Biochimica et Biophysica Acta, 358 (1974) 308–318

BBA 67278

STUDIES ON HUMAN POLYMORPHONUCLEAR LEUKOCYTE ENZYMES IV. INTRACELLULAR DISTRIBUTION AND PROPERTIES OF α-L-FUCOSIDASE

JOSÉ LUIS AVILA AND JACINTO CONVIT

Instituto Nacional de Dermatología, Apartado 4043, Caracas 101 (Venezuela)

(Received January 30th, 1974)

SUMMARY

1. The location and characteristics of the α-L-fucosidase (EC 3.2.1.–) activity in human polymorphonuclear leukocytes were studied.

2. There was a fairly strong α-L-fucosidase activity in human leukocytes. The pH–activity curve showed a broad peak with highest activity between pH 5.4 and 5.6, using *p*-nitrophenyl-α-L-fucoside as substrate. The apparent K_m and V were 0.168 mM and 149 nmoles/mg protein per h. The temperature optimum was 45 °C.

3. The enzyme was stable during preincubation at 37 °C in 0.1 M acetate buffer pH 5.5, up to 2 h, and was also extremely sensitive to thiol-blocking agents.

4. Despite the use of several different biochemical approaches (gel filtration, thermostability, pH stability and subcellular fractionation) no clear evidence for the presence of several forms of α-L-fucosidase was obtained.

5. In cytoplasmic extracts, about 30% of the activity of α-L-fucosidase is latent, and is unmasked by a number of treatments (detergents, sonication, low osmotic pressure, Waring–Blendor) that unmask other lysosomal enzymes. On the other hand, the distribution pattern of α-L-fucosidase in subcellular fractions of polymorphonuclear leukocytes is closely similar to that reported for other acid hydrolases of this tissue. These results support the concept of an association of α-L-fucosidase with primary granules.

INTRODUCTION

L-Fucose is frequently located on the non-reducing termini of the carbohydrate moiety of blood group substances, glycoproteins and immunoglobulins [1]. An enzyme which catalyzes the hydrolysis of the α-fucoside bond has been purified from a variety of biological sources [2–5]. Detailed specificity studies indicate that it is highly specific for non-reducing terminal L-fucose residues [2, 3].

The lysosomal intracellular location of α-fucosidase has been demonstrated by Conchie and Hay [6], while two distinct α-fucosidases have been found in pig kidney [2], in abalone liver [7], in *Trichomona Fetus* [8] and in human placenta, amniotic fluid, liver and kidney [4].

The importance of α-fucosidase in mucopolysaccharide metabolism was

strikingly shown in fucosidosis, a progressive degenerative disease of the central nervous system associated with an accumulation of fucose [9, 10] and ceramide tetra- and pentahexoside [11] and a profound deficiency of α-fucosidase activity [12, 13].

This report deals with studies on the α-L-fucosidase (EC 3.2.1.–) of human polymorphonuclear leukocytes. The results obtained indicate the presence of a fairly strong α-L-fucosidase activity. The intracellular distribution and kinetic properties are discussed.

EXPERIMENTAL PROCEDURES

Materials

p-Nitrophenyl-α-L-fucoside, *p*-nitrophenyl-β-D-fucoside, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide and *p*-nitrophenyl-α-D-mannoside and phenolphthalein glucuronic acid, sodium salt were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

All solutions were prepared in deionized water.

Methods

Leukocytes were isolated from the blood of fasting normal subjects and processed as previously described [14]. About 92–95% of the cells in the final suspension were polymorphonuclear leukocytes.

Unless otherwise stated homogenates were prepared in ice-cold 0.34 M sucrose–50 mM KCl.

Intracellular distribution studies. For these studies homogenates were prepared in ice-cold 0.34 M sucrose–50 mM KCl/50 units heparin per ml. Fractionation experiments were carried out exactly as described previously [15].

Latency studies. These studies were performed on cytoplasmic extracts as reported by Avila and Convit [16]. Total release of α-fucosidase was insured by pretreating the tissue preparations with a Waring–Blendor homogenizer for 80 s at 4 °C.

Enzyme assays. α-L-Fucosidase (EC 3.2.1.–) was determined in a final volume of 1 ml of 0.1 M acetate buffer, pH 5.5, with 1 mM *p*-nitrophenyl-α-L-fucoside as substrate. The reaction was stopped by the addition of 3.0 ml of a solution containing glycine (133 mM), NaCl (67 mM) and Na_2CO_3 (83 mM), pH 10.5. Readings were taken at 400 nm. Other enzymes were determined as described before [17, 18]. Except in the free activity experiments, all other results represent total activity for which Triton X-100 was added at 0.04% to the incubation mixture of the enzymes.

Protein content was measured according to Lowry et al. [19] with bovine serum albumin as standard.

RESULTS

Enzyme activity in human polymorphonuclear leukocytes

As can be seen in Table I there was a fairly strong α-fucosidase activity in polymorphonuclear leukocytes. Activity was, however, less than that found in the same

TABLE I

ACID HYDROLASE ACTIVITIES IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

All results given as mean ± S.D. in nmoles/mg protein per h. Numbers in parentheses indicate the number of different individuals studied.

Enzymes	Activity
α-L-Fucosidase (24)	60 ± 37
α-D-Mannosidase (23)	326 ± 140
β-Glucuronidase (30)	118 ± 11
N-Acetyl-β-glucosaminidase (34)	223 ± 156

preparations for *N*-acetyl-β-glucosaminidase, β-glucuronidase and α-mannosidase activities. No β-fucosidase activity was detected when assayed under the same conditions as α-fucosidase.

pH optimum

The effect of pH on the catalytic activity of the enzyme was studied by using *p*-nitrophenyl-α-L-fucoside in 0.1 M acetate buffers ranging from pH 4.2 to 6.0. The pH–activity profile shown in Fig. 1 indicates a broad peak with highest activity at pH values between 5.4 and 5.6. However, in some experiments a small shoulder in the pH–activity curve was found at about pH 4.8.

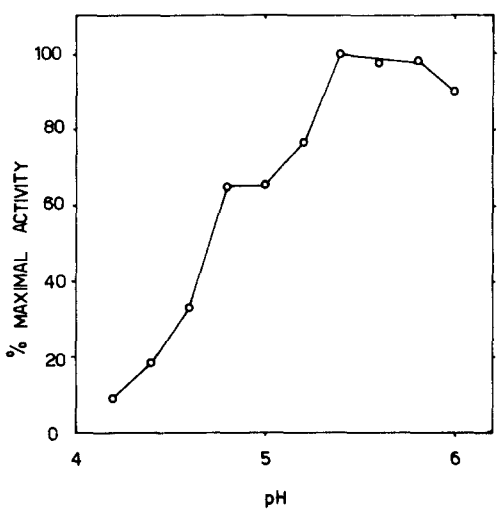

Fig. 1. Effect of pH on the reaction velocity of human polymorphonuclear α-L-fucosidase. *p*-Nitrophenyl-α-L-fucoside was used as substrate. The pH values reported were those measured electrometrically at 20 °C.

The optimum pH of the human polymorphonuclear α-fucosidase is thus very close to that reported for the enzyme present in several tissues [2, 20], except in the case of *Aspergillus niger* [3], which showed an optimum at pH 3.8. It is also different from the optimum pH found for human kidney α-fucosidase I, but similar to that

reported for human kidney α-fucosidase II [4], human skin [21] and human normal serum [22] α-fucosidase activities.

On the other hand, in contrast to the results obtained for human normal serum [22] no difference in activity was found by incubating a total homogenate preparation with 0.1 M acetate, 0.1 M citrate or 0.1 M citrate–phosphate buffer in the pH range 5.0–5.8.

Effect of substrate concentration on enzyme activity

Using the above substrate at concentrations varying from 0.1 to 2 mM in 0.1 M acetate buffer, pH 5.5, a Lineweaver–Burk plot was obtained. The plot of $1/v$ versus $1/S$ showed a straight line relationship. The value for the apparent K_m as computed from the plot was 0.168 ± 0.057 mM (four experiments) and that of V 149 ± 33 nmoles *p*-nitrophenol/mg protein per h (four experiments ± S.D.) (Fig. 2).

The K_m value is thus very close to the Michaelis constant of α-fucosidase activity of normal human serum [22] and rat epididymus and ox liver [23].

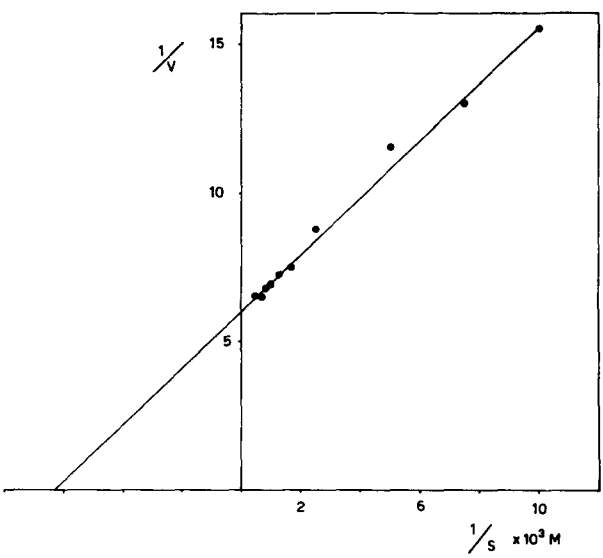

Fig. 2. Lineweaver–Burk plot of α-L-fucosidase activity.

Effect of enzyme concentration and time of incubation on α-fucosidase activity

A linear increase in hydrolysis was obtained up to at least 2 mg of homogenate and 8 h of incubation.

Entirely different results have been reported for normal human serum [22] α-fucosidase activity in which hydrolysis of substrate was linear up to 90 min of incubation at 37 °C.

Effect of preincubation

When preincubated at 37 °C in 0.1 M acetate buffer, pH 5.5, no loss in enzyme activity was found at the end of 2 h.

The preincubation of homogenates at different temperatures up to 20 min in 0.1 M acetate buffer, pH 5.0 or pH 5.5, showed a strong stability of α-fucosidase activity in the range 4–55 °C with a loss of 60% of enzyme activity at 70 °C (Fig. 3).

When preincubations were carried out at different H^+ concentrations by using 0.1 M citrate buffers in the pH range 3.0–5.8 for 30 min at 37 °C, again no loss in enzyme activity was found when compared with the same enzyme preparations pre-

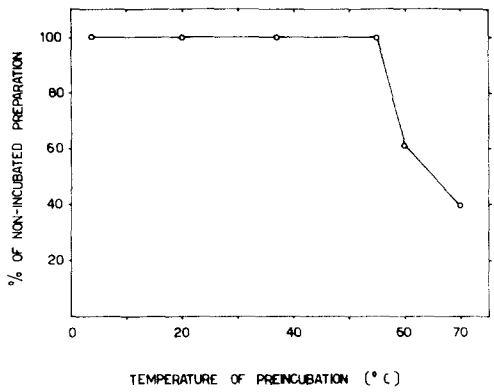

Fig. 3. Thermal inactivation of the α-fucosidase. The homogenate was incubated in 0.1 M acetate buffer (pH 5.5) for 20 min a tthe indicated temperature. After the mixture had been cooled on ice, substrate was added and the remaining enzyme activity was determined at 37 °C. The results are given as percentages of the unincubated control value for each enzyme.

incubated at same pH values but at 4 °C. This finding correlates well with the stability–pH curve found for α-fucosidase I activity of human kidney, but is quite different from results for α-fucosidase II [4]. Our results are, however, quite similar to that reported for human kidney total α-fucosidase activity. In other experiments the preincubation of a total homogenate at 60, 65 and 70 °C for different lengths of time induced a progressive inactivation of the α-fucosidase activity, reaching about 22% of the control activity after preincubation at 70 °C for 20 min (Fig. 4).

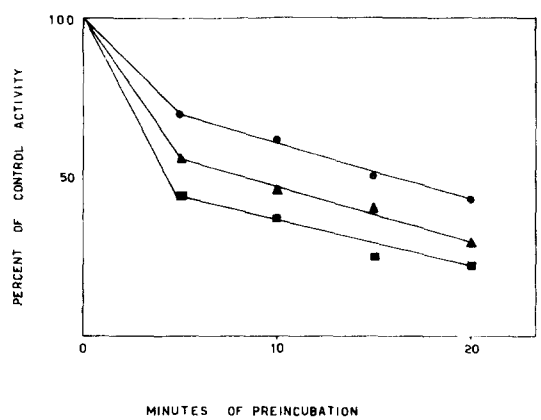

Fig. 4. Effect of preincubation at various temperatures in 0.1 M acetate buffer, pH 5.5, for different lengths of time on thermolability of α-L-fucosidase, ●—●, 60 °C; ▲—▲, 65 °C; ■—■, 70 °C.

Stability

No loss of α-fucosidase activity was detected when homogenates were stored at −10 °C for 24 h; after 3 days 3–6% losses were observed. Repeated freezing and thawing resulted in 5–10% inactivation.

Effect of different substances on leukocytic α-fucosidase

Table II shows that NH_4^+, Na^+, Ca^{2+}, Zn^{2+} and Mg^{2+} had virtually no effect at the concentrations used. This behaviour contrasts with that reported for the α-fucosidase of *Clostridium perfringens* [20] which is activated by Na^+, Mg^{2+}, Ca^{2+} and

TABLE II

EFFECT OF VARIOUS COMPOUNDS ON THE α-FUCOSIDASE ACTIVITY OF HUMAN POLYMORPHONUCLEAR LEUKOCYTE HOMOGENATE

Assays were carried out with *p*-nitrophenyl-α-L-fucoside for 6 h in 50 mM acetate buffer, pH 5.5, at 37 °C. Values are the means of four different experiments, each carried out in duplicate. PCMB, *p*-chloromercuribenzoate.

Compounds	Percent of control
None	100
0.1 mM $HgCl_2$	8
0.1 mM PCMB	8
2 mM $CuSO_4$	12
3 mM AgCl	41
2 mM $FeCl_3$	60
2 mM $FeCl_2$	112
6 mM EDTA	95
30 mM cysteine	80
15 mM ascorbate	92
2 mM $CaCl_2$	103
2 mM $(NH_4)_2SO_4$	102
2 mM $ZnSO_4$	90
2 mM NaCl	91
2 mM $MnSO_4$	74
2 mM $MgCl_2$	103
2 mM KCl	100
2 mM $SnCl_2$	76
160 mM formaldehyde	43
6 mM glucose	79
6 mM galactose	98
6 mM arabinose	104
0.1% Triton X-100	104
75 mM citrate	95
300 mM KCl	117
300 mM NaCl	102
0.012% deoxycholate	64
0.012% taurocholate	100

NH_4^+. On the other hand, simular to the bacterial enzyme, *p*-chloromercuribenzoate, Hg^{2+}, Ag^+ and Cu^{2+} induced a strong inhibition of the α-fucosidase activity, suggesting the presence of an SH group.

No effect was found by EDTA in concentrations up to 6 mM, or by the ionic strength up to 300 mM.

Gel filtration on Sephadex G-200

When a total homogenate preparation was submitted to gel filtration on Sephadex G-200 only one peak of α-fucosidase activity was found (Fig. 5). This corresponds to an activity of 620 nmoles/mg protein per h which represents a 10-fold purification relative to the specific activity of the total homogenate.

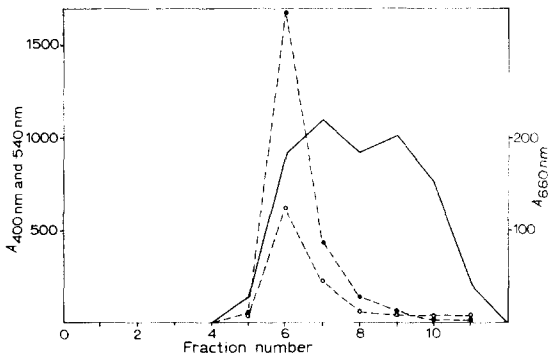

Fig. 5. Chromatography on Sephadex G-200 of a human polymorphonuclear leukocyte total homogenate. 250 mg of tissue were treated at 4 °C for 30 min with 0.2% Triton X-100 and then centrifuged at 500 000 × g·min. The supernatant was separated and 4.5 ml was applied to a column (3.6 cm × 44 cm) equilibrated with 0.1 M acetate buffer (pH 4.0)–0.2% Triton X-100. This buffer was also used for elution. Fractions (10 ml) were collected and assayed for absorbance at 400 nm (α-L-fucosidase; ○—○), 540 nm (β-glucuronidase; ●—●) and 660 nm (total proteins; ———).

Intracellular localization

Fig. 6 shows the distribution observed for α-L-fucosidase, peroxidase, acid β-glycerophosphatase, alkaline phosphatase, acid phenylphosphatase, following fractionation of a whole human polymorphonuclear leukocytes homogenate by differential centrifugation. It can be seen that the distribution of α-fucosidase in subcellular fractions is similar to that of the lysosomal enzymes, with a high percent of the activity present in Fraction I, and with only about 18% of the total activity in the supernatant fraction.

Latency of α-fucosidase

When assayed under proper conditions, this enzyme displayed an important degree of latency in cytoplasmic extracts: its free activity (see Avila and Convit [16]) measured at pH 5.5 was 30 ± 7% of its total activity (average of four determinations ± S.D.).

Partial or full activation of α-fucosidase could be achieved by several of the treatments that release the other acid hydrolases in human polymorphonuclear cytoplasmic extracts (Table III).

Influence of temperature on enzyme activity

The dependence of enzyme activity on temperature at pH 5.5 is seen in Fig. 7, which shows that this preparation was more reactive at 45 °C, and that a linear increase of activity occurred in the range 23–45 °C. It has also been reported that abalone liver α-fucosidase [7] is most reactive at 55 °C.

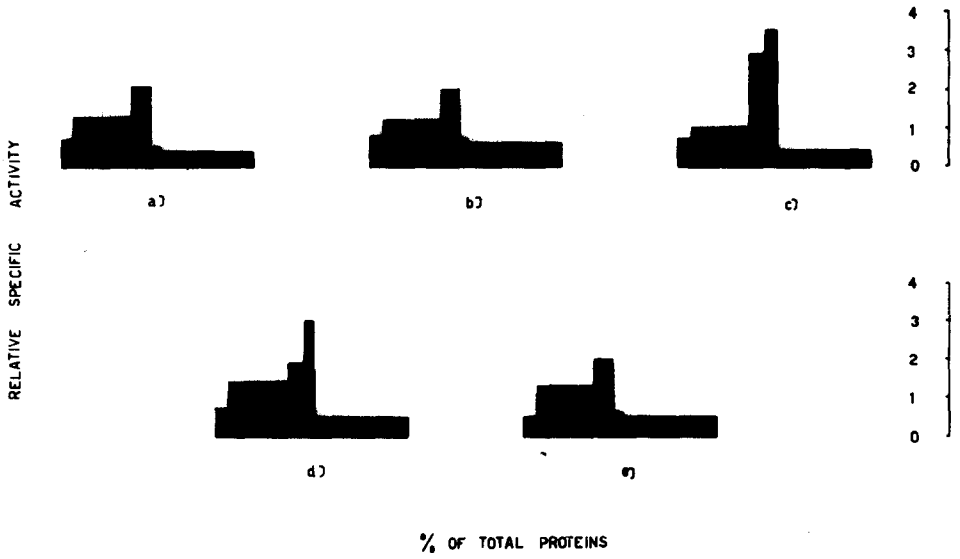

Fig. 6. Intracellular distribution of enzymes. (a) α-L-fucosidase (4), (b) acid β-glycerophosphatase (4), (c) alkaline phosphatase (4), (d) acid phenylphosphatase (3), (e) peroxidase (5). The numbers in parentheses refer to number of experiments. The ordinate shows the mean relative specific activity of the fractions. On the abscissa the fractions are represented by their relative protein content, in the order in which they are isolated, i.e. from left to right Fractions I, II, III, IV and Fraction S.

TABLE III

INFLUENCE OF THE TREATMENT OF CYTOPLASMIC EXTRACT WITH SEVERAL ACTIVATING PROCEDURES ON FREE ACTIVITIES OF α-FUCOSIDASE, β-GLUCURONIDASE AND β-*N*-ACETYLGLUCOSAMINIDASE

Cytoplasmic extracts containing the equivalent of 40 mg fresh human polymorphonuclear leukocytes per ml, were subjected to the experimental conditions described below. Appropriate amounts of the homogenate were then used for the measurement of free activity as in Avila and Convit [16], for 60 min at 37 °C. Total activities were measured on extracts incubated for 60 min in the presence of 0.04% Triton X-100. Results are expressed as percentages of the corresponding total activities. Sonication was carried out with the Branson sonifier (Model W185) set in Position 5 (output 65 W) and with the standard probe for 30 s. In the detergent experiments, they were present in the incubation mixture.

Experimental conditions	Free activity (% of total)		
	α-Fucosidase	β-Glucuronidase	β-*N*-Acetyl-glucosaminidase
0.34 M sucrose	26	16	11
0.08 M sucrose	69	78	70
0.04% Triton X-100	100	100	100
0.1% deoxycholate	92	100	94
Waring–Blendor, 80 s	81	70	84
Sonication	51	57	51
0.1 mM $HgCl_2$	89	92	91
0.1% taurocholate	100	100	88
0.04% Tween 20	88	84	56
0.04% Tween 40	79	55	42
0.04% Tween 60	44	21	30

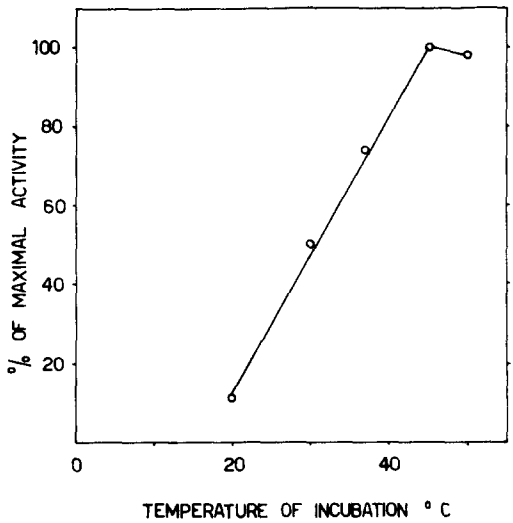

Fig. 7. Activity–temperature curve for a human polymorphonuclear homogenate with *p*-nitrophenyl-α-L-fucoside as substrate. The experimental conditions are those of the enzyme assay described in the text. Reaction time: 6 h.

Effect of dialysis on α-fucosidase activity

Dialysis against phosphate buffer, pH 7.0, for 48 h at 4 °C did not alter the α-fucosidase activity of a total homogenate. However, when dialysis was carried out under the same experimental conditions but against citrate buffers in the pH range 3.0–6.0 important losses of α-fucosidase activity were found (Table IV).

TABLE IV

EFFECT OF DIALYSIS ON THE α-FUCOSIDASE ACTIVITY PRESENT IN A TOTAL HUMAN POLYMORPHONUCLEAR HOMOGENATE

Dialysis was carried out for 48 h at 4 °C against several changes of the indicated buffer or water.

Conditions	Percent of non-dialyzed homogenate
0.1 M citrate buffer, pH 3.0	51
0.1 M citrate buffer, pH 4.0	76
0.1 M citrate buffer, pH 5.0	71
0.1 M citrate buffer, pH 6.0	82
Water	100
0.1 M phosphate buffer, pH 7.0	100
Non-dialyzed preparation	100

DISCUSSION

Our present results do not reveal the presence in human polymorphonuclear leukocytes of two forms of α-fucosidase activity, as has been reported in several other human tissues [4]. Thus, as in some experiments we got a very slightly biphasic pH–activity curve (with optimum pH values at 5.2 and 5.6) and as by preincubation at different temperatures we also found a biphasic curve (Fig. 3), we performed several

experiments to obtain more information concerning the existence of two α-fucosidase activities in human polymorphonuclear leukocytes. Consequently we studied the influence of preincubation at 65 °C for 30 min on the pH–activity profile. After this treatment the residual α-fucosidase activity was only about 8% of the untreated preparation and again the optimum pH remained between 5.2 and 5.6. Furthermore, when a total homogenate was preincubated in 0.1 M acetate buffer, pH 5.5, at 60, 65 and 70 °C for different lengths of time up to 20 min (Fig. 4), no evidence was found for the presence of two different forms of α-fucosidase. In other experiments, gel filtration of a total homogenate on Sephadex G-200 did not reveal the presence of two peaks of α-fucosidase activity (Fig. 5).

It has been reported that in the case of the α-mannosidase [24] and neuraminidase [25] activities of rat and chick liver, respectively, the presence of two different forms of enzyme activities can be explained by a different subcellular localization, the enzyme present in the soluble fraction differing from that present in the lysosomal fraction. We performed experiments separating granular and supernate fractions and studying the pH-activity profile and thermostability of the α-fucosidase activity present in each fraction. Again no difference was found between the α-fucosidase activity present in each fraction.

From our present data it is not possible to identify the α-fucosidase activity present in human polymorphonuclear leukocytes with the forms I and II reported to be present in several other human tissues [4]; although the leukocytic enzyme showed an optimum pH of 5.4 which corresponds to that of the form II, it also had pH–stability and thermostability profiles similar to that reported for form I. In spite of the application of several biochemical approaches we have not been able to confirm the presence of two forms of α-fucosidase activity in human polymorphonuclear leukocytes, although they can perhaps be revealed by more sensitive biochemical methods.

Finally, the sedimentation pattern obtained by differential centrifugation and the similarities of behaviour of α-fucosidase with respect to several other latent acid glycosidases suggest that they all are situated in one population of lysosomes, which in polymorphonuclear leukocytes correspond to primary granules.

ACKNOWLEDGEMENTS

We are indebted to Mrs Mariela de Luna and Mrs María Argelia de Casanova for their excellent technical assistance and for Mrs Candelaria de Aranguren for her secretarial help.

This work was supported by a grant from C.O.N.I.C.I.T. (Consejo Nacional de Investigaciones Científicas y Tecnológicas, Proyecto DF 0125).

We are grateful to the Unidad del Banco de Sangre del Hospital Vargas (Caracas) for having kindly given us fresh blood.

REFERENCES

1 Gottschalk, A. (1966) in Glycoproteins, p. 362, American Elsevier Publishing Co. Inc., New York
2 Wiederschain, G. Y. and Rosenfeld, E. L. (1971) Biochem. Biophys. Res. Commun. 44, 1008–1014
3 Bahl, O. P. (1970) J. Biol. Chem. 245, 299–304
4 Wiederschain, G. Y., Kolibaba, L. G. and Rosenfeld, E. L. (1973) Clin. Chim. Acta 46, 305–310

5 Wiederschain, G. Y. and Rosenfeld, E. L. (1969) Bull. Soc. Chim. Biol. 51, 1075–1084
6 Conchie, J. and Hay, A. J. (1963) Biochem. J. 87, 354–361
7 Tanaka, K., Nakano, T., Noguchi, S. and Pigman, W. (1968) Arch. Biochem. Biophys. 126, 624–633
8 Stealey, J. R. and Watkins, W. M. (1972) Biochem. J. 126, 16P–17P
9 Durand, P., Phillipart, M., Barrone, C., Della Cella, G. and Bugiani, O. (1967) Minerva Pediatr. 19, 2187–2196
10 Durand, P., Barrone, C. and Della Cella, G. (1969) J. Pediatr. 75, 665–674
11 Durand, P., Barrone, C., Della Cella, G. and Philippart, M. (1968) Lancet 1, 1198
12 Van Hoof, F. and Hers, H. G. (1968) Lancet 1, 1198
13 Van Hoof, F. and Hers, H. G. (1968) Eur. J. Biochem. 7, 34–44
14 Avila, J. L. and Convit, J. (1973) Biochim. Biophys. Acta 293, 397–408
15 Avila, J. L. and Convit, J. (1973) Clin. Chim. Acta 44, 21–31
16 Avila, J. L. and Convit, J. (1973) Biochim. Biophys. Acta 293, 409–423
17 Avila, J. L. and Convit, J. (1970) Int. J. Lepr. 38, 359–364
18 Avila, J. L. and Convit, J. (1973) Clin. Chim. Acta 47, 335–345
19 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
20 Aminoff, D. and Furukawa, K. (1970) J. Biol. Chem. 245, 1659–1669
21 Ockerman, P. A. (1969) Acta Derm. Venereol. 49, 139–141
22 Zielke, K., Okada, S. and O'Brien, J. S. (1972) J. Lab. Clin. Med. 79, 164–169
23 Levvy, G. A. and McAllan, A. (1961) Biochem. J. 80, 435–439
24 Marsh, C. A. and Gourlay, G. C. (1971) Biochim. Biophys. Acta 235, 142–148
25 Tulsiani, D. R. P. and Carubelli, R. (1972) Biochim. Biophys. Acta 284, 257–267