β-MANNOSIDASE OF HUMAN TROPHOBLAST AND SKIN FIBROBLASTS

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A new hereditary disease of man connected with β-mannosidase deficiency has recently been discovered [4]. β-D-mannosidase is considered to be the chief enzyme involved in exoglycosidase degradation of oligosaccharide units of glycoproteins. A hereditary storage disease linked with β-mannosidase deficiency has been identified in animals [5], but the enzyme itself in animal tissues has virtually not been studied, and all that was known was the fact that the ratio of α-mannosidase to β-mannosidase activity is very high in most tissues. β-Mannosidase has been purified from marine mollusks [8] and the chicken oviduct [9]; its pH-optimum was found to be 4.5 (a broad peak from 4.0 to 5.0) and 4.6, respectively.

It was shown previously that most lysosomal hydrolases have high activity in the trophoblast, and for that reason they could be used for the early detection of a deficiency of lysosomal hydrolases [1, 7, 10]. No data on the level of β-mannosidase activity in the chorionic villi of the human fetus could be found in the literature.

The aim of this investigation was to study the properties of human trophoblastic β-mannosidase and to compare them with those of the enzyme from skin fibroblasts, using a synthetic fluorogenic substrate.

METHODS

The substances used and their sources were as follows: aqueous extracts of chorionic villi and of a culture of human skin fibroblasts (HSF); 4-methylumbelliferyl-β-D-mannopyrazide (4-MUP) was synthesized and generously provided by Senior Scientific Assistant Ya. M. Voznyi; 4-methylumbelliferyl-α-D-mannopyranoside was obtained from Koch-Light (Great Britain), Triton X-100 from Merck (Germany).

An aqueous homogenate of chorionic villi (8th-10th weeks of pregnancy), obtained after termination of pregnancy on medical grounds, was prepared as described previously [2]. HCF from healthy donors and a patient with α-mannosidosis were obtained from the cell bank of the Institute of Molecular Genetics, Academy of Medical Sciences of the USSR. The cells were grown in Eagle's medium with a 10% mixture of bovine and umbilical serum (1:1), 0.03% L-glutamine, and 100 U/ml of antibiotics. Monolayer cultures were used for determination. The aqueous extract of HSF was prepared as described previously [3].

The incubation mixture for determination of β-mannosidase activity contained 5 μliters of enzyme preparation (25-65 μg protein), 2 μliters Triton X-100 (final concentration 0.1%), and 18 μliters of a 2.5 mM solution of substrate, made up in 0.15 M phosphate—citrate buffer, pH 4.67. The samples were incubated for 1 h at 37°C. The reaction was stopped by the addition of 0.4 M glycine buffer, pH 10.4 (to a final volume of 4 ml) and fluorescence was measured on a BIAN-130 fluorometer. Activity of β-mannosidase was expressed in mmoles of substrate degraded per hour per milligram protein. α-Mannosidase activity was determined as described previously [1]. Protein in the homogenates was determined by Lowry's method [6].

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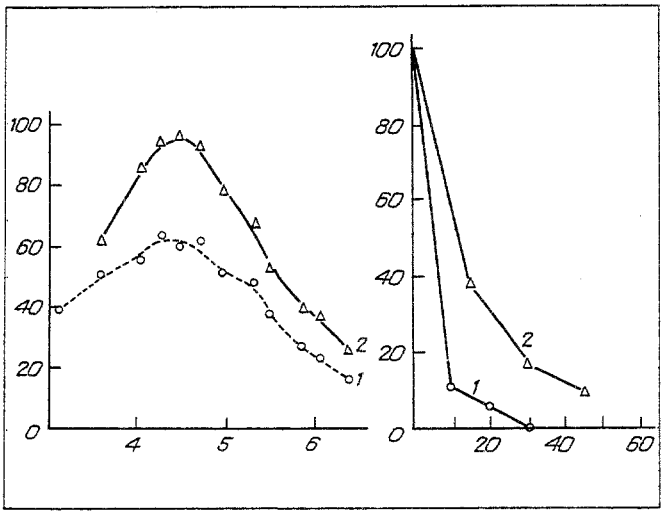

Fig. 1 Fig. 2

Fig. 1. Effect of pH on β-mannosidase activity of human trophoblast (1) and skin fibroblasts (2). Abscissa) pH; ordinate) specific activity (in mmoles 4-MUP/mg protein·h).

Fig. 2. Thermal inactivation of β- (1) and α-mannosidase (2) of human trophoblast at 56°C. Abscissa) duration of preincubation at 56°C (min); ordinate) enzyme activity (% of initial value).

TABLE 1. β-Mannosidase and α-Mannosidase Activity (in mmoles 4-MUP/mg protein·h) in Human Trophoblast and Skin Fibroblast Culture

Enzyme	Trophoblast	Fibroblasts
β-mannosidase	77,7 (58,8—115,1)	95,7 (76,2—156,4)
α-mannosidase	150,0 (57,0—244,1)	155,9 (66,0—265,7)

Parameters of the Michaelis—Menten equation were found by minimization of the sum of the squares of deviations by Markwardt's method by microcomputer.

RESULTS

As already stated, β-mannosidase in human and animal tissues has virtually not been characterized. All that is known is that activity of this enzyme in mammals is very low, many times less than α-mannosidase activity. It was first necessary, therefore, to determine total β-mannosidase activity in the chorionic villi and to compare it with activity of the enzyme in HSF. We found that β-mannosidase activity must be determined under the same conditions as the majority of other lysosomal hydrolases, i.e., in aqueous tissue homogenates, and the nonpolar detergent Triton X-100 in a final concentration of 0.1% was used as the solubilizing agent. However, Triton X-100 has a weak effect on β-mannosidase activity of aqueous homogenates of the chorion and HSF. The increase in activity under our conditions was not more than 15%. Table 1 gives β- and α-mannosidase activity in human chorionic villi and HSF. Trophoblastic β-mannosidase has quite high activity, virtually the same as its activity in HSF. It is important to emphasize that β-mannosidase activity in the tissues studied differs only a little from their α-mannosidase activity. Moreover, high β-mannosidase activity of HSF under normal conditions is a characteristic feature of that enzyme, for β-mannosidase activity has values of the same order in a preparation of HSF from a patient with α-mannosidosis (α-mannosidase deficiency).

Under our chosen experimental conditions of measurement of β-mannosidase activity the reaction velocity in the investigations was linear for 120 min within the protein concentration range up to 70 mg.

Investigation of the effect of pH on β-mannosidase activity in the chorionic villi and HSF showed that the shape of the curve of reaction velocity versus pH for the enzyme was the same, with a wide pH-optimum within the 4.2-4.7 interval (Fig. 1). The value of the pH-optimum obtained for β-mannosidase of chorionic villi and HSF, determined with the aid of the synthetic fluorogenic substrate, was close to the value of the pH-optimum of 4.5 and 4.6, found with a synthetic nitrophenyl substrate for the enzyme in marine mollusks [8] and the chicken oviduct [9]. However, it differed a little from the pH-optimum of 5.0 described for β-mannosidase of goat kidney [5].

The reaction velocity within the concentration range of substrate from 0.37 to 3.50 mM is described by a saturation kinetics, with KM = 0.66 mM for β-mannosidase of chorionic villi, which was close to the value of KM for HSF (0.62 mM).

The β-mannosidase of human chorionic villi and HSF was found to be sufficiently stable in an aqueous tissue homogenate and it preserved its activity for 2 weeks when kept at 4°C. However, heat treatment of the chorionic enzyme at 56°C for only 10 min led to virtually complete loss of its activity. As Fig. 2 shows, chorionic β-mannosidase was more sensitive to heat treatment than the α-mannosidase from the same tissue. Closely similar results were obtained in a study of the effect of preincubation at 55°C on the enzyme from mollusks [8].

According to [9], Zn^{2+} stabilizes α-mannosidase activity but does not affect β-mannosidase activity from chicken oviduct. It was found that Zn^{2+} ions have virtually no effect likewise on activity of this enzyme from human chorionic villi: in the presence of 1 mM $ZnCl_2$, β-mannosidase activity was 94% of its initial value.

Like the α-mannosidase from various tissues, trophoblastic β-mannosidase binds completely with concanavalin A-sepharose.

Like other lysosomal hydrolases, β-mannosidase of chorionic villi thus exhibits its activity in the early stages of embryogenesis (the first term of pregnancy), its activity is quite high and virtually indistinguishable from β-mannosidase activity in the postnatal period, and it has values of the same order of magnitude as the α-mannosidase from various human and animal tissues. The high activity, and also the close similarity between the properties of trophoblastic β-mannosidase and the enzyme from human skin fibroblasts leads to the conclusion that biopsy material from the chorion can be used for the antenatal diagnosis of hereditary β-mannosidase deficiency.

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