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MORQUIO DISEASE, TYPE B: ACTIVATION OF G_{M1}-β-GALACTOSIDASE BY G_{M1}-ACTIVATOR PROTEIN

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SUMMARY: Residual G_{M1}-β-galactosidase activity of Morquio B fibroblasts increases from 4 % to at least 20 % of the respective normal value in the presence of partially purified G_{M1}-activator protein. The activator does not lead to a stimulation of keratan sulfate-degrading β-galactosidase. There is no significant difference between normal and Morquio B fibroblasts in the rates of synthesis of pro-β-galactosidase (M_r = 85.000) and in conversion to a mature form (M_r = 68.000). The absence of neurological symptoms in Morquio B patients may therefore be explained by a specific activation of G_{M1}-β-galactosidase *in vivo*.

Acid β-galactosidase (EC 3.2.1.23) is a heterocatalytical enzyme liberating D-galactose from a variety of synthetic and naturally occuring β-D-galactosidases including G_{M1} ganglioside (1), lactosyl ceramide (2,3), glycoproteins, and keratan sulfate (4,5). Inborn diseases caused by its inactivity exhibit a great phenotypic variability:

In the infantile and in the less severe juvenile type of G_{M1} gangliosidosis the patients suffer from neurological deterioration mainly due to glycolipid accumulation as well as from dysostosis multiplex due to glycoprotein and keratan sulfate accumulation (6). In other patients neurological symptoms predominate (7,8). A further group of patients shows no psychomotor deterioration, but all signs of keratan sulfate accumulation (9-12). In order to indicate their resemblance with a disorder caused exclusively by an impaired

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degradation of glycosaminoglycans, these patients were classified as suffering from Morquio disease, type B.

In two such cases the residual activity of β-galactosidase against aryl β-D-galactosides, G_{M1} ganglioside, keratan sulfate and asialofetuin was depressed to a similar extent when measured under standard assay conditions. The mutant enzyme behaved as a K_m variant towards p-nitrophenyl-β-D-galactoside (9) and showed an increased aggregation tendency at pH 7.0 (13). Nevertheless, the properties of mutant β-galactosidase could not be correlated with the phenotypic expression of the disease.

In the present paper we will show that Morquio dissease, type B, does not result from reduced synthesis, anomalous processing or increased catabolism of β-galactosidase protein. Its residual activity towards G_{M1} ganglioside, however, can be increased 5-fold by G_{M1}-activator protein. G_{M1}-Activator is claimed to act in vivo by forming stoichiometric complexes with G_{M1} ganglioside, thus allowing the watersoluble enzyme to interact with its glycolipid substrate (14).

MATERIALS AND METHODS

Skin fibroblasts were obtained from two patients with Morquio disease, type B (9) and from a patient with typical symptoms of infantile G_{M1} gangliosidosis. These and control cell lines were maintained in culture as described (15). Fibroblast homogenates for protein (16) and enzyme activity determinations were prepared from confluent cultures.

The preparation of [^{3}H]galactose-labeled G_{M1} ganglioside and of [^{3}H]galactose-labeled keratan sulfate as well as the conditions for measuring β-galactosidase activities against these and synthetic substrates have been described (17). G_{M1}-Activator protein was purified approximately 240-fold with 18 % yield from boiled human liver homogenate according to Li and Li (14) by two consecutive cycles of chromatography on DEAE-Sephadex A-50 and CM-Sephadex C-50, respectively. The activator was heterogenous in charge (14). Only protein peaks containing the bulk of activity were subjected to further purification. Purification was followed by determining the amount of protein required to achieve the same stimulation of G_{M1}-β-galactosidase as caused by 14 mM taurocholate.

An antiserum against β-galactosidase purified from human liver (17) was raised in guinea pigs. 36 µg of enzyme protein (specific activity 8.3 U/mg) in complete Freund's adjuvans were given to each animal intradermally. After injecting the same amount of protein in

incomplete Freund's adjuvans on days 21, 42, and 53, the animals were bled on day 63. The antiserum was diluted with an equal volume of 0.01 M Tris/HCl buffer, pH 7.0, containing 0.15 M NaCl, and dialyzed against the same buffer.

Labeling of fibroblasts with L-[4,5-^{3}H]leucine and preparation of cell and medium extracts was done according to the protocol of Hasilik and Neufeld (18). For immunoprecipitation, 250 µl of cell or medium extracts were mixed with 30 µl (25 mU) carrier β-galactosidase, 30 µl antiserum, and 103 µl of a solution containing 4 % Triton X-100, 1.6 M KCl and 0.4 M Tris/HCl buffer, pH 7.4. The mixtures were incubated for 1 h at 37° C and for 48 h at 4° C. Collection of immunoprecipitates, reduction, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, fluorography and molecular weight determinations were performed as described (18).

RESULTS

Pulse-chase labeling of β-galactosidase

β-Galactosidase is synthesized at similar rates by normal and Morquio B fibroblasts as a species of molecular weight of 85,000

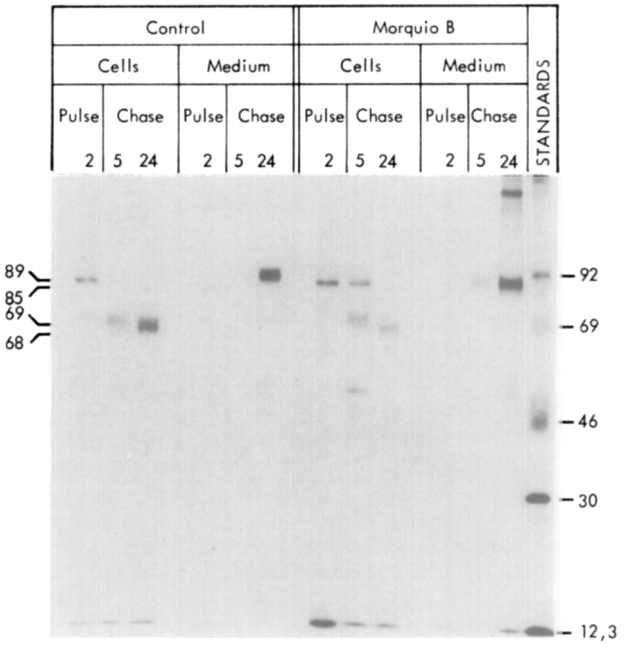

Fig. 1: Pulse-chase labeling of normal and Morquio B-β-galactosidase

Normal and Morquio B fibroblasts grown to confluency in three 75 cm^2 flasks each, were labeled with 0.2 mCi [^{3}H]leucine for 2 h. One flask each was then harvested; others were supplemented with non-radioactive medium for additional 5 h and 24 h, respectively. Mobility of molecular weight standards in thousands is indicated on the right margin.

(Fig. 1). Within 24 h intracellular pulse-labeled β-galactosidase is completely converted to a protein of M_r = 68,000 that remains stable for at least 53 h (not shown). An intermediate form of M_r = 69,000 is seen after chase periods of 5 h or 7 h. [^{3}H]Leucine-labeled β-galactosidase was not secreted into the culture medium during a pulse period of 2 h. During chase periods of up to 24 h increasing amounts of a protein of M_r = 89,000 could be immunoprecipitated from the medium. In several experiments no significant differences in the labeling pattern of normal and Morquio B fibroblasts were found. It was therefore concluded that the mutant enzyme was of normal stability and synthesized, processed and secreted at a normal rate.

Activation of G_{M1}-β-galactosidase by G_{M1}-activator protein

β-Galactosidase activity against G_{M1} ganglioside is usually measured in the presence of taurocholate. At a 14 mM concentration of the detergent both normal and Morquio B-β-galactosidase was optimally active (results not shown). To measure β-galactosidase under more physiological conditions taurocholate was replaced by partially purified G_{M1}-activator protein. As seen in Fig. 2, addition of G_{M1}-activator results in a dose-dependent increase of β-galactosidase activity. At the highest activator concentration tested the normal enzyme was 2.5-fold, but the mutant enzyme 20-fold more active than in the presence of the detergent. Similar results were obtained with fibroblasts from a second Morquio B patient.

Under the same conditions, homogenates of G_{M1} gangliosidosis fibroblasts exhibited neither in the presence of 14 mM taurocholate nor in the presence of activator (0.75 mg/ml) measurable G_{M1}-β-galactosidase activities. Keratan sulfate-β-galactosidase activity of normal fibroblasts was slightly reduced by addition of 0.75 mg

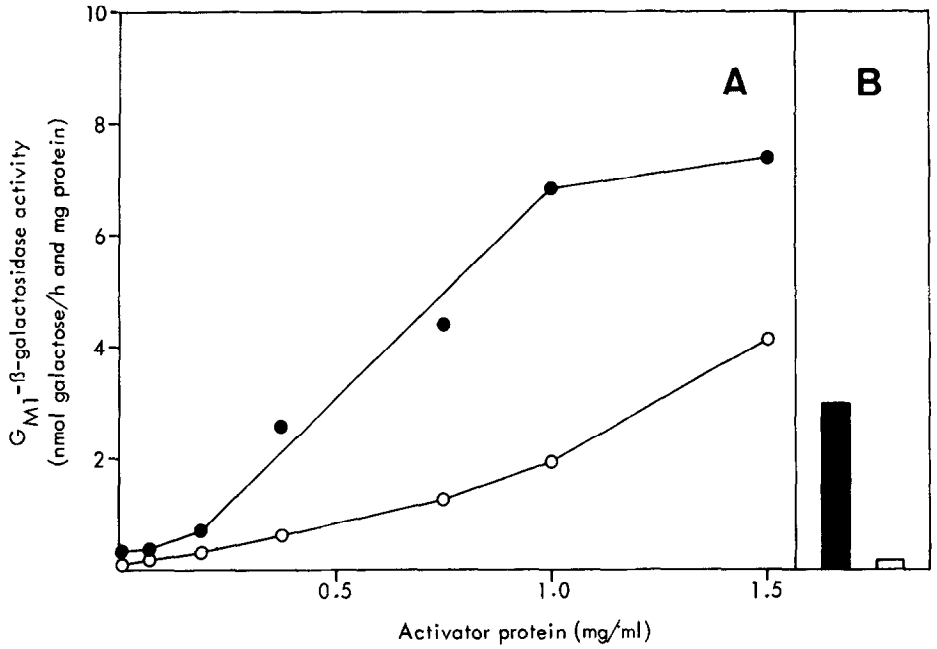

Fig. 2: A: Influence of activator protein on the activity of G_{M1}-β-galactosidase of normal (●) and Morquio B fibroblasts (o)

15 µg of cell protein were incubated with 3.2 nmol G_{M1} ganglioside for 60 min. Total protein concentration was kept constant by addition of bovine serum albumin.

B: G_{M1}-β-D-galactosidase activity of normal (black column) and Morquio B fibroblasts (open column) in the presence of 14 mM taurocholate instead of activator protein.

activator/ml, whereas that of Morquio B fibroblasts remained unchanged.

The stimulatory effect of G_{M1}-activator protein on β-galactosidase activity (Fig. 2) had been determined under conditions where the enzyme reaction did not follow zero order kinetics. Enzyme was therefore incubated with increasing amounts of G_{M1} ganglioside and activator at a constant molar ratio (Fig. 3). After converting the curves according to Lineweaver and Burk the following values were calculated: V_{max} of normal β-galactosidase increased from 6.6 to 121 nmol/h and mg protein after replacing taurocholate by activator; for the Morquio B enzyme an increase from 0.25 to 27 nmol/h and mg protein was found. Normal and mutant enzymes exhibited similar

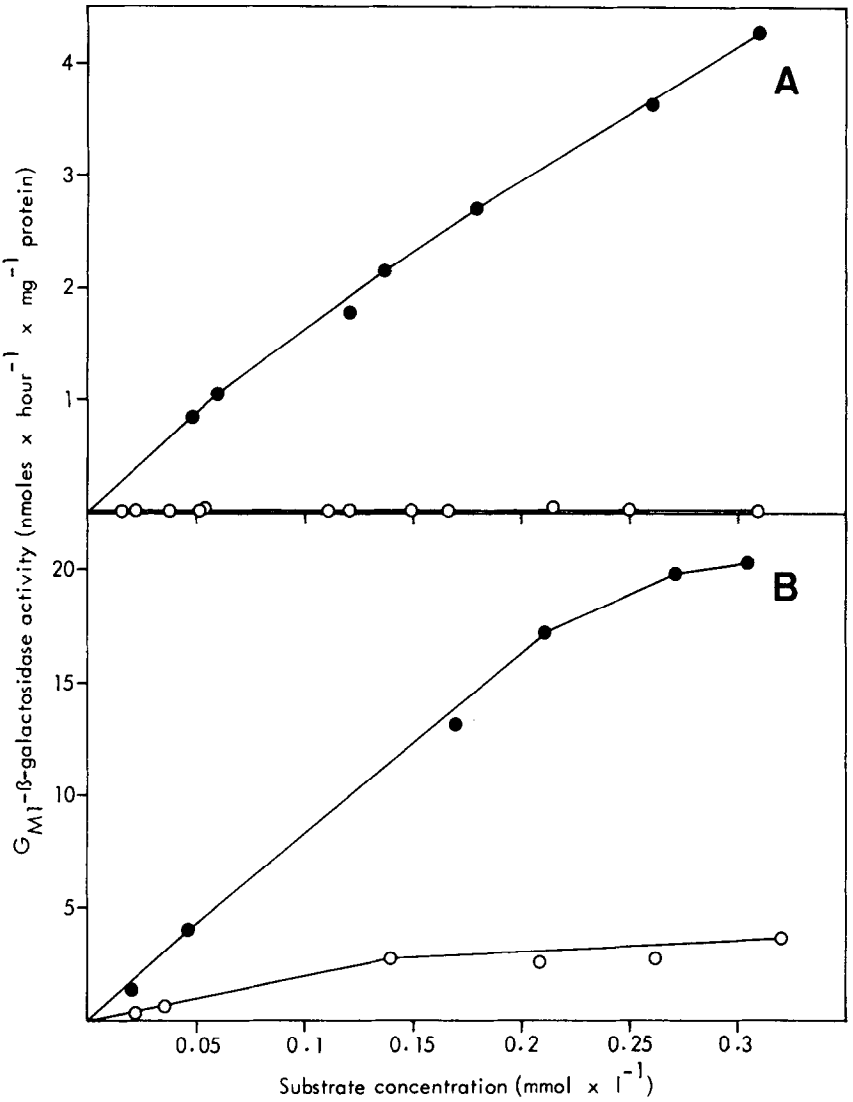

Fig. 3: Effect of substrate concentration on G_{M1}-β-galactosidase activity of normal (●) and Morquio B (○) fibroblasts in the presence of 14 mM taurocholate (A) or activator protein (B)

15 µg cell protein were incubated for 2 h. A constant ratio of 100 µg of partially purified activator protein/ nmol G_{M1} ganglioside was used. Total protein concentration was kept constant by addition of bovine serum albumin.

apparent K_m-values of approximately 0.25 mmol/l and 1.5 mmol/l in the presence of taurocholate and activator, respectively.

DISCUSSION

From our results and those of others (19) β-galactosidase must be considered as a further example of a human lysosomal hydrolase

that is synthesized as a precursor of higher molecular weight (18). Similar findings have been reported for β-galactosidase from mouse macrophages, the proenzyme having an M_r = 82,000, the mature form an M_r = 63,000 (20). For reasons not yet investigated, human β-galactosidase differs from most other lysosomal enzymes, since the intracellular proenzyme has a somewhat smaller size than the protein appearing in the medium.

As normal and Morquio B fibroblasts did not differ significantly in the metabolism of β-galactosidase protein, Morquio disease, type B must be due to a β-galactosidase protein of reduced catalytical activity. The residual activity of this protein towards G_{M1} ganglioside is enhanced in the presence of G_{M1}-activator protein from 4 % to 22 % of the activity of a normal control. Since keratan sulfate-β-galactosidase was not influenced by the activator, it seems unlikely that the effect was caused by an increased stability of the mutant enzyme. Similar activation of residual β-galactosidase activity was found with the urinary enzyme from one of the patients.

On the basis of our findings one might speculate that the absence of neurological symptoms is caused by a specific, though not yet explainable activation of G_{M1}-β-galactosidase in vivo by the activator protein. However, the concentrations of G_{M1} ganglioside and activator in the tissues of the patients are not known. If G_{M1}-activator functions in a similar manner as G_{M2}-activator (21), the formation of stoichiometric complexes of activator and ganglioside would be a prerequisite for intralysosomal ganglioside degradation. Activation of residual G_{M1}-β-galactosidase could then be of physiological significance.

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