The intrinsic viscosity of β-lactoglobulin during proteolysis as the initial step of enzymed enduced gelation

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ABSTRACT

Partial enzymatic hydrolysis by a specific protease isolated from *Bacillus licheniformis* can induce gelation in whey protein, and measurements of the intrinsic viscosity provides information on the changes occuring before gelation, but must be supplemented by other methods in order to quantify these changes.

INTRODUCTION

Measurement of intrinsic viscosity, [η], has practical application when determining molecular weight of polymers. It can also be of use in characterizing the linear polymers, e.g induced by thermal denaturation, which occur prior to gelation in many food systems[1]. [η] can thus possibly provide information on the initial stages of food protein gelation, such as the reported enzyme induced gelation of whey protein[2].

THEORY

[η] is defined as the limiting value, at zero concentration, c, of the reduced viscosity, η_{red}:

$$\eta_{red} = \frac{(\eta_{rel} - 1)}{c}$$

$$[\eta] = \lim_{c \to 0} [\eta_{red}]$$

where η_{rel} indicate the relative viscosity.

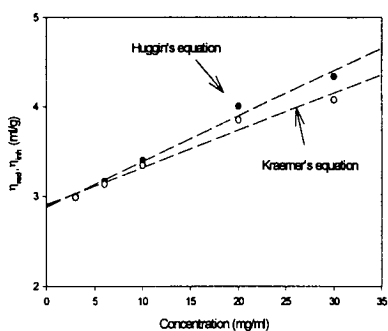In practice, [η] is found by measuring the viscosity at a number of concentrations and then extrapolate to a concentration of 0, using either Huggin's or Kraemer's equations (or both) in a double extrapolation plot.

Huggin 's equation : $\eta_{red} = [\eta] + k'[\eta]^2 c$

Kraemer 's equation : $\eta_{inh} = [\eta] + k''[\eta]^2 c$

η_{inh} is the inherent viscosity ($\ln(\eta_{rel})/c$), and k' and k" denote the Huggin's and Kraemer parameters respectively.

The theory connecting [η] with structure makes a number of assumptions concerning the shape and properties of the molecules involved. The simplest models relate [η] of flexible polymers to the relative molecular mass and the axial ratio of the molecules.

However, the polydispersity of molecular weight, the effect of chain branching and the complex hydrodynamics of semi-flexible chains makes it difficult to make deductions from measurements of [η] to molecular shape. It has nonetheless been possible to link changes in the intrinsic viscosity of bovine serum albumin with the persistence length and the diameter[1], but this requires the use of additional measurements, such as lightscattering in order to characterize the molecular weight distribution.

Figure 1: Double extrapolation plot showing Huggin's and Kraemer's plot for β-lacto-globulin.

MATERIALS AND METHODS

3% solutions of β-lactoglobulin (prepared according to reference 3) were made in 0.075 M tris-HCl buffer, pH 7.5. A serine protease from *Bacillus licheniformis*, specific for Glu-X and Asp-X bonds (obtained from Novo Nordisk A/S, Bagværd, Denmark) was added in an amount equal to an enzyme/substrate ratio of 0.01. The intrinsic viscosity was measured after 0, 5 and 10 minutes of hydrolysis at 50°C using a capillary viscosimeter (Y-501 IV from Viscotek, Houston, USA). Measurements were made at concentrations of 3, 6, 10, 20 and 30 mg/ml, and the intrinsic viscosity extracted from a double extrapolation plot, as illustrated in figure 1.

Samples were kept in ice until analysis, and measurements were performed at 30°C at a flow of ~ 1 ml/min, which is equivalent to an apparent wall shear rate of 160 sec^{-1} in the capillary.

RESULTS AND DISCUSSION

Results are summarized in figure 2, and show an increase in the intrinsic viscosity after 5 and 10 minutes of hydrolysis. Samples taken after 20 minutes could not be analyzed due to sedimentation. The increase in [η]
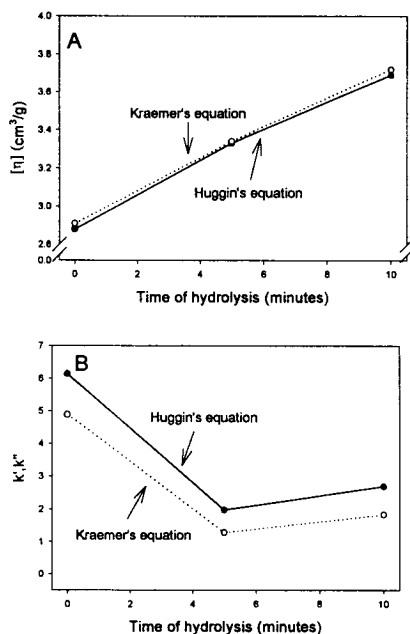

Figure 2: Intrinsic viscosity,[η] (A) and Huggin's and Kraemer's parameter (B) of β-lactoglobulin after different times of hydrolysis.

indicates protein unfolding and/or aggregation.

The Huggins and Kraemer parameters are independent of molecular weight, and the substantial decrease after 5 minutes of hydrolysis is perhaps as the result of formation of double molecules (or aggregates), as this can decrease these parameters in the presence of a good solvent[4].

CONCLUSION

Although measurements of intrinsic viscosity provides indication of changes (unfolding and/or aggregation) taking place in β-lactoglobulin in the initial steps of gelation, additional experiments and information on the molecular weight distribution (i.e. by light scattering, size ex-

clusion chromatography or sedimentation analysis) are necessary in order to quantify these changes.

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