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α-MANNOSIDASE IN HUMAN RED CELLS

L. POENARU and J.C. DREYFUS
With the technical assistance of M.C. VINET

Institut de Pathologie Moléculaire, 24 rue du Faubourg Saint-Jacques, 75674 Paris Cedex 14 (France)

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Summary

1. A search for lysosomal hydrolases and related enzymes has been made in hemolysates from human and rabbit red cells. Apart from acid phosphatases, significant activities were found only for α-mannosidase, neutral α-glucosidase and β-hexosaminidase.

2. α-Mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24) activity per cell in human red blood cells was 200-times lower than in white cells. The optimal pH was 5.5—6.0. Electrophoresis on cellulose acetate showed three bands.

Hemolysates from four patients with mannosidosis were not deficient in α-mannosidase. pH activity curves and elctrophoretic pattern were similar to those of controls.

From its biochemical and genetic properties, it is concluded that red cell mannosidase differs from the lysosomal acid mannosidase.

Introduction

Mature red blood cells possess no lysosomes. They are, therefore, not expected to contain active enzymes of lysosomal origin. Some lysosomal glycosidases might however remain in the red cells after the disappearance of the organelles themselves, or non lysosomal glycosidases might be present. In a preliminary investigation, we found that most activities of human and rabbit red cells were very low, and that only three (in addition to acid phosphatase) were relatively high, e.g. β-glucosaminidase, α-glucosidase, α-mannosidase (Table I). The latter enzyme showed the highest activity and we concentrated on its study.

α-D-Mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24) has been detected in several human tissues and fluids [1—8]. Three types of α-D-mannosidase activity have been found in normal human liver: acidic activity with a pH optimum between 3.8 and 4.5 [9]; neutral activity with a pH optimum

TABLE I

ACTIVITY OF GLYCOSIDASES IN HUMAN AND RABBIT RED CELLS AT 37° C

The assay of α-mannosidase has been performed at pH 4 and 6.5 and at optimal pH 5.5 in the other experiments.

Enzymes	Glycosidase activity (nM/h per ml blood)	
	Man (n = 10)	Rabbit (n = 10)
α-Glucosidase, pH 4	0.035 (0—0.035)	0.96 (0.74—1.85)
α-Glucosidase, pH 6.5	11 (4.4—14.8)	3.7 (2.5—4.8)
β-Glucosidase	0	0
α-Galactosidase	0	0.07 (0—0.07)
β-Galactosidase	0.14 (0.07—0.36)	0.96 (0.07—1.48)
α-Mannosidase, pH 4	3.2 (2.9—4.4)	7.5 (1.9—14.8)
α-Mannosidase, pH 6.5	71.5 (26.6—102.6)	33.3 (5.1—77)
β-Glucosaminidase	7.1 (4.9—18.8)	6.7 (5.2—12.4)
α-Fucosidase	1.5 (0.59—2.6)	3.9 (2.9—5.2)
β-Glucuronidase	0.22 (0.05—0.28)	2 (0.7—2.9)

between 6 and 6.5 and activity with an intermediate pH optimum of 5.5.

The interconvertible components A and B of acidic α-mannosidase have been recognised as lysosomal components [10], neutral form C has been localized in cytosol [11] and intermediate pH forms found in Golgi membranes of rat liver [12].

Mannosidosis, a lysosomal storage disease with an accumulation of mannose-rich oligosaccharides in the tissues, is attributed to a drastic reduction of acidic activity of α-mannosidase [13]. The neutral activity is poorly affected in mannosidosis, being presumably under separate genetic control [9]. The possible function of neutral and intermediate types is obscure. The present work describes some properties of human red cell mannosidase.

Experimental procedure

Our biological material was human erythrocytes obtained from heparinized blood samples after elimination of leucocytes and platelets by dextran sedimentation. The red cells were washed twice with saline and were hemolyzed by freezing and thawing. A one to one dilution in water was used for assays and electrophoresis.

The substrate used was 1 mM 4-methylumbelliferyl-α-D-mannopyranoside (Koch-Light), in citrate/phosphate buffer at various pH values. Because of the strong fluorescence quenching effect of hemoglobin, the enzyme assay involved a precipitation with trichloroacetic acid, after incubation, to eliminate hemoglobin. 2 ml 1 M glycine buffer (pH 10) were added to the supernatant fraction in order to develop fluorescence. Other glycosidases were determined using the same fluorimetric technique and the appropriate substrates (Koch-Light). Fluorescence was determined in an Aminco-Bowman spectrofluorimeter (366 nm excitation and 446 nm emission). Electrophoresis was made on Cellogel (Chemetron). Details of the electrophoretic procedure have been described previously [7].

Electrofocusing runs were made on horizontal ampholine polyacrylamide gel plates (L.K.B.) pH range 3.5—9.5, then plates were stained with the same substrate.

Results

Enzyme activity level and localization

The assay made on 10 samples of rabbit hemolysates demonstrated that the presence of α-mannosidase is not specific for human red cells. A bimodal distribution has been observed in rabbit samples (Fig. 1). Sample activities were either below 10 units (5 rabbits) or over 60 units (5 rabbits). In human samples the variation of α-mannosidase activity was not very wide (Fig. 1). The incubation was performed at pH 5.5 because acidic pH 4 precipitates hemoglobin and probably disturbs substrate-enzyme interaction giving results which cannot be reproduced. A mixture of leucocyte extract and hemolysate was compared with one of leucocytes and saline. α-Mannosidase activity at pH 4 was reduced in the former. For subcellular location, the assays were done on cytosol and stroma which were separated by centrifugation and on purified red cells membranes. α-Mannosidase activity was found only in cytosol. No significant dif-

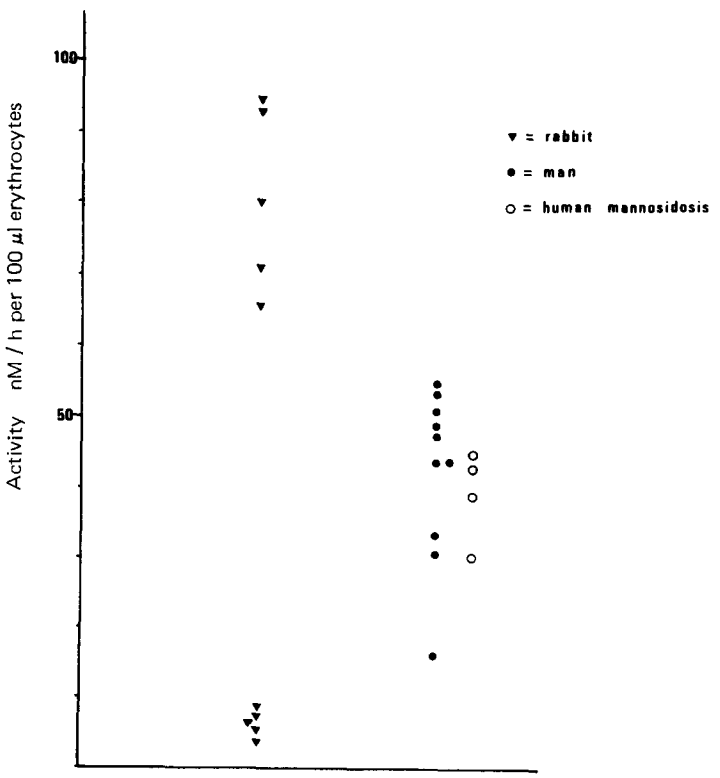

Fig. 1. Distribution of α-mannosidase activity at pH 5.5 in nM/h per 100 µl of erythrocytes, from man and rabbit.

ference was found in the level of α-mannosidase activity either between normal and reticulocytosis hemolysates, or between young and old erythrocytes separated by centrifugation in hematocrite tubes. α-Mannosidase activity was present also in erythrocytes from four patients with mannosidosis in the same range as in controls (Fig. 1). Two of these hemolysates had been kept frozen for two years and two others were fresh samples.

Effect of pH

The variations of red cell, leucocytes and liver α-mannosidase activity between pH 3.5 and 7.0 are shown in Fig. 2. Different profiles were found in the three tissues. The pH activity curve showed two maxima in the liver at pH 4.0 and 6.5. Only the acidic maximum was clearly visible in the leucocyte extracts (pH 4). Red cell extracts showed no maximum in the acid range but only at pH 5.5—6. No significant difference with controls was found in samples from mannosidosis patients.

Electrophoresis

The electrophoretic pattern of erythrocyte α-mannosidase showed three bands with a different anodal migration. Compared to the leucocyte pattern, the slowest band migrated to the same position as the acidic isozyme; the intermediate and strongest band occupied the position of the neutral band of leucocytes; the third, weak band was still more anodal and had no counterpart in other tissues. Similar patterns were obtained whether incubation with the substrate took place at pH 4, 5.5 or 6.5 with an identical ratio between bands, but with a different global intensity, a maximum staining being obtained at pH 5.5. The pattern of the fresh mannosidosis samples was identical to those of the controls.

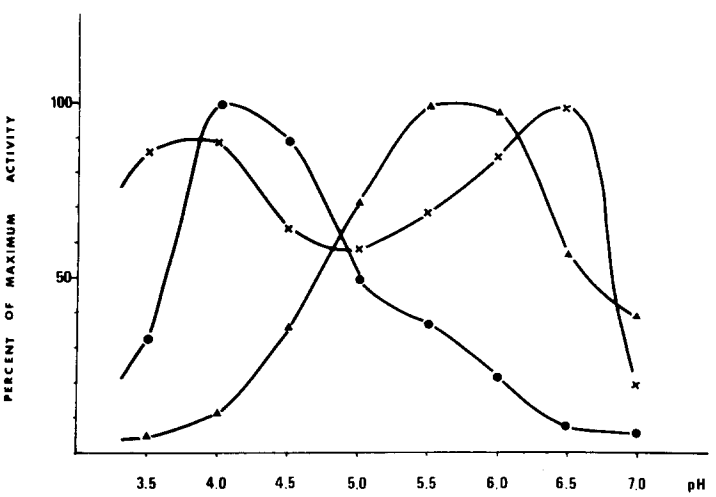

Fig. 2 α-Mannosidase activity as a function of pH. Incubation were performed for 1 h in a citrate/phosphate buffer (0.1 M) at pH values from 3.5 to 7.0. The scale is given as percentage of the maximal activity observed for each tissue. X———X, liver; ●———●, leucocytes; ▲———▲, erythrocytes.

Isoelectric focusing

Analysis by isoelectric focusing demonstrated the presence of one major band with an apparent p*I* of 5.8—6.0 which was found at the same position as kidney neutral α-mannosidase.

Discussion

This work demonstrates the presence of α-mannosidase activity in red cells, at a relatively high level, contrasting with the very low activities of all other tested glycosidases.

Calculated per cell, mannosidase is 200 times lower in erythrocytes than in leucocytes. Referred to whole blood, α-mannosidase activity at optimal pH values is about 205 nM/h per ml blood from erythrocytes and 48 nM/h per ml blood for leucocytes.

Since red blood cells synthesize no protein, the presence of mannosidase must be due to its synthesis in precursors of the red cells, erythroblasts and reticulocytes. The decay of mannosidase activity during the life of the red cells is very slow since the difference between hemolysates from reticulocytes, young and old cells is not significant. This is not a unique example of α-mannosidase persistence in old tissues since we have already described a neutral α-mannosidase with relatively high activities in the central parts of the lens [14].

The electrophoretic pattern, identical for mannosidosis and controls, permits the inference that the red cell α-mannosidase is of a different genetic origin from that of acidic α-mannosidase, the deficiency of which is responsible for the disease, mannosidosis.

The general characters of red cell α-mannosidase are the same as those of neutral mannosidase [15]: cytosolic location, pH activity curve, isoelectric migration, and normal activity level in mannosidosis.

Acknowledgement

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