Characterization of microbial transglutaminase

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Introduction

Transglutaminase catalyzes cross-linking of proteins by formation of isopeptide bonds (ε-(γ-Glu)Lys bonds) between protein-bound glutamine and lysine residues. Microbial transglutaminase (TG) from *Streptoverticillium sp.* is available as an additive for meat products to improve firmness. Here we present first data on the characterization of this enzyme.

Materials and methods

A “ready-to-use” preparation for the production of frankfurter type sausage („Activa WM“, AJINOMOTO Europe Sales GmbH, Hamburg) was used for the studies. Protein was determined by a modified method according to Bradford [1]. Results were calculated using the absorbance quotient of A_{590nm} and A_{450nm}. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli [2] on a gel with 14 % T (concentration of acrylamide and bisacrylamide) and 0,4 % C (concentration of bisacrylamide) at a constant current of 20 mA. Enzyme activity was determined by a colorimetric hydroxamate procedure described by Folk *et al.* [3].

Results and Discussion

Protein determination

The commercial available transglutaminase preparation consists of a sugar matrix in mixture with the enzyme to simplify dosage and processing. The determined protein content was only 0,25 %.

Molecular Weight Determination

On a 14 % SDS polyacrylamide gel 40 µg respectively 80 µg (0,1 µg and 0,2 µg protein) of the TG preparation were separated (Fig. 1) into two bands. The band at 10 kD was not identified, but its concentration was much lower than that of the main component showing a band at 42 kD. The latter value corresponds with the published molecular weight of transglutaminase.

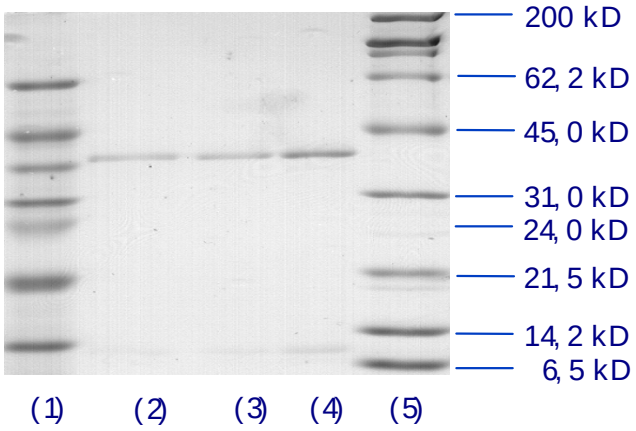

Figure 1: SDS-PAGE of the TG preparation for frankfurter type sausage on a 14% gel, stained with „Coomassie Brilliant Blue". (1) Molecular weight marker I; (2) molecular weight marker II; (3) 40 µg TG preparation; (4) 40 µg TG preparation; (5) 80 µg TG preparation; (6) molecular weight marker I; (7) molecular weight marker II

Activity Determination

The TG activity test consists of two reactions. First the enzyme catalyzes the reaction of carbobenzoxi-L-glutaminglycine and hydroxylamine to hydroxamic acid. Together with Fe(III) the hydroxamate forms in a second step a red-coloured complex, which was determined at 502 nm. (Fig. 2).

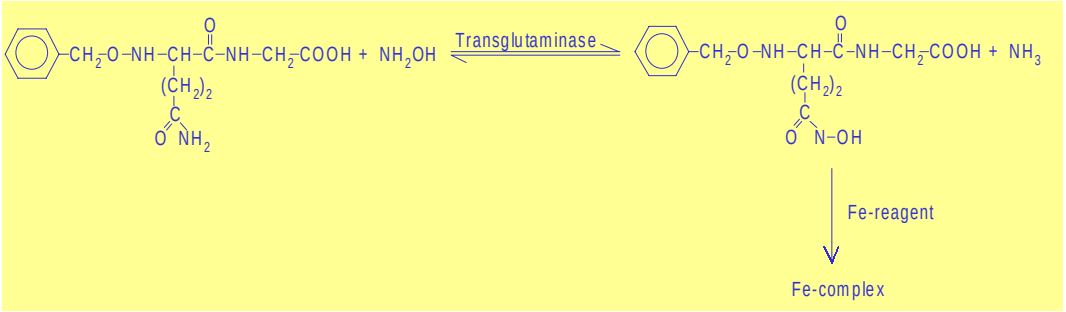

Figure 2: Reaction system for the determination of TG activity.

The Reaction rate was calculated from the quotient of the difference of the absorbance at 502 nm and a defined reaction time. The resulting reaction rates were plotted against substrate concentration (Fig 3). 1 ml test volume contained 250 µg of TG preparation (0,625 µg protein), 50 µmol hydroxylamine in 100 mM TRIS-buffer pH 8.0 at 37 °C. Incubation time for enzyme reaction was 10 minutes. Under this conditions a linear dependence between reaction rate and substrate concentration was measured between 0,5 µmol/ml and 5 µmol/ml.

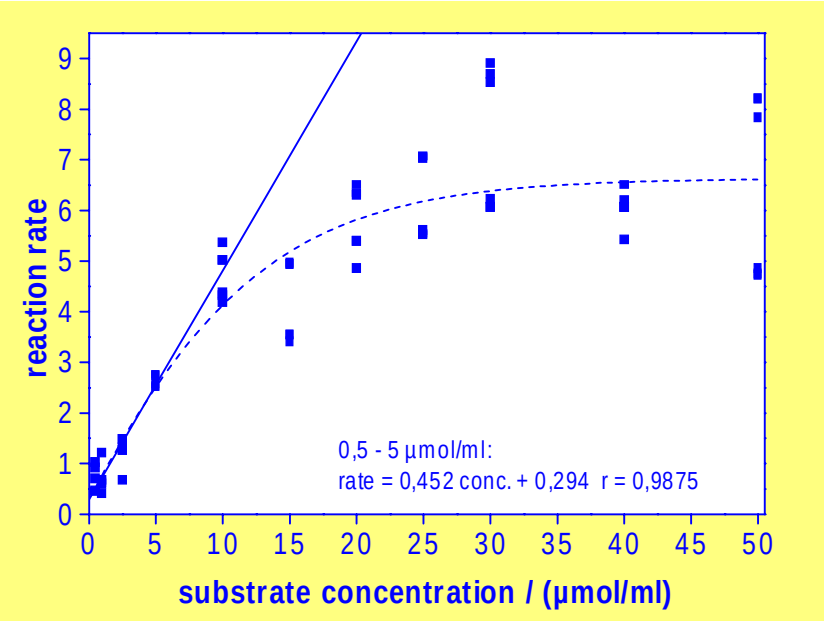

Figure 3: TG reaction rate in dependence of substrate concentration.

The influence of different parameters on TG activity e. g. temperature, pH, and salt concentration will be investigated in future experiments. The enzyme preparation will be characterized also by other kinetic data (V_{max}, Michaelis-Menten-Constant). The aim of further studies is to develop a method allowing the determination of TG activity in meat products.

References

1. Bradford, M. 1976, Anal. Biochem. **72**, 248-254
2. Laemmli, U.K. 1970, Nature **227**, 680-685
3. Folk, J.E., Cole, P.W. 1965 J. Biol. Chem. **240**, 2951-2960