Characteristics of thermal gels set from β-lactoglobulin hydrolysed to various degrees by a specific protease

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ABSTRACT

β-lactoglobulin was partially hydrolysed and subsequently heated to form gels. Partial hydrolysis led to coarser microstructure and higher water mobility in the gels. The total gel stiffness was at first slightly increased and then decreased with increasing hydrolysis.

INTRODUCTION

Previous studies have shown that partial enzymatic hydrolysis of whey proteins with a protease from *Bacillus licheniformis* leads to increased strength of gels set by subsequent heat treatment at neutral pH[1]. This effect is exerted through formation of aggregates[2,3]. Since β-lactoglobulin (β-LG) constitutes ap-proximately half of the protein in whey protein isolate, this protein may be largely responsible for the effect observed.

The purpose of the present study was to assess the effect of partial hydrolysis on thermal gelation of pure β-LG and on some properties of the resulting gels.

MATERIALS AND METHODS

Pure β-LG B (5% w/v) in Tris buffer, pH 7.5 was hydrolysed with immobilized enzyme for various times (0 to 6 h) at 40°C. The *Bacillus licheniformis* enzyme (from Novo Nordisk A/S) is specific for Glu and Asp residues.

Gelation was monitored by dynamic oscillation using a Bohlin VOR Rheometer. The temperature gradient was 50-80°C at 5°/min, 80° for 1 h, cooling to 25° at 2.5°/min. Gel time was recorded as the time when the phase angle remained below 45°. Total gel stiffness was measured as G* just after reaching 25°C.

Water mobility was determined by pulsed low resolution NMR. Microstructure was evaluated by transmission electron microscopy (TEM) as described in [3], but using a cacodylate buffer for postfixation.

RESULTS

During hydrolysis for 0-6 h by the immobilized enzyme, 0 to 30% of the β-LG was degraded (not shown). Partial hydrolysis had a significant influence on the gel time (not shown), essentially inversely proportional to the effect on the final gel strength (Fig. 1). The gel stiffness was markedly increased after 1 h of hydrolysis and decreased after longer hydrolysis times. The effect of partial hydrolysis on the gel stiffness of pure β-LG, however, was less than previously observed for whey protein isolate at pH 7.0 [1].

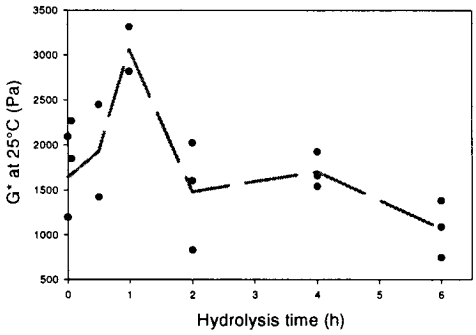

Figure 1. Complex modulus of gels made from β-LG hydrolysed by *Bacillus licheniformis* protease for various times.

Opposed to the effect on gel strength, there was a high linear correlation between the time of hydrolysis before gelation and the T_2-relaxation times of the resulting gels (Fig. 2). With increasing hydrolysis time the T_2 -values increased indicating an increased water mobility and a less tight water binding.

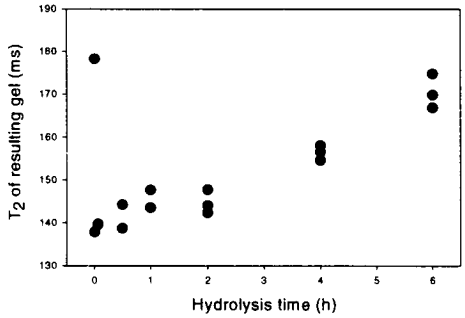

Figure 2. T_2 relaxation times of gels made from β-LG hydrolysed by *Bacillus licheniformis* protease for various times.

The decreased water binding as measured by NMR (Fig. 2) was correlated with a coarser microstructure of the gels from hydrolysed β-LG (Fig. 3). With increasing hydrolysis the protein strands became thicker and the pores wider.

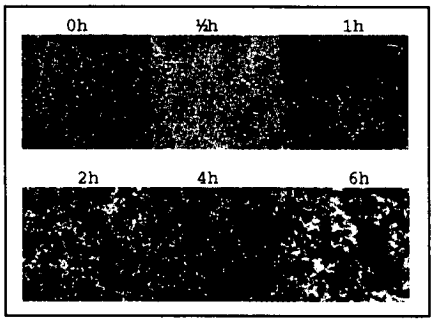

Figure 3. Microstructure of gels made from β-LG hydrolysed by *Bacillus licheniformis* protease for various times.

It is not immediately apparent from Fig. 3 why the gel made from β-LG hydrolysed for 1h was much stronger than the other gels.

CONCLUSIONS

- Partial hydrolysis of β-LG B before thermal gelation leads to first increased, and then decreased gel stiffness. The microstructure became coarser with decreased water binding.
- Pulsed low-resolution NMR appears to be a highly repeatable, quick and non-destructive method for detection of water mobility in whey protein gels.
- Differences in microstructure of gels made from partially hydrolysed β-LG can be revealed by TEM.

REFERENCES

1. Ju, Z.Y. et al. (1995), "Effects of limited proteolysis on gelation and gel properties of whey protein isolate", *J. Dairy Sci.* **78**, 2119-2128.
2. Otte, J. et al. (1996), "Protease-induced aggregation and gelation of whey proteins", *J. Food Sci.* **61**, 911-915+920.
3. Otte, J. et al. (1996), "Effects of limited proteolysis on the microstructure of heat-induced whey protein gels at varying pH", *J. Dairy Sci.* **79**, 782-790.