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HUMAN α-FUCOSIDASE

SINGLE RESIDUAL ENZYMATIC FORM IN FUCOSIDOSIS

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Summary

Four major forms of α-fucosidase (EC 3.2.1.51) activity were separated by isoelectrofocusing from sera of normal control individuals. All forms shifted towards less acidic pI values after neuraminidase treatment. In two patients affected with fucosidosis, only a single major acidic peak was observed and this was affected to a lesser degree by neuraminidase treatment. The kinetics of heat inactivation of the residual activity found in these two patients showed two decay rates while the controls showed only one rate.

These data are considered in relation to the hypothesis of the existence of interconvertible thermolabile and thermostable forms of the enzyme which has been discussed in the preceeding paper. The residual α-fucosidase found in patients could be structurally altered so that its ability to form the thermostable higher moleculer weight aggregates is impaired.

Introduction

Fucosidosis, an autosomal recessive inborn error of metabolism [1], is characterized by a deficiency of α-fucosidase (EC 3.2.1.51) and by the accumulation of fucose containing glycolipids [2] and glycoproteins [3]. Several forms of α-fucosidase have been identified by starch gel electrophoresis and by isoelectrofocusing, from extracts of different human tissues [4—6]. None have been detected in liver, lymphoid cells, serum or cultured fibroblasts from patients affected with fucosidosis [4,5].

In the present study we show by isoelectrofocusing that in sera of two patients with fucosidosis a single band of α-fucosidase activity is observed, whereas multiple bands are found in sera of normal controls. Neuraminidase treatment of the sera from the patients modified the pI of this residual peak, but much less than with the multiple peaks of controls. In addition, the kinetics of thermal inactivation, and the K_m values for the fluorogenic substrate in sera

and fibroblasts from patients and controls have been analyzed, and show a significant difference in the rate of thermal decay and slightly different K_m values. These data, together with the different behaviour after neuraminidase treatment, could suggest a structural alteration of the enzymatic molecule in fucosidosis.

Materials and Methods

Sera of two patients, brother and sister, 18 and 6 years-old, respectively, already described elsewhere from a clinical point of view [4] and of normal controls were kept frozen for 1—2 weeks until analyzed. The two patients studied are the only living affected members of a large family originating from Calabria (Southern Italy). The two patients originally described as affected with the infantile form of fucosidosis [1] belonged to this same family. Isoelectrofocusing, thermal inactivation, and K_m determinations were performed as described previously [8,9]. The pH range for the Ampholine solution used in these experiments was 4—6 and 5—7, respectively, for untreated sera and for sera treated with neuraminidase. The assays for α-fucosidase activity in the fractions collected after the isoelectrofocusing of control sera were performed as already described [8], while aliquots of 200 μl of sera from patients, fractionated by the same technique, were incubated with 50 μl of 1 mM 4-methylumbelliferyl-α-fucoside (Me-Umb-Fuc) in 0.5 M citrate buffer pH 6.0 for 48 h.

Neuraminidase purified by affinity chromatography [10] was incubated with sera of patients and controls as described elsewhere [8]. Fibroblast cultures were established and grown in Dulbecco modified Minimal Essential Medium plus 10% fetal calf serum [11].

Results

Activity of α-fucosidase in serum

A marked deficiency of α-fucosidase was found in sera of the two patients affected with fucosidosis with respect to normal controls, using the Me-Umb-Fuc as substrate (Fig. 1). However, neither of the two obligate heterozygotes, (father and mother of the two patients) could be distinguished from controls on the basis of this assay in serum, whereas their carrier state could be demonstrated in leukocytes (Fig. 2). In addition, in the sera of 7 out of 59 healthy control individuals a deficiency of α-fucosidase activity was found (see Fig. 1, Group B). However, this group of seven controls (Fig. 2, Group B) showed values of α-fucosidase activity in leukocytes similar to those found in 33 controls (Fig. 2, Group A). This indicated that the serum assay cannot be used as the only means of diagnosis for heterozygotes or homozygotes. The serum specific deficiency of α-fucosidase seems to behave as an autosomal dominant character in one family examined, where the mother and two children had low serum activity, but normal values in their leukocytes.

Isoelectrofocusing in sera of patients and controls

The isoelectrofocusing pattern in the sera of the six controls showed four major acidic peaks and some other minor peaks in the cathodic region of the

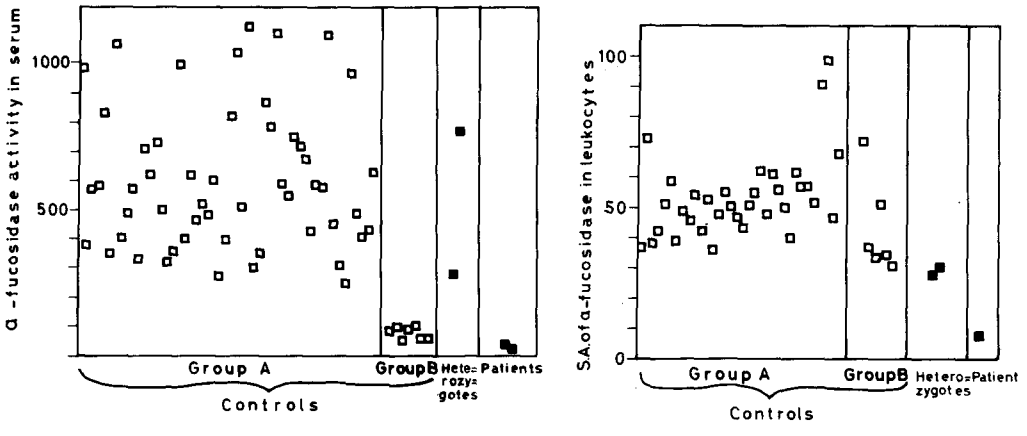

Fig. 1. Activity of α-fucosidase (nmol of Me-Umb-Fuc hydrolyzed per ml/h) in sera of controls, heterozygotes and patients. The control group has been subdivided in groups A and B, the latter being represented by individuals with low serum activity.

Fig. 2. Specific activity of α-fucosidase (nmol of Me-Umb-Fuc hydrolyzed/mg protein per h) in leukocytes of some control individuals of groups A and B (see Fig. 1) and of heterozygotes and patients.

gradient (Fig. 3). The p*I* values of the major peaks corresponded respectively to 4.56, 4.80, 5.01 and 5.15. The p*I* of the major peaks in the different individuals were strikingly similar and only small differences in their relative amount could be noticed (Table I). On the other hand, the sera of the two patients affected with fucosidosis showed by isoelectrofocusing, one major peak of activity only with a p*I* of 4.5 and some other minor peaks (Fig. 4).

Neuraminidase treatment of the sera of normal controls gave a reproducible shift of the four major peaks towards the cathode, as shown in Fig. 3, with p*I* values corresponding to 5.35, 5.53, 5.80 and 6.25, respectively. With respect to the four major peaks found before neuraminidase treatment, the shifts in pI were respectively of 0.79 (5.35—4.56), 0.73 (5.53—4.8), 0.79 (5.80—5.01) and 1.1 (6.25—5.15). By the same neuraminidase treatment, the single major peak present in the two patients was transformed into several less acidic peaks (Fig. 5 and Table I), which did not correspond to the peaks found in controls after neuraminidase treatment.

The isoelectrofocusing of sera of two individuals showing a serum specific deficiency of α-fucosidase did not show any change in the pattern of peaks with respect to normal controls.

Characterization of α-fucosidase from patients

The K_m values with respect to Me-Umb-Fuc of the four major peaks obtained from one normal control corresponded approximately to 0.3 mM. The K_m values of the single major peak of the two patients was 0.91 and 1.0 mM, respectively. In addition, a marked difference was found in the kinetics of heat inactivation at 51°C of the α-fucosidase activity in the two patients with respect to controls (Fig. 6). While the α-fucosidase activity of controls showed a single rate of thermal decay up to 120 min, the serum residual activity of the patients showed a double rate of decay, indicating the presence of a thermolabile and a thermostable component. A difference in thermal inactivation,

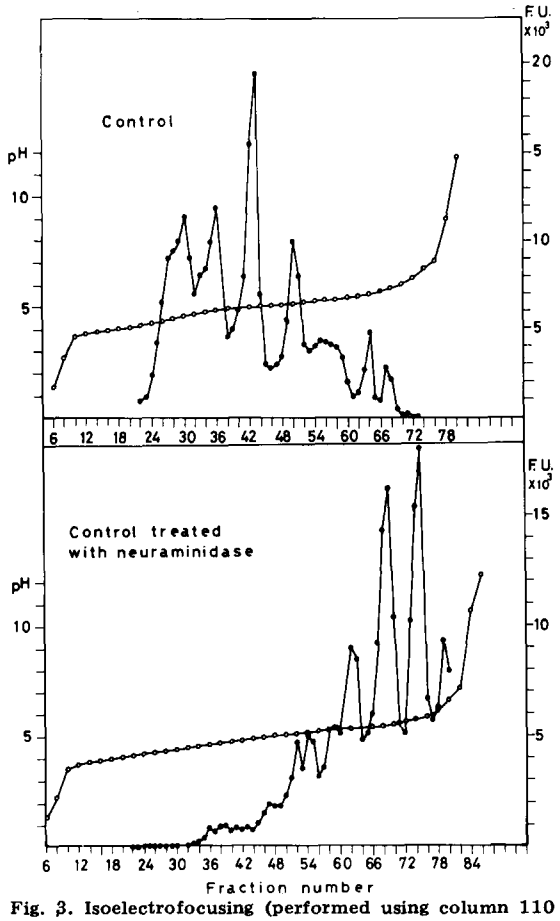

Fig. 3. Isoelectrofocusing (performed using column 110 — LKB equipment) on serum of one control individual before and after treatment with neuraminidase. Fluorescence units (F.U.), ●———●; pH ○———○. In this experiment the column was loaded with 3 ml of serum sontaning 18 mU of activity and 200 mg of protein.

TABLE I

SHIFTS IN p*I* OF SERA OF ONE REPRESENTATIVE CONTROL AND THE TWO PATIENTS BEFORE AND AFTER NEURAMINIDASE TREATMENT.

The percent of the total activity of each peak after electrofocusing was calculated graphically.

Control serum		Control serum +neuraminidase		Patient no. 1		Patient no. 1 +neuraminidase		Patient no. 2		Patient no. 2 +neuraminidase	
p*I*	%	p*I*	%	p*I*	%	p*I*	%	p*I*	%	p*I*	%
4.56	27.8	—	—	4.35	9.2	—	—	4.35	8.4	—	—
4.8	24.4	—	—	4.42	17.3	—	—	4.42	24.7	—	—
5.01	24.4	5.0	2.3	4.55	43.3	4.55	6.8	4.50	39.4	4.55	31.0
5.15	11.4	5.15	10.1	4.66	14.9	4.65	13.7	4.64	13.7	4.67	24.5
5.3	5.9	5.35	16.2	4.76	8.9	4.8	23.3	4.80	9.4	4.8	18.5
5.58	3.7	5.53	30.0	4.95	6.4	4.92	27.0	4.92	4.4	4.92	12.0
5.8	2.4	5.86	29.2			5.02	17.0			5.06	4.2
		6.25	13.2			5.1	7.9			5.4	2.2
						5.28	2.5			5.6	3.5
						5.8	1.7			5.9	3.9

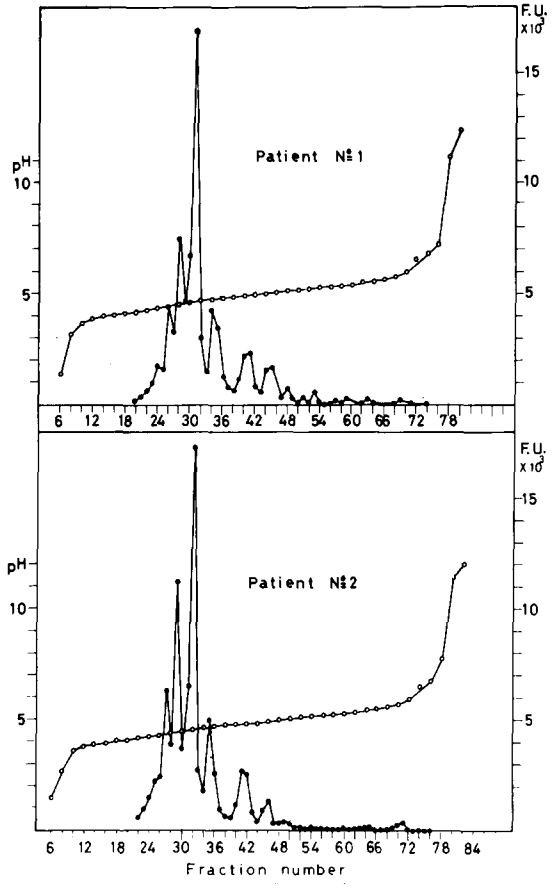

Fig. 4. Isoelectrofocusing (performed as in Fig. 3) on serum of patients no. 1 and 2. In this experiment the column was loaded with 3 ml of serum containing 0.05 mU of activity and 200 mg of protein. The assay was run as described under Methods, for 48 h. Fluorescence units (F.U.), ●———●; pH, ○———○.

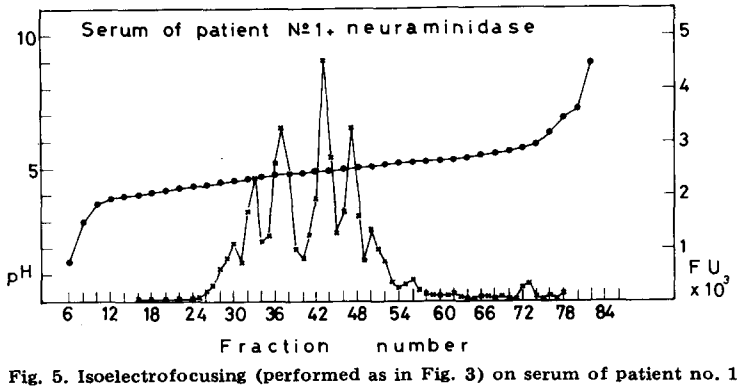

Fig. 5. Isoelectrofocusing (performed as in Fig. 3) on serum of patient no. 1 after treatment with neuraminidase Fluorescence units (F.U.), X———X; pH, ●———●.

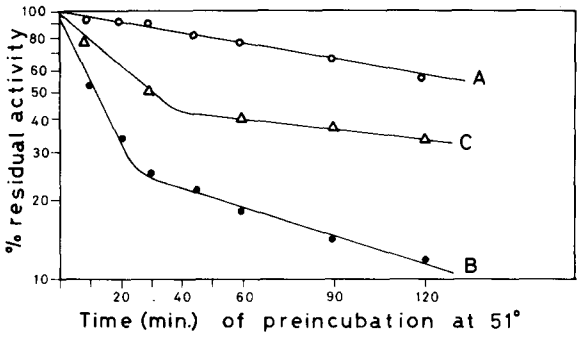

Fig. 6. Kinetics of thermal inactivation of α-fucosidase in serum of one representative control (A) and of patients no. 1 (B) and no. 2 (C).

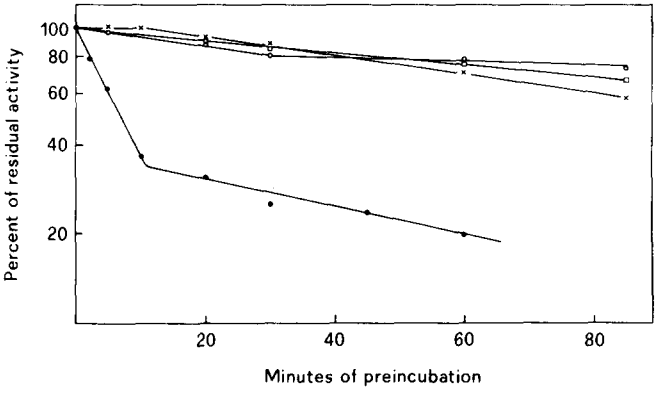

Fig. 7. Kinetics of heat inactivation of α-fucosidase in extracts of fibroblasts from 3 controls (single rate of decay) and from patient no. 1 (double rate of decay).

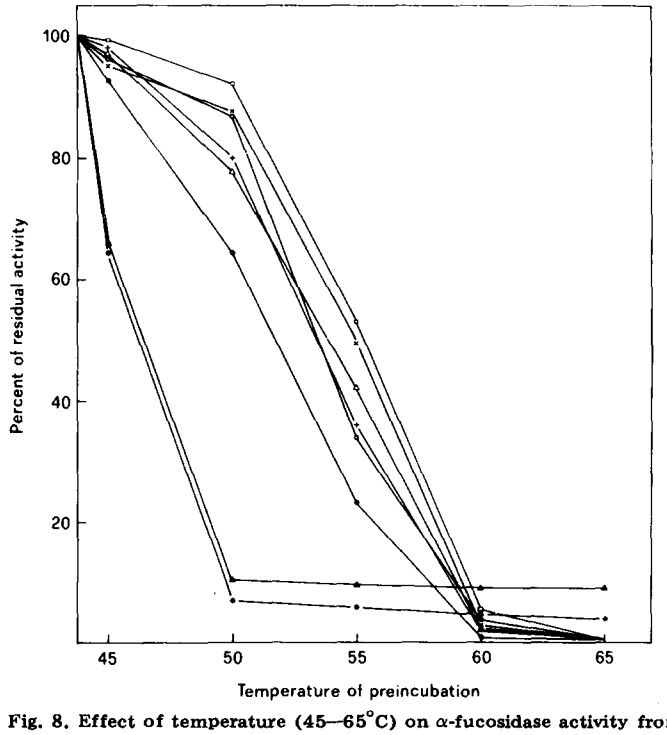

Fig. 8. Effect of temperature (45—65°C) on α-fucosidase activity from sera of patient no. 1 and 2 (* and △) and controls. Preincubation time at the temperatures indicated was 5 min.

completely similar to that found in sera, has been noticed in fibroblast extracts from patient n° 1 and controls (Fig. 7).

The rate of thermal inactivation tested at different temperatures (45—70°C) showed a similar behaviour, the sera from patients being more thermolabile than those from controls (Fig. 8). Mixing experiments between the controls and patients sera did not indicate the presence of an inhibitor for α-fucosidase activity in the latter sera.

Discussion

The deficiency of α-fucosidase activity can be assayed in patients affected with fucosidosis using both the Nph-Fuc (*p*-NO_2-phenyl-α-fucoside) or the Me-Umb-Fuc. However, one should be aware that neither the homozygotes nor the heterozygotes can be properly detected with serum assays only, since a marked deficiency of serum α-fucosidase is found in some controls (Fig. 1, Group B). The serum assays can therefore constitute only a rough screening procedure for detection of patients affected with fucosidosis, provided that the leukocytes of those subjects showing low serum activity are also tested. The same hereditary deficiency present in serum only has already been noticed in the assaying the activity of α-fucosidase with the Nph-Fuc substrate [12]. On the other hand, this kind of serum specific deficiency cannot be ascribed to the heterozygous state for fucosidosis, since these individuals consistently show normal activity in leukocytes. Its nature is therefore still obscure and could be related to an alteration of the physiological mechanism by which this enzyme is secreted into the extracellular compartment.

The behaviour of serum α-fucosidase after neuraminidase treatment is interesting, when compared to that of other tissues. The four major peaks found in controls closely correspond, after neuraminidase treatment, to the four major ones (2—5) of placenta [8] having p*I* values of 5.39 (±0.04), 5.63 (±0.06), 5.94 (±0.07) and 6.24 (±0.07). On the contrary, no noticeable shift in p*I* occurs in the major peaks of placenta [8] liver [4] and lymphoid cells [5] when treated with neuraminidase. This finding suggests that at least the heterogeneity of α-fucosidase observed between serum and other tissues can be due to the binding of sialic acid residues. The two patients of this study are related to the first two patients described as affected with fucosidosis [1]. They do show, however, a milder course of the disease and one of them, the 18 year-old boy, has angiokeratoma [4]. Since it is highly unlikely that two independent rare mutations affecting the same gene are present in one pedigree, this kind of clinical heterogeneity could be caused by the interaction with other loci, in particular with those responsible for the synthesis of fucose containing blood-group substances (H and Lewis). This hypothesis is now being investigated.

The single major peak found in the two patients has a p*I* corresponding to that of the most acidic peak observed in controls. Even after neuraminidase treatment, the peaks found in the two patients did not correspond to those of the controls treated in the same way. A similar finding has been reported for the residual activity of α-galactosidase in Fabry's disease, which appears as a single

acidic peak on isoelectrofocusing in contrast to the multiple peaks found in controls [13].

The hypothesis of interconversion of the thermolabile and thermostable forms of α-fucosidase discussed in the preceeding paper [8] could also help in explaining the data presented here. Following such a hypothesis, the α-fucosidase found in patients could structurally be so altered that the higher molecular weight aggregates would form in a relative lesser proportion with respect to the enzyme found in control individuals. In this way, one could justify why the kinetics of heat inactivation show a double rate of decay in patients affected with fucosidosis. In addition, the slightly different K_m values found in sera of the two patients with respect to controls, together with the different behaviour after neuraminidase treatment, could also be suggestive of structural alteration of the enzymatic molecule.

References

1 Durand, P., Borrone, C. and Della Cella, G. (1966) Lancet ii, 1313
2 Dawson, G. and Spranger, J.W. (1971) N. Engl. J. Med. 285, 122
3 Chen Tsay, G. and Dawson, G. (1975) Biochem. Biophys. Res. Comm. 63, 807—814
4 Alhadeff, A.J., Miller, A.L., Wenger, A.D. and O'Brien, J.S. (1974) Clin. Chim. Acta 57, 307—313
5 Turner, B.M., Beratis, N.G., Turner, S.V. and Hirschhorn, K. (1974) Clin. Chim. Acta 57, 29—35
6 Thorpe, R. and Robinson, D. (1975) FEBS Lett. 54, 89—92
7 Borrone, C., Gatti, R., Trias, X. and Durand, P. (1974) J. Pediatrics 84, 727—730
8 Di Matteo, G., Orfeo, A. and Romeo, G. (1976) Biochim. Biophys. Acta 000, 000—000
9 Romeo, G., Di Matteo, G., D'Urso, M., Su-Chen Li and Yu-Teh Li (1975) Biochim. Biophys. Acta 391, 349—360
10 Cuatrecasas, P. and Illiano, G. (1971) Biochem. Biophys. Res. Comm. 44, 178—184
11 Romeo, G. and Migeon, B.R. (1970) Science 170, 180—181
12 Ng, W.G., Donnel, G.N. and Kock, R. (1973) Pediatr. Res. 7, 391—163 (Abs)
13 Ho, M.W., Beutler, S., Tennant, L. and O'Brien, J.S. (1972) Amer. J. Human Gen. 24, 256—266