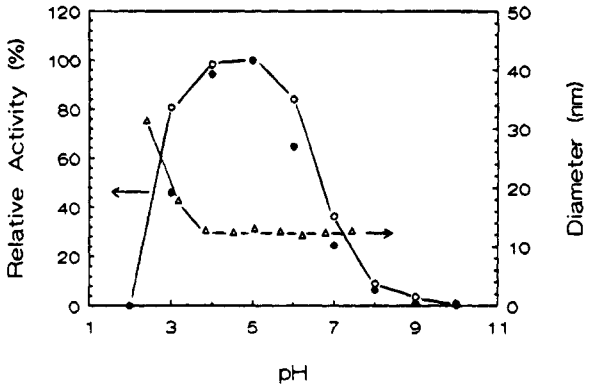

Figure 4. Effects of pH on protein particle size and enzyme activity, with 0.135 M lactose and 1 M NaCl in solution.

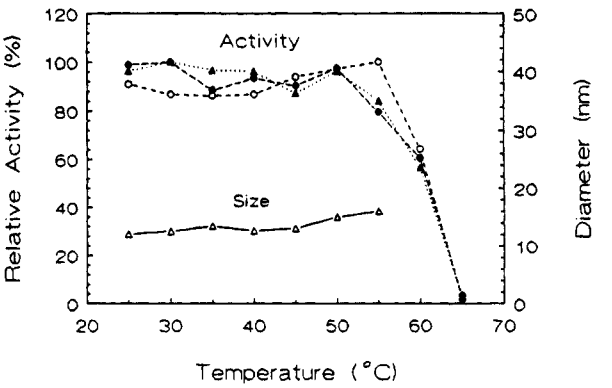

Figure 5. Effects of temperature on protein particle size and enzyme activity (in pH 5.5, 1 M NaCl solution; DLS measurements were made after 10 min of exposure to the temperature).

protein size was observed to increase from ~15 nm for native protein at 0 M urea to ~170 nm for unfolded protein at 8 M urea, with rapid change (unfolding) occurring as the urea concentration increased from 3 to 4 M. As also shown in this figure, the size change as the protein was being unfolded by the chaotropic agent was accompanied by activity loss. Since the size change corresponded well with the activity loss, this size information may be used in studying protein denaturation and refolding (Wang et al., 1980).

There is conflicting evidence in the literature regarding the subunit structure of *A. oryzae* lactase, one study suggesting a dimeric structure (Mega and Matsushima, 1979) and another suggesting monomeric (Ogushi et al., 1980). The particle size change during unfolding, shown in Figure 2, does not appear to support the existence of a dimeric structure. However, additional study is necessary before making any conclusion regarding the subunit structure.

Effects of pH. The effects of pH on protein structure and enzyme activity are demonstrated in Figure 3. Depending on the pH range, two different phenomena were observed with the size change. First, at pH values below 5, approximately the isoelectric pH, protein size increased to ~140 nm as the pH decreased to 3, despite the presence of 1.0 M NaCl to insure against aggregation. This increase in size could be due to either aggregation, denaturation, or a combination of these two effects. The observed size of 140 nm was in the size range for an unfolded protein (see Figure 2). Also, the samples with a pH of less than 4 were turbid and contained a solid precipitate the following day. The second effect is apparent at pH values above 8. The particle size falls to a value of 2.9 nm. This roughly corresponds to a molecular weight of 10 000, which is about 9 times less than that of the native protein. The large size reduction in this pH range is more than what can possibly be explained by the dissociation of an oligomer (dimmer) to monomers, suggesting that degradation has occurred.

The effect of pH on the enzyme activity is also shown in Figure 3. It appears that the pH optimum for enzyme activity does not correspond well with the protein size. There is a shift of about 2 pH units when comparing the particle size curve with the activity curve. However, this inconsistency can be attributed to the stabilization effect of enzyme substrate (Mahoney et al., 1988; Mahoney and Wilder, 1989; Izutsu et al., 1991). Lactose was present in all enzyme solutions used for the activity assay, but not in this size study. A comparison of particle size and activity as a function of pH in the presence of lactose is shown in Figure 4. With lactose, the protein size remained unchanged until the pH was below 3.5, which corresponded well with the activity curve. Thus, the decrease in activity at low pH values is clearly due to unfolding and perhaps aggregation too. At pH values above 8, the degradation of the protein results in a decrease in enzyme activity. The DLS technique has demonstrated that a change in protein structure with pH is at least partially responsible for the loss in activity.

Effects of Temperature. Two different experiments were performed in investigating the effects of temperature on the structure and activity of the enzyme. In the first study, the protein was exposed to various temperatures for a very short period of time (10 min) before particle size determination. A comparison of the particle size as a function of temperature with the relative enzyme activity at various temperatures is shown in Figure 5. The relative enzyme activity was determined by the relative amount of reaction product formed after allowing the enzyme reaction to take place at various temperatures for three given time periods (5, 10, and 15 min). As shown in Figure 5, this enzyme is quite stable at temperatures below 55 °C. The protein size remained under 15 nm for temperatures up to 55 °C. At temperatures higher than 55 °C, the size increased rapidly (data not shown), indicating the onset of denaturation and aggregation. The onset of size increase and aggregate formation at 55 °C compares favorably with the activity curves. This is also consistent with the reported optimum temperature for enzyme activity at ~55 °C (Tanaka et al., 1975).

In order to investigate the thermal stability of the enzyme, the experiment in the preceding paragraph was repeated for longer periods of time at 50 and 70 °C. The experiment was discontinued when the enzyme activity was completely lost or the particle size no longer changed significantly. As shown in Figure 6, at 70 °C the protein denatured and aggregated very rapidly. The solution became turbid almost immediately after submersion in the constant temperature water bath, and the particle

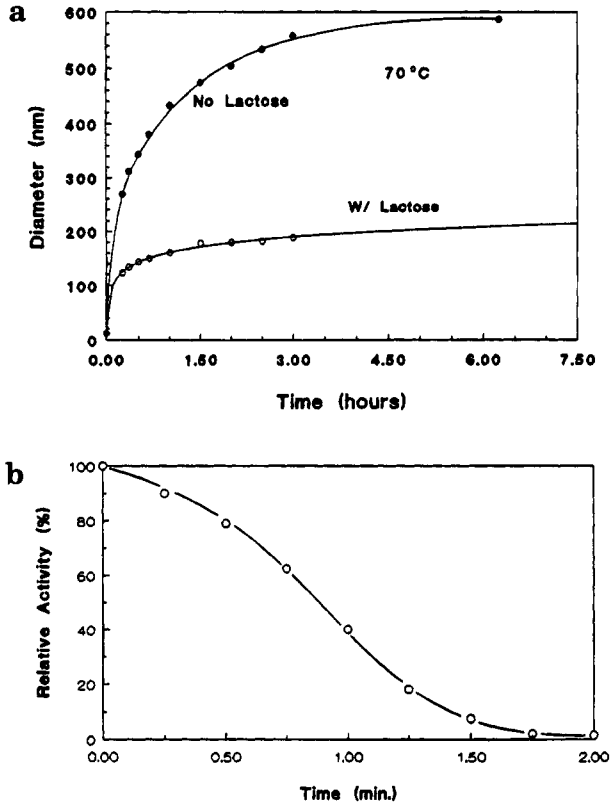

Figure 6. Thermal aggregation and deactivation of lactase at 70 °C. (a) aggregation; (b) deactivation (in pH 5.5, 1 M NaCl solution).

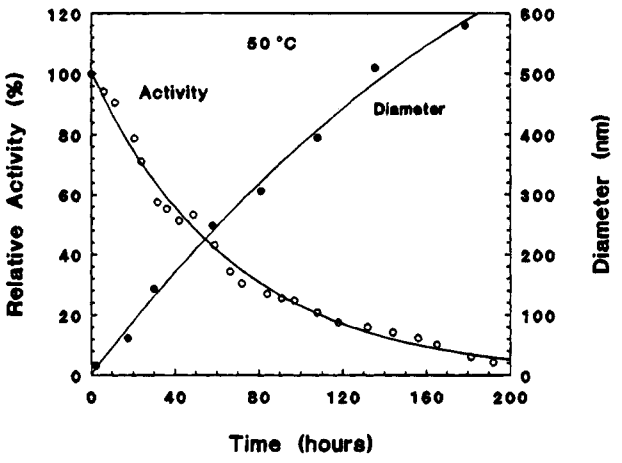

Figure 7. Thermal aggregation and deactivation of lactase at 50 °C (in pH 5.5, 1 M NaCl solution).

size reached 275 nm within just a couple of minutes. The enzyme was also completely deactivated within 2 min (Figure 6b). The aggregate size leveled off at 600 nm after just 7 h. At 50 °C, the aggregate size increased much more slowly and was still increasing 6 days later, albeit not at the same large size observed at 70 °C. The slower aggregation at 50 °C was also accompanied by a slower deactivation (Figure 7). It is clear that protein denaturation to the unfolded state and aggregation contribute to enzyme deactivation at high temperatures. The parallel trends between enzyme activity loss and particle size increase in these experiments indicate that DLS can be used to study enzyme stability.

Stabilization Effect of Lactose. As discussed earlier, the presence of lactose in the pH-aggregation profile is shown to stabilize the protein until the pH falls below 3.5 (Figure 4). This is in contrast to the aggregation observed below a pH of 5.0 when no lactose is present

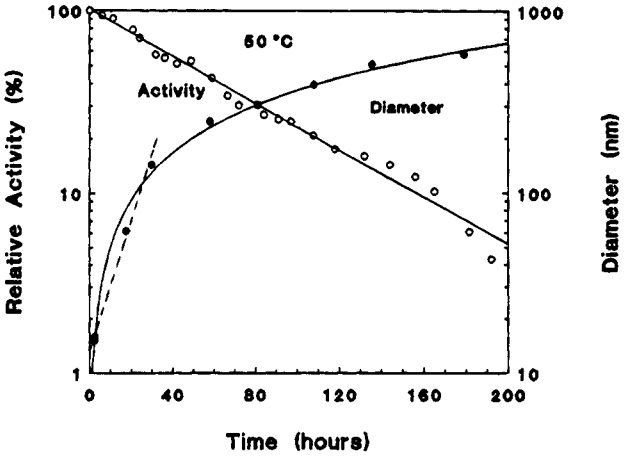

Figure 8. Kinetics of thermal deactivation of lactase at 50 °C.

(Figure 3). In addition, the final aggregate size was found to be much smaller than the samples without lactose.

The stabilizing effect of lactose on the thermal aggregation (deactivation) of β-galactosidase was also shown in Figure 6, where the particle size as a function of time at 70 °C is shown for samples with and without lactose. The particle size reached equilibrium sooner and the resultant particle size was smaller when lactose was added. Although the lactose is stabilizing the protein, the high temperature used in this study resulted in significant aggregation that could not be totally eliminated by the addition of lactose. However, the reduction in particle size upon the addition of lactose clearly demonstrates the stabilizing effect of the substrate on the enzyme.

Thermal Deactivation and Aggregation Kinetics. Thermal deactivation of lactase follows a first-order reaction mechanism and can be modeled by the following equation (Yang & Okos, 1989):

$$E = E_0 e^{-k_d t} \tag{6}$$

where E_o is the initial enzyme activity, E is residual enzyme activity at time t, and k_d is the thermal deactivation rate constant. The k_d value can be determined from the semilogarithmic plot of E versus t (Figure 8) and was found to be 0.0148 h^{-1} at 50 °C and 3.28 min^{-1} at 70 °C. The deactivation rate constant for *A. oryzae* lactase has been previously reported to be 0.0160 h^{-1} at 50 °C (Illanes et al., 1990).

The effects of temperature on protein structure during thermal inactivation include denaturation (unfolding) and aggregation (Volkin and Middaugh, 1992), and the process may be represented by a two-step reaction as follows:

$$N \rightleftarrows U \rightarrow A$$

where N represents the native (folded) structure, U represents the unfolded or denatured structure, and A represents the aggregate. When the second step is much faster than the first step, the overall aggregation rate would be controlled by unfolding, which follows first-order reaction kinetics as already shown in deactivation. Also, since the particle size and enzyme activity are related to each other (Figures 2, 4, and 6), the particle size change during thermal deactivation (unfolding) may be modeled using the following equation:

$$d = d_0^{k_a t} \tag{7}$$

where d_o is the initial particle diameter, d is particle diameter at time t, and k_a is a rate constant. As also

shown in Figure 8, the particle size change follows eq 7, with $k_a = 0.082\ h^{-1}$, only for particle sizes smaller than 200 nm. Presumably, under this particle size most protein molecules are in either the unfolded (U) or folded (N) state. At larger particle sizes, significant amounts of protein are present as the aggregate (A), and the size increase did not follow first-order kinetics.

Aggregation is much more complicated kinetically than deactivation or denaturation. The aggregation of unfolded proteins has been shown to follow second-order reaction kinetics (Kiefhaber et al., 1991). A much more complicated model involving dissociation and hydrophobic interactions has also been proposed in a recent study of insulin aggregation (Sluzky et al., 1992). Various aggregation models have also been proposed from protein precipitation studies (Glatz, 1992; Przybycien & Bailey, 1989). In general, when aggregation is controlled by Brownian diffusion in the absence of a force field, the Smoluchowski second-order collision theory can be applied and the rate of disappearance of similarly sized particles is

$$\frac{dN}{dt} = -k_n N^2 \tag{8}$$

where N is number concentration. Equation 8 can be expressed in the following integrated form:

$$\frac{1}{N} - \frac{1}{N_0} = k_n t \tag{9}$$

When the total mass of the particles in solution remains unchanged, N can be related to the number-average particle weight (M) or effective diameter (d) as $N = N_0M_0/M = N_0d_0^m/d^m$. Equation 9 thus can be rewritten as:

$$d^m - d_0^m = CN_0M_0k_at \tag{10}$$

where C is a coefficient relating the particle size and mass. The parameter m is dependent on the shape or compactness of the particle, and $m = 3$ if the particle is a hard sphere. When d_0 is much smaller than d, a logarithmic plot of d versus t should give a line with its slope equal to m^{-1}. As shown in Figure 9, the size data fit well with this model. The slopes from these plots are different, suggesting that the aggregate structure obtained from different thermal aggregation conditions is also different. At 50 °C, $m \approx 1.2$, suggesting that the aggregate was not spherical. The larger aggregates (in the micrometer size range) showed elliptic and short rod shapes under a phase-contrast microscope.

Concluding Remarks

Very little is known about the structure of *A. oryzae* lactase, which is quite different from the relatively well-studied *Escherichia coli* lactase (Auersch et al., 1991; Shifrin & Steers, 1967; Wickson & Huber, 1969). This study provides useful information about the structure (size) of *A. oryzae* lactase under various conditions. The particle size measured with dynamic light scattering correlates well with the enzyme activity. DLS measurement also provides the necessary information to explain the deactivation of lactase due to extreme physical conditions, including very acidic pH and high temperatures. The pH and temperature stability determined by DLS compared very favorably with enzyme activity profiles. In addition, aggregation was observed at low salt concentrations, the so-called salting-in effect, and confirmed the salt performance predicted by the Hofmeister series. The addition of lactose in amounts equivalent

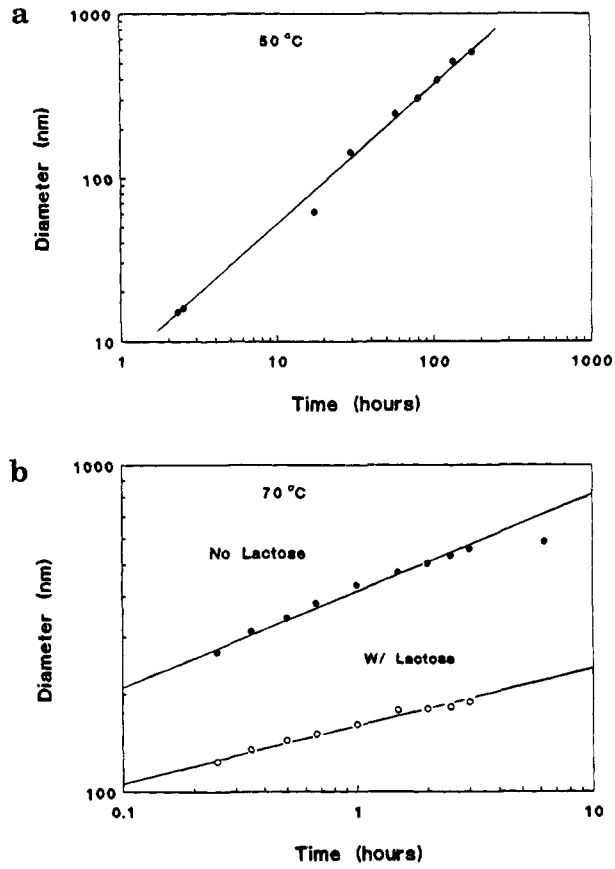

Figure 9. Kinetics of thermal aggregation of lactase at (a) 50 °C and (b) 70 °C.

to those of milk and whey had a significant stabilizing effect on the enzyme subject to extreme pH and temperature conditions. Finally, a large change in protein structure was observed as the urea concentration increased from 3 to 4 M. This size change profile is typical when the protein is unfolded by a chaotropic agent. DLS thus may be used to study protein folding kinetics.

It should be mentioned that the diameter measured for the native protein seems to be higher than expected. For a compact, globular protein with a molecular weight of 100 000, the particle diameter would be ~7 nm. The diameter of this protein has not been reported before. Estimation errors could be from the n and η values used in size calculation. Also, the effective diameter measured with DLS can be affected by the particle shape and might have been overestimated if a few large particles were present. The enzyme used in this study was not further purified and might contain a significant amount of impurity biopolymers that might have interfered with the DLS measurement. This possibility was checked with the CONTIN method in data analysis, which reports the size distribution of the sample. In general, a monodisperse size distribution was obtained from the native protein sample and the results from this method were consistent with those from cumulant method. Thus, there is no need to further purify the protein sample for DLS analysis. It should be mentioned, however, that the protein sample may be purified using liquid chromatography or gel electrophoresis, but the purified protien may be present in (partially) denatured form.

This study shows that the size information (more of the relative size, less of the absolute size) obtained from the DLS analysis gives useful indications of protein conformation (change) and its activity. The DLS technique presents little or no perturbation on the system investigated and yields both structural and dynamic information on small quantities of material. Since the

functional properties of a biologically active protein are largely dependent on its tertiary structure or conformation (size), DLS can be a useful technique in monitoring, either on-line or off-line, the structural and biological properties of a protein. Also, it may be used to replace some tedious bioassays in bioprocess research.

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