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ALPHA GLUCOSIDASES IN WHITE BLOOD CELLS, WITH REFERENCE TO THE DETECTION OF ACID α 1-4 GLUCOSIDASE DEFICIENCY

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SUMMARY. The isozymes of α glucosidases have been studied in human white blood cells by immunologic and electrophoretic techniques. Three isozymes have been found in leucocyte extracts :
a) Lysosomal α glucosidase (acid maltase, EC 3.21.3)
b) Cytoplasmic neutral α glucosidase and
c) An enzyme immunologically identical to renal maltase (EC 3.2.1.20).
In Pompe's disease and other types of acid maltase deficiency, the deficiency is as complete in leucocytes as in other tissues, and the residual activity is due to "renal" maltase.

INTRODUCTION

The detection of enzymatic deficiencies in lysosomal diseases is generally performed on extracts from white blood cells, which express all these lysosomal enzymatic activities. Difficulties, however, are encountered in the diagnosis of the deficiency of α glucosidase (amylo 1-4 α glucosidase, or acid maltase, EC 3.2.1.3), which is responsible for glycogen storage disease, type II (1). A residual activity at pH 4 is generally found, so that the reliability of the use of leucocytes is questioned, especially in the late form of the disease (2-9). This makes it necessary to resort to more complicated techniques in order to ensure the diagnosis, e.g. muscle biopsy or cultured fibroblasts obtained after a skin biopsy. The residual activity in white cell extracts may be due to two different mechanisms : a) The gene for acid maltase, while being inactive in most tissues, might be expressed in leucocytes : b) White blood cells might contain another type of α glucosidase, that would possess some activity at acid pH ; such an activity is known to be present in amniotic fluid (5, 10) and in kidney extracts (5).

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In the present work we demonstrate that : 1) an additional isozyme of α glucosidase is actually present in white blood cell extracts 2) this isozyme is immunologically identical to the specific "renal maltase" 3) the "renal maltase" isozyme accounts for the residual α glucosidase activity at pH 4 in leucocyte extracts from deficient patients.

MATERIAL AND METHODS

MATERIAL

Human tissues were obtained either by biopsy or on autopsies performed less than 8 hours after death. Leucocytes were prepared as previously described (11). Mononuclear and polymorphonuclear cells were separated by a gradient technique (11). Cultured fibroblasts were obtained from Drs A. and J. Boué and Drs M.C. Meienhofer and C. Guillouzo. Amniotic fluid was obtained by amniocentesis at 17 weeks of pregnancy (Dr R. Henrion). Three patients were available during the course of the study. Patient 1 was a 5 month infant with a typical picture of Pompe's disease. The two others were adults (ages 30 and 50) with myopathic symptoms (Dr M. Fardeau).

METHODS

Extraction medium was distilled water containing 0.1% Triton X 100. Tissues were ground in a Potter-Elvehjem apparatus, and the homogenates were centrifuged at 10,000 g for 10 minutes. Cells were extracted by four cycles of freezing and thawing, and centrifuged.

Enzyme determination. We employed essentially the fluorimetric technique, using 1 mM 4-methyl umbelliferyl α-glucoside (Koch-Light) as a substrate, as described (12). Fluorescence was read in an Aminco-Bowman spectrofluorimeter (excitation 366 nm, emission 446 nm). The pH activity curves were made in 0.04 M citrate-phosphate buffer, pH 3.5 to 7.0.

Electrophoresis was performed on Cellulose acetate strips (Cellogel, Chemotron) as described earlier (13). Staining took place at pH 4.0. After development of fluorescence the spots were visualized in a UV cabinet and photographs were taken with a Polaroïd camera.

Immunologic techniques. Two antisera that had been raised in rabbits against human enzymes were used.
a) An antiserum prepared against purified acid maltase, in the form of purified γ globulin was kindly donated by Dr Tager (Jansen Institute, Amsterdam, Nederland).
b) An antiserum prepared against pure renal maltase (14, 15), kept as whole serum, was kindly donated by Drs de Barlet and Sudaka (Faculté de Médecine, Nice, France).

We used the immunoprecipitation technique since these antisera are only partly inhibitory. Complete precipitation of the antigen-antibody complex was ensured by the addition of polyethylene glycol (PEG) to a final concentration of 5%, which had no effect on the activity of the enzyme itself. Extracts were incubated with antiserum or normal serum overnight at 4°. After addition of PEG they were left at 4° for one hour, then centrifuged at 10,000 g for 15 min. The supernatant was used. In the presence of PEG a straight line was obtained for the decrease of enzyme activity in supernatants when increasing amounts of antiserum were added. In the absence of PEG small amounts of antiserum showed no effect.

RESULTS.

Effect of anti acid maltase antiserum.

Three types of effects could be observed when an extract was treated with an excess of antiserum and assayed at pH 4.0.

a) In most tissue extracts the immunoprecipitation was complete and no activity remained. These tissues included : liver, muscle, heart, brain, placenta, spleen, cultured fibroblasts and cultured amniotic cells.(fibroblastic type)

b) In amniotic fluid and extracts from small intestine the antiserum showed no effect.

c) A decrease with persistence of residual activity was observed only in kidney and white blood cell extracts. When a separation of leucocytes was effected, both mononuclear and polymorphonuclear cells showed the same phenomenon. No attempt was made to further separate lymphocytes from other mononucleated cells.

Effect of anti renal maltase antiserum.

This antiserum had no effect at any pH on any extract except those from kidney and leucocytes. The combination of the effect of the two antisera on leucocyte extracts is shown of fig. 1. Curve 1 shows the pH activity curve of the crude extract. It shows a broad maximum around pH 6. Curve 2 shows the difference between this curve and that obtained after addition of anti acid maltase antiserum ; this curve corresponds to the classical pH activity curve of acid maltase, with a maximum at pH 4 and a residual activity of about 10% at a neutral pH. Curve 3 shows the difference between curve 1 and that obtained after addition of anti renal maltase antiserum. This pH activity curve of renal maltase displays a broad maximum at pH 6, and is, therefore a "neutral maltase". Its activity at pH 4, however, amounts to more than 50% of that found at pH 6. This pH activity curve is similar to that described by de Burlet and Sudaka for pure human renal maltase (15). Finally curve 4 pictures the residual activity of the extract after addition of both antisera. Its maximum is also at pH 6.0 but no activity is found below pH 5.0.

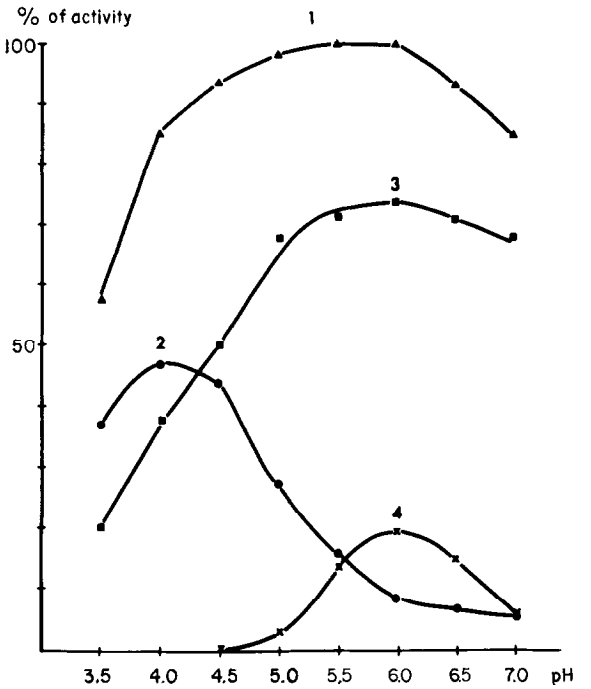

Fig. 1 : pH activity curves of α glucosidases in a leucocyte extract.

Curve 1	▲——▲	Crude extract
Curve 2	●——●	Activity removed by anti acid maltase antiserum
Curve 3	■——■	Activity removed by anti renal maltase antiserum
Curve 4	x——x	Residual activity after combined action of the two antisera.

This is the typical pH activity curve of the cytoplasmic neutral maltase. The same residual pH activity curve is found after treating, for example, extracts from liver or fibroblasts with anti acid maltase antiserum (not shown).

The percentage of renal maltase activity at pH 4 can be evaluated by residual activity of the extracts after addition of anti acid maltase antiserum. This percentage was measured on 10 leucocyte extracts and was found to be 37% as a mean, with values ranging from 20 to 52%. Quite analogous results were obtained with kidney extracts. The percentage of the two activities at pH 4.0 was similar in whole kidney and leucocyte extracts.

From the above data it appears that the overall activity at acid pH is the result of the combined action of "acid" and "renal" maltases, while the activity at neutral pH results from the combined action of "renal" and "neutral" maltases. The ratio of activity pH 4.0/6.5 has been computed from 80 leucocyte extracts from various control patients seen during the last two years. The mean is 0.60 with a standard deviation of ± 0.13 and an overall range 0.37-0.95. During the course of this study three patients with a deficiency in α glucosidase were examined. The general results are summarized in fig. 2. The patients showed activity ratios of 0.48, 0.40 and 0.28. Only the last one showed a ratio outside the limits of the controls (fig. 2 A).

The effects of anti acid maltase antiserum were assayed in two patients (fig. 2 B). Contrasting with residual activity of 20-52% in the controls, 90% of initial activity remained present in the patients'extracts, showing the very low activity of acid maltase in these patients. The effects of antirenal maltase antiserum were evaluated in three controls and one patient : residual activity was 56, 60 and 72% in the controls, against less than 10% in the patient. This confirmed that in the patient the residual activity at pH 4 was almost completely due to renal maltase.

Electrophoresis. Cellogel electrophoresis showed only one band of acid glucosidase in extracts from most tissues, as described previously (16). Fig. 3 compares the results obtained by staining at pH 4 of extracts from placenta, normal leucocytes and deficient leucocytes. Placenta is taken as a model of an organ devoid of renal maltase. The activity (line 1) disappears completely after incubation with anti acid maltase antiserum (line 2). In normal leucocyte extracts, the activity is diminished but not abolished by each of the two antisera. The two isozymes are not completely separated but it can be seen from lines 4 and 5 that the renal isozyme runs slightly faster. Deficient leucocyte extracts are unaffected by anti acid maltase antiserum, while the spot disappears completely after incubation with anti renal maltase antiserum.

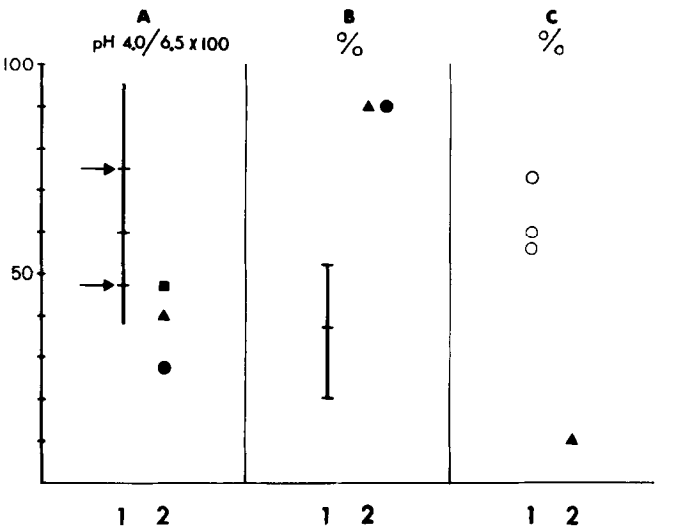

Fig. 2 : Properties of α glucosidases in white blood cell extracts from controls and patients deficient in acid maltase.

A : Ratio of α glucosidase activity $\frac{pH4}{pH\ 6.5} \times 100$

Column 1 : controls (N = 80) - Arrows : limits of standard deviation. Column 2 : patients.

B : Percentage of residual activity at pH 4.0 after addition of anti acid maltase antiserum. First column : controls (N=10). Second column : patients B.M. and B.G.

C : Percentage of residual activity at pH 4.0 after addition of anti renal maltase antiserum. First column : three controls. Second column : patient B.G.

Symbols for patients : D.J. ■
B.M. ●
B.G. ▲

These results are in complete agreement with those obtained by immunological titration.

DISCUSSION.

This work demonstrates the presence of three isozymes of 1-4 α glucosidase in human white blood cells. In addition to the ubiquitous acid and neu-

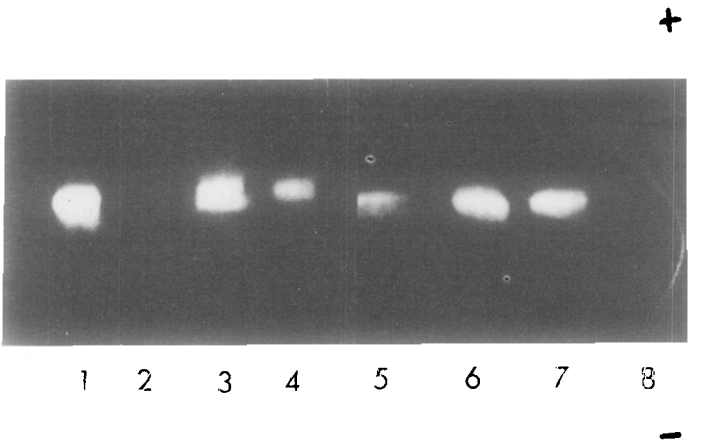

Fig. 3 : Cellogel electrophoresis of α glucosidase. Electrophoresis : pH 6.5
Staining : pH 4.0.
Lines 1 and 2 : Placenta
3 to 5 : Leucocytes, control
6 to 8 : Leucocytes, patient n°3
Lines 1, 3, 6 : Normal serum
2, 4, 7 : Anti acid maltase antiserum
5, 8 : antirenal maltase antiserum.

tral maltases, they contain the renal type of maltase which is known to be present in the membranes of kidney proximal tubules (17). This membranous localization probably explains why the use of a detergent is necessary for complete extraction. Like acid maltase, renal maltase is twice as active when extraction is performed in the presence of Triton than without. The percentage of renal maltase in white cells is as high as in an extract of human whole kidney. The results of combined immunologic and electrophoretic experiments allow a clear separation of the three maltases, which form the bulk of α glucosidase activity of all examined tissues or humors, except small intestine and amniotic fluid. The other major result of this work is the demonstration that patients with a deficiency of α glucosidase (even of the adult type) express the defect also in leucocytes, but that the

deficiency may be masked by the presence of renal maltase, especially when the ratio renal/neutral maltase, which is variable from one subject to another, is high. The practical conclusion is that the diagnosis of α glucosidase deficiency can be made safely on leucocytes, provided one of the two antisera is available. Otherwise it remains safer to ask for a muscle biopsy or for a culture of skin fibroblasts.

It is to be noted that most authors claim that the residual activity in leucocytes is lower in the infantile form (Pompe's disease) than in the later type. This is not substantiated by our results, since even in our adult patients the residual acid maltase activity is very low. It could, however, be explained if the renal activity, compared to the neutral activity, were lower in infants than in adults. Our present results do not support this hypothesis. Our patient 1, a typical case of Pompe's disease, could not be diagnosed by activity determinations on leucocytes (fig. 2), and results obtained with leucocytes prepared from three cord bloods did not show a lower percentage of renal maltase than adult samples.

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