The Synthesis of Cross-Linked Dextran and its Enzymatic Hydrolysis

Dextran with an approximate average molecular weight of 250,000 (Dextran 250) was cross-linked under alkaline conditions with 1,4-butandiol-glycidether. This gave an insoluble substance which becomes swollen on addition of water. A colour marker was then covalently attached. This substance could be hydrolyzed by the enzyme dextranase isolated from various sources. Hydrolysis yielded water-soluble blue dextran split products which were easily separated from the unhydrolyzed and water-insoluble blue dextran. The amount of enzymatically released water-soluble blue dextran oligosaccharides was, due to the attached blue marker, easily determined by measurement in a spectrophotometer. The release of blue dextran split products was found to be related to the activity of the dextranase used.

The preparation of cross-linked and coloured dextran was similar to that of blue starch polymer[1]. 20 g of Dextran 250 (Pharmacia Fine Chemicals) was solubilized in 100 ml of distilled water at 95 °C. After cooling to 25 °C, 5 ml of 10 *M* NaOH was added with constant stirring. During a 5-h-period, 3 ml of 1,4-butan-diolglycidether was added dropwise. The gel was then suspended in distilled water, neutralized with concentrated acetic acid and lyophilized. The ball-milled material was suspended in 325 ml of distilled water, 7.5 g of NaCl and 10 g of Cibacron blau F3 G-A was added. The suspension was mixed at 25 °C for 2 h and then 5 ml of 10 *M* NaOH was added and mixed for a further 2 h. The coloured gel was left at 4 °C for about 24 h and was then neutralized with acetic acid. After careful washing with distilled water, the polymer was dried, ball-milled and sieved. To the pre-incubated testtubes containing dextranase, 1 ml aliquots of blue dextran polymer suspended in 0.1 *M* potassium phosphate buffer, pH 6.0 (containing 0.02% sodium azide) were added. After a given time of incubation at 45 °C, the reaction was terminated by the addition of 0.5 ml of 0.5 *M* sodium hydroxide. The coloured supernatant was separated from the unhydrolyzed polymer by centrifugation. The extinction of the supernatant was determined in a Zeiss PMQ II spectrophotometer at 620 nm.

Figure 1 shows the enzymatic hydrolysis of the synthetic blue dextran by dextranase (Worthington, 150 U/mg protein) after various incubation periods. It can be observed that under the experimental conditions used, the hydrolysis of the synthetic substrate is a linear function of time of incubation up to 30 min. Thereafter the hydrolysis levels off, as the enzyme is probably no longer working under substrate saturation conditions. Figure 2 shows the hydrolysis of various amounts of blue dextran polymer by dextranase. The Michaelis-Menten type of curve is observed.

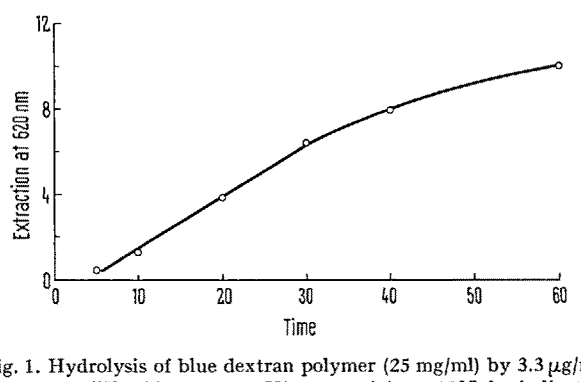

Fig. 1. Hydrolysis of blue dextran polymer (25 mg/ml) by 3.3 µg/ml dextranase (Worthington, 150 U/mg protein), at 45 °C for indicated time intervals.

The amount of 1,4-butandiolglycidether used for the cross-linking of dextran is critical. Higher amounts of diepoxide will yield rigid water-insoluble dextran polymers which are degraded by dextranase to a lower degree. On the other hand, lower amounts of diepoxide will not facilitate the synthesis of water-insoluble dextran gels.

A similar substance was previously synthesized for the determination of α-amylase, using soluble starch as starting material. Such a cross-linked and coloured insoluble starch material was successfully used for the determination of α-amylase activity in human fluids[2,3].

The water-insoluble dextran polymer prepared as described was found to be a very suitable substrate for dextranase of endo type. Using this substrate for the determination of dextranase activity offers a number of advantages compared to the saccharogenic method[4] or to methods using soluble coloured dextrans[5,6]. The present method is sensitive, very simple to perform and useful particularly in cases where a large number of samples have to be tested.

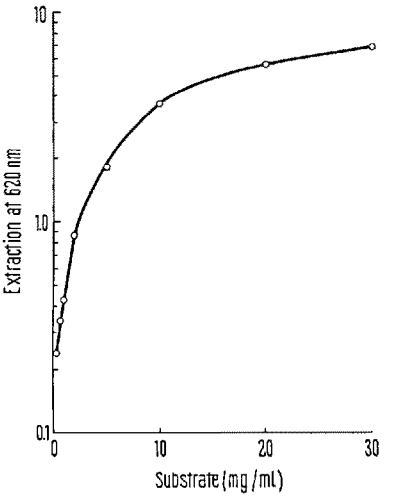

Fig. 2. Hydrolysis of various amounts of blue dextran polymer by 4.17 µg/ml dextranase (Worthington, 150 U/mg protein), for 30 minutes at 45 °C.

Zusammenfassung. Durch Vernetzung von Dextran mit Diepoxid wurden wasserunlösliche Substanzen synthetisiert, welche nach Farbstoffbindung mit Cibacronblau ein besonders geeignetes Substrat für das Enzym Dextranase ergaben.

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