TRANSFERASE ACTIVITY OF ENDO-β-1,6-GLUCANASES FROM THE CRYSTALLINE STALKS OF BIVALVE MARINE MOLLUSCS

L. A. Elyakova, V. Ya. Rudakova,
E. V. Sundukova, and V. V. Isakov

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It has been shown that endo-β-1,6-glucanases from marine molluscs perform a transglycosylation reaction. When o-nitrophenyl β-D-glucopyranoside (Np glucoside) was used as acceptor, among the newly formed products Np gentiobioside, -trioside, and -tetraoside with a total yield of up to 20% on the initial Np glucoside were detected.

We have previously [1] developed a method for obtaining a β-1,6-glucanase from the crystalline stalks of marine molluscs forming industrial wastes in the Far East, have studied some of their physicochemical properties, and have determined that their action is of the endo type. In the present paper we report the presence in this enzyme of a capacity for transglycosylation and we characterize the products obtained.

EXPERIMENTAL

The transglycosylation reaction was perofrmed under conditions optimum for the action of endo-β-1,6-gluconases: in 0.05 M acetate buffer, pH 5.0, with 0.1 M NaCl, using as a donor a β-1,6-glucan – pustulan, which we isolated from the lichen Umbellicaria rustica – and as an acceptor – o-nitrophenyl β-D-glucopyranoside (Np glucoside) in concentrations of 10 mg/ml each, in the presence of 0.008 U/ml of the enzyme. The reaction products obtained were separated first by gel filtration on Bio-Gel P-6 to eliminate high-molecular-weight reaction products and the excess of Np glucoside taken in the reaction, and then on Bio-Gel P-2 (400 mesh, column 1.5 × 130 cm) in the manner described in [2], the issuance of the aryl glucosides obtained being recorded from their absorption at 280 nm. Under these conditions, newly formed substances (peaks II and III, [1]) issuing from the P-2 column after the glucose, and also minor amounts of substances in peaks IV, V, and VI, were detected.

In the PMR spectrum (taken on a WM-250 spectrometer at 30°C in D_2O with methanol as internal standard, δ = 3.47 ppm relative to TMS) of compound (II) it was possible unambiguously to isolate the signals of the protons of the o-nitrophenyl residue: δ = 7.38, 7.60, 7.80, and 8.06 ppm. The observed chemical shift of the signals of an anomeric proton at δ = 4.56 ppm is characteristic for β-1,6-bound glucose residues [3]. The signals of the protons at anomeric C atoms – δ 5.38 ppm, J_{12} = 7.3 Hz, and δ = 4.56 ppm, J_{12} = 7.9 Hz – were present in an integral ratio of 1:1. These facts showed that the substance (II) under study was o-nitrophenyl β-gentiobioside.

It might be assumed that peaks (III, IV, V, and VI) corresponded to a series of higher homologues of Np gentiooligosides. In actual fact, when the values of $-\log K_d$ for substances (II), (III), (IV), and (V) were plotted against their assumed degrees of polymerization [4] a straight line was obtained the angle of slope of which was 0.08 (for comparison: the angle of slope of p-nitrophenyl laminarioligosides is 0.067) (Fig. 2). Consequently, the whole series of substances was homologous, and substances (III), (IV), and (V) were o-nitrophenyl tri-, tetra-, pentagentiooligosides, respectively.

So far as we know, the transglycosylation reaction characteristic for endo-glycanases has not been invesetigated in its application to endo-β-1,6-glucanases. The total yield of the products of transglycosylation by the endo-β-1,6-glucanases under the given reaction conditions amounted to 15-20% on the initial Np glucoside.

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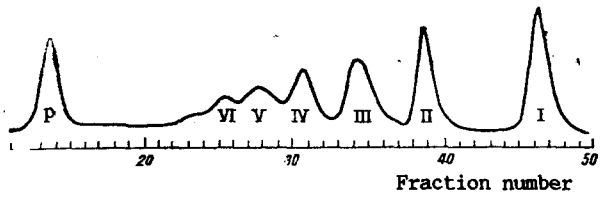

Fig. 1. Separation of the transglycosylation products on Bio-Gel P-2: I) initial Np glucosides; II) Np gentiobioside, etc; P – initial pustulan.

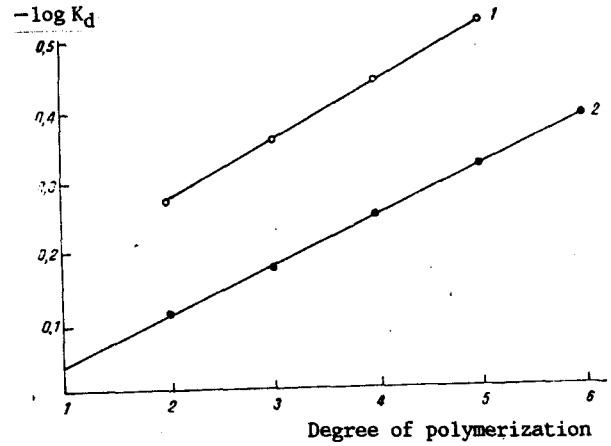

Fig. 2. Dependence of $-\log K_d$ on the degree of polymerization of the aryl oligosides (separation on Bio-Gel P-2): 1) series of o-nitrophenyl gentiobiosides; 2) series of p-nitrophenyl laminarioligosides, $K_d = \frac{V_e - V_0}{V_w - V_0}$ where V_e is the elution volume of the given oligomer, V_0 is the free volume of the column, and V_w is the volume of gel in the column.

The proposed simple method may be promising for the preparative production of aryl gentiooligosides, the usual route for the synthesis of which is fairly complicated [5] and requires a preliminary preparation of gentiooligosaccharides. The use of the newly obtained substances as colored substrates for β-1,6-gluconases as is done, for example, in the testing of amylases [6], is possible.

SUMMARY

It has been shown that endo-β-1,6-glucanases from marine molluscs perform the transglycosylation reaction. When o-nitrophenyl β-D-glucopyranoside was used as acceptor, among the newly formed products were detected Np gentiobioside, -trioside, and -tetraoside in a total yield of up to 20% on the initial Np glucoside.

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