ELECTROPHORETIC CHARACTERIZATION OF ACIDIC AND NEUTRAL AMYLO 1-4-GLUCOSIDASE (ACID MALTASE) IN HUMAN TISSUES AND EVIDENCE FOR TWO ELECTROPHORETIC VARIANTS IN ACID MALTASE DEFICIENCY.

by

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SUMMARY

Acidic and neutral amylo 1-4-glucosidase activities were clearly separated by cellulose acetate electrophoresis. An extra isozyme was found in kidney extracts. Two variants, one fast and one slow, were detected in two cases of amylo glucosidase deficient patients.

The enzyme, amylo 1-4-glucosidase (E.C. 3.2.1.20) or acid maltase is normally present in lysosomes. Its absence (Hers 1963) results in a generalized glycogen storage disease (type II in Cori's classification). This disease is rapidly lethal in its typical form (Pompe's disease) while a milder form follows a slow course and gives rise to myopathic symptoms (Courtecuisse et al 1965, Engel 1970). No evidence is at hand to explain these differences.

The pH activity curve of α amylo glucosidase in tissue extracts shows two maxima, one at pH 4 and the other at pH 6.5. The acidic activity peak disappears in deficient patients while the neutral peak persists in most but not all cases (Angelini and Engel 1972). It is not yet known whether "acid" and "neutral" activities are borne by the same or different molecules. In the

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present communication, we demonstrate an electrophoretic separation of acid and neutral glucosidases, and we show that some deficient mutants can be characterized by electrophoresis. This work was made possible by the availability of an artificial substrate, 4-methyl-umbelliferyl α-glucoside. Cleavage of the glucosidic bond by the enzyme generates free 4-methyl umbelliferone which is fluorescent.

MATERIAL AND METHODS

The work was performed on human tissues. Liver, kidney and muscle samples came from biopsies taken at surgery. Placenta was obtained as soon as possible after delivery and was not perfused. White blood cells were prepared after sedimentation in polyvinyl pyrrolidone followed by differential hemolysis. Cell cultures were grown from embryonic or adult skin samples, and from one case with late α glucosidase deficiency. Tissue samples were extracted in a glass homogenizer with four volumes of water containing 0.1 p. 100 (V/V) Triton X 100 to ensure the destruction of the lysosomes. The homogenate was centrifuged at 20 000 g for 15 minutes and the supernatant was immediately submitted to electrophoresis.

Electrophoresis was carried out according to the technique devised by Fluharty et al (1971), for the detection of β galactosidase with minor modifications. Aliquots of extracts were applied onto strips of Cellogel. The buffer for electrophoresis was potassium phosphate 0.04 M, pH 6.5, which seemed to give sharper bands than sodium phosphate. The separation was carried out in the cold room for two hours at 200 V, resulting in a current of 3 mA per strip. At the end of the run the strip

was placed on Whatman 3 MM filter paper strips saturated with a solution containing 0.5 mM of 4-methyl-umbelliferyl α glucoside (obtained from Koch-Light). The best results were obtained with sodium acetate buffers 0.20 M at pH's 4.0 and 6.5. The paper strips were kept sandwiched between glass plates (Fluharty et al 1971). In the case of pH 4.0 it was necessary to wet first the cellulose acetate paper with the buffer alone to be sure to obtain the correct pH in the incubation reaction. After 60 minutes at room temperature, a Whatman paper strip saturated with 0.2 M glycine-sodium carbonate buffer pH 10.0 was substituted for the reaction strip. This alkaline treatment stops the enzymatic reaction and converts the liberated umbelliferone to the strongly fluorescent anionic form. The resulting fluorescence pattern was viewed in a Chromatovue cabinet and photographs were taken immediately using a Polaroid Land camera, since diffusion is very fast after alkalinization.

RESULTS

As shown in Fig. 1 (channels 5 to 8) α glucosidase activity at pH 6.5 displayed a two-banded pattern. The two bands were generally sharp except in extracts from white blood cells, for which the slow spot may be either doubled or not visible (fig. 1, channel 7). At pH 4.0 only one band could be seen. In the case of most organs (fig. 1, channels 1 to 4) the α glucosidase band was exactly at the same place as the slower band obtained at pH 6.5. Electrophoresis at other pH's (from 6.0 to 8.0) did not modify this result. In addition, at both pH's of incubation, kidney extract displayed a weaker band with a very slow migration or no migration at all (fig. 1, channels 1 and 5).

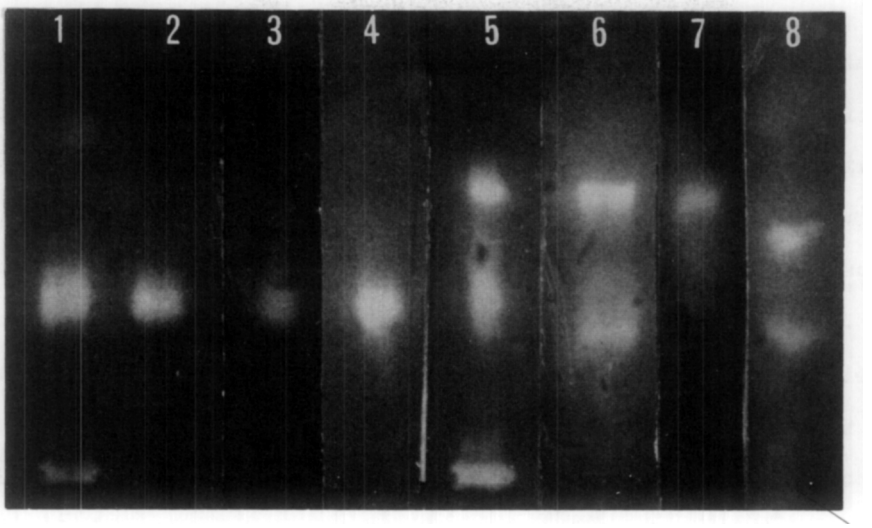

Fig. 1.

α GLUCOSIDASE

Channels	1	to 4	:	pH 4.0
Channels	5	to 8	:	pH 6.5
Channels	:			
	1	and 5	:	kidney
	2	and 6	:	liver
	3	and 7	:	leucocytes
	4	and 8	:	placenta

By contrast, muscle extracts showed a very peculiar situation, since the position of the pH 4.0 band was not identical to that of the slow pH 6.5 spot but slightly more anodic (fig. 2, channels 1 to 4). In one case of Pompe's disease (courtesy of Dr. Van Hoof) a residual activity was found with a definitely slow migration rate (fig. 5, channel 5), while incubation at pH 6.5 after electrophoresis of several samples of extracts from deficient muscles and livers showed no consistent departure from normal (channel 6). Finally, α glucosidase was also studied in extracts from cell cultures (fig. 3). The pattern was analogous to

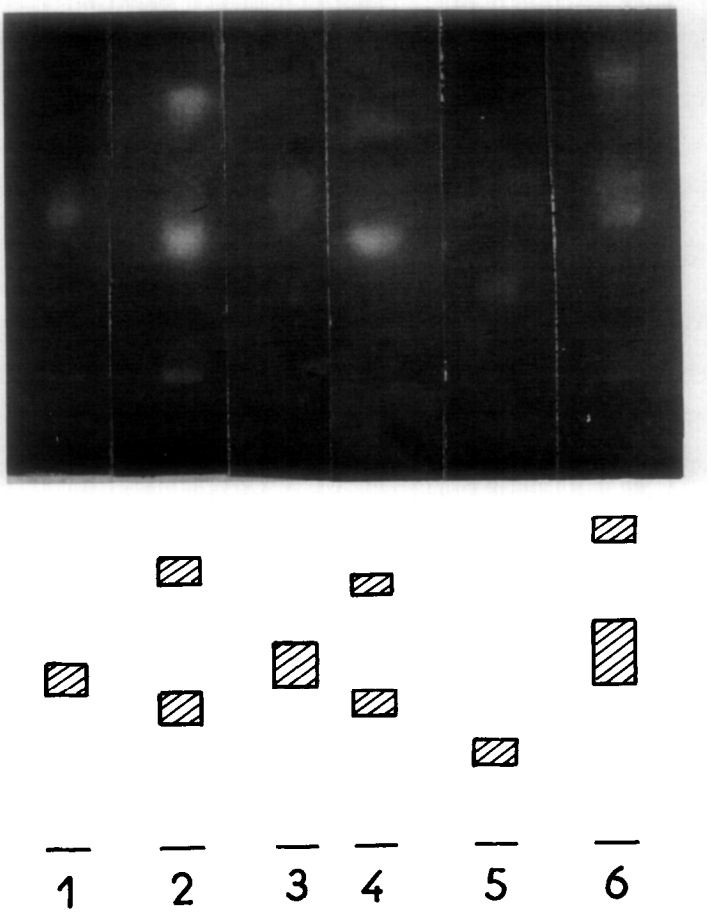

Fig. 2.

α GLUCOSIDASE MUSCLE

Channels	1, 3 and 5	:	pH 4.0
Channels	2, 4 and 6	:	pH 6.5
Channels	1 and 2 3 and 4	:	normal muscle
Channels	5 and 6	:	α glucosidase deficiency

that of other tissues, but for a somewhat diffuse picture and a more predominant fast spot at pH 6.5. In a case of juvenile α glucosidase deficiency (Drs Rossier and Lesage) no abnormality

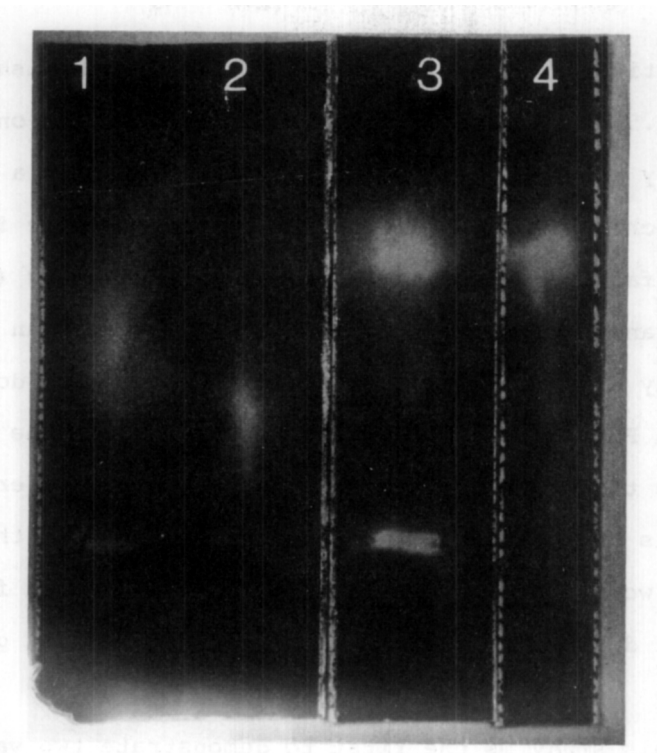

Fig. 3.

α GLUCOSIDASE FIBROBLASTS

Channels	1 and 2	:	pH 4.0
Channels	3 and 4	:	pH 6.5
Channels	1 and 3	:	α glucosidase deficiency of the late type
Channels	2 and 4	:	control

Protein concentration was twice as high in the extract from deficient fibroblasts than in the control.

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was found at pH 6.5 (channel 3). Activity at pH 4.0 was very low but a faint fluorescence was observed, well ahead of that of the control (channel 1), using concentrated extracts.

DISCUSSION AND CONCLUSIONS

Our results on α glucosidase are of interest on several grounds.

Activity at pH 4.0 can be readily distinguished from that at pH 6.5, but additional facts can be observed on two tissues. Kidney extracts contain an extra isozyme with a very slow mobility which is active at both pH's. This specific isozyme may explain the fact recognized by Steinitz and Rutenberg (1967), and by Salafsky and Nadler (1971) that kidney possesses an α glucosidase activity which is not sensitive to turanose and does not disappear in Pompe's disease. Muscle extracts, on the other hand, clearly show that the active enzyme at pH 4.0 is different from the two bands which stain at pH 6.5 since it has not the same mobility. This work, therefore, gives strong evidence in favor of the existence of different molecules for acid and neutral glucosidase activity.

This study is the first to demonstrate two variants in α glucosidase deficiency : one faster and one slower than normal. To date we can only remark that the fast variant was associated with a relatively mild type of the disease. Results obtained at pH 6.5 show that neutral α glucosidase is not affected by the deficiency, although the persistence of the glucosidase activity at a neutral pH does not prevent the clinical manifestations of the disease.

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