THE ACTION OF RENNIN ON β-CASEIN

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ABSTRACT

A study was made of the action of the enzyme rennin on β-casein. Hydrolysis of β-casein initially at a single sensitive bond under controlled conditions of temperature, pH and relative enzyme and substrate concentrations, formed the basis of the investigation. Information on the hydrolysis of this sensitive bond was gained from the isolation of a small peptide produced and from a study of the effect of several parameters on the rate of hydrolysis. Evidence obtained from electrophoresis and gel filtration allowed the assumption that attack on the sensitive bond resulted in a macropeptide and a small peptide of molecular weight about 2000. The small peptide was isolated and partially characterised. As a result it appears that the small peptide is derived from the C-terminal end of the β-casein molecule.

A polyacrylamide electrophoresis technique was used to study the effect of ionic strength and calcium ions on the rate of hydrolysis and the rate of appearance and disappearance of

degradation products at 10°, 25° and 37°C. It was found that an increase in ionic strength retarded the reaction and the addition of calcium ions at a constant ionic strength further retarded the reaction. Also, the rate of appearance and disappearance of degradation products was found to increase with increasing temperature. A development of the polyacrylamide technique into a quantitative one enabled the determination of the Michaelis constant at pH 6.50 and 37°C for the rennin hydrolysis of β-casein as 9.59 g/l. This technique was also used to study the rate of hydrolysis at pH 6.12, 6.50 and 6.94 where an optimum rate occurred at pH 6.50.

Finally, assuming that the small peptide is derived from the C-terminal end of the β-casein molecule and allowing for the sequential degradation elucidated by the temperature studies, alternative courses of rennin degradation of the β-casein molecule have been proposed.

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