Biochimica et Biophysica Acta, 403 (1975) 473—476

BBA 67619

THE OCCURRENCE OF LOW α-L-FUCOSIDASE ACTIVITIES IN NORMAL HUMAN SERUM

PETER RAMAGE* and WILLIAM L. CUNNINGHAM†

Department of Brewing and Biological Sciences, Heriot-Watt University, Chambers Street, Edinburgh (U.K.)

(Received May 30th, 1975)

Summary

The existence of very low activities of α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) in some normal human sera is reported. The mean value for these sera is less than 10% of the corresponding mean for other normal sera. A preliminary experiment suggests that the low-activity sera do not contain inhibitors of the enzyme. Activities in sera prepared from the same individuals on separate occasions are remarkably consistent.

During studies on the activities of human serum acid-hydrolases in mucopolysaccharidosis, the establishment of appropriate normal ranges revealed remarkable and hitherto unreported variations in the activity of α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51). Several biochemical studies on mucopolysaccharidosis have involved the comparative analysis of serum acid-hydrolase levels, often including α-L-fucosidase [1,2]. At least one inherited storage disorder, Fucosidosis, exists in which this enzyme is deficient both in the liver [3] and in serum [4]. Consequently, we feel that attention should be drawn to the occurrence of unusually low levels of activity in the sera of normal individuals.

Blood samples from fasting donors were collected into glass tubes and sera were separated from the blood clots within 3 h of collection. Sera were subdivided into several aliquots and stored in glass vials at −10°C for up to two weeks without significant loss of activity, in agreement with the observations of Zielke et al. [4]. Enzyme assays were carried out within one to two weeks on sera which had been frozen only once using a modification of the procedure described by Ockerman [1]. Each incubation mixture contained 0.1 ml of 10

* Present address: Department of Agricultural Science, University of Oxford, Oxford, U.K.
† Present address: Department of Medicine, Queens University, Kingston, Ontario, Canada.

mM *p*-nitrophenyl-α-L-fucopyranoside (Sigma); 0.1 ml of 1.0 M sodium acetate buffer, pH 5.5; and 0.05 ml of serum. After 2 h at 37 °C the reaction was stopped by the addition of 0.2 ml of 5% (w/v) trichloroacetic acid and precipitated proteins were removed by centrifugation. To 0.2 ml of the supernatant solution was added 0.1 ml of 0.5 M NaOH and 0.7 ml of sodium glycinate buffer, pH 10.7 [1] and the absorbance read at 400 nm. Calibration graphs were prepared from *p*-nitrophenol (Sigma) dissolved in the sodium glycinate buffer. Reaction rates were directly proportional to serum concentration over the range employed. α-D-Mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24) was measured using the same method except that *p*-nitrophenyl-α-D-mannopyranoside was used as substrate. The assay of *N*-acetyl-β-D-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase EC 3.2.1.30) was accomplished essentially by the procedure of Ockerman [1] except that the acetate buffer was replaced with sodium citrate, pH 5.0 (final concentration 0.15 M). These enzymes were also stable during extended periods of storage at −10 °C. Enzyme activity units are expressed as μmol *p*-nitrophenol released per l serum per min.

Values given in Table I include those of three siblings suffering from a variant of mucopolysaccharidosis (Morquio syndrome) and that of one patient with Downe's syndrome. They are included because the activities fall within the normal spectrum. However, even if the donors with clinical disorders are excluded then three completely normal individuals from a total of 35 possess a

TABLE I

ACID-HYDROLASE ACTIVITIES IN HUMAN SERA

The two series of values (I and II) represent activities in sera collected from the donors on two separate occasions. Excluding the sera with the low α-L-fucosidase activities, those of 32 remaining donors produced mean values (± S.E.) of 4.16 ± 0.30, 1.10 ± 0.03 and 15.49 ± 0.63 for α-L-fucosidase, α-D-mannosidase and *N*-acetyl-β-D-glucosaminidase, respectively. Mean for the low activity sera given in the table = 0.32 ± 0.05.

Donor	Sex	Activity (μmol/min)					
		α-Fucosidase		α-Mannosidase		*N*-Acetyl-β-D-glucosaminidase	
		I	II	I	II	I	II
1	F	8.28	7.79	0.88	1.07	16.30	20.15
2	F	2.28	2.38	0.91	0.87	11.82	14.73
3	F	2.63	1.94	0.91	0.83	11.82	10.93
4	F	4.66	5.26	0.90	1.03	17.08	20.06
5	F	6.16	5.62	1.20	1.09	14.59	16.00
6*	F	2.92	3.05	0.76	0.90	13.10	12.59
7*	M	9.32	10.01	0.67	ND	14.04	ND
8*	F	0.32	0.07	0.83	1.04	13.56	14.22
9	F	0.42	0.41	1.21	1.21	16.91	15.43
10	F	0.31	ND	1.50	ND	18.91	ND
11	M	0.28	ND	1.22	ND	21.40	ND
12**	F	0.41	ND	1.60	ND	13.59	ND

* Mucopolysaccharidosis.
** Downe's Syndrome.
ND, Not determined.

TABLE II

EFFECT OF MIXING SERA WITH LOW AND HIGH α-L-FUCOSIDASE ACTIVITIES

25 μl each of a and b were assayed separately. Equal volumes of the two sera were mixed and 50 μl assayed (a + b).

Serum	Vol. per assay (μl)	*p*-Nitrophenol released (μg)
a	25	0.01
b	25	2.84
a + b	50	2.78

mean serum α-L-fucosidase activity less than 10% of that of the other normals. Attempts to correlate these low activities with age, sex, ABO/Rhesus blood groupings or even with transient infections were unsuccessful. When different aliquots of the same serum sample were analysed, identical values were obtained. This provides some evidence against errors in experimental manipulation, spurious inactivation of sera in particular. In addition, and of greater significance, is the observation that individuals of both normal groups maintained very consistent activities in sera collected on separate occasions, also seen with α-D-mannosidase and *N*-acetyl-β-D-glucosaminidase. Furthermore, the latter enzymes did not reflect the unusual distribution of α-L-fucosidase activities. The α-D-mannosidase values are of particular importance in this respect since the assay procedure was the same as that described for α-L-fucosidase. The possible existence of inhibitors of the latter enzyme in low-activity serum was investigated by assaying mixtures of low and high-activity sera and comparing the values obtained with those of appropriate controls (Table II). Although the activity of the mixture in this typical experiment is slightly less than the sum of the individual activities, the degree of inhibition apparent is not significant. Consequently it is unlikely that the low activities found in some normal sera reflect the presence of inhibitors.

It is not possible to reconcile a marked general deficiency of α-L-fucosidase in normal individuals with the existence of an inherited storage disorder caused by the absence of this enzyme, i.e. Fucosidosis [3]. Two isoenzymes of α-L-fucosidase have been detected in normal human liver and serum [5,6]. Of the total enzyme activity isolated from liver about 75% is represented by the higher molecular weight component, Fucosidase I, and 25% by Fucosidase II, whereas in serum the activities of the two isoenzymes are about equal [6]. Consequently, if most cells of the body possess similar ratios of the two isoenzymes to that of liver, it seems likely that they are not released into the bloodstream with equal facility. This suggests in the first instance that the rate of release of these enzymes into the blood is some function of their structure. Hence one interpretation of our results is that structural alterations to one or both of the isoenzyme components, or to the cellular machinery involved in their transport, in some way interferes with their release, at least into serum, in a small proportion of normal humans. The extent to which the phenomenon is associated with the blood coagulation event is not clear, but the α-L-fucosidase activity in the plasma of one donor (in the higher normal group) was identical with the serum value (cf. ref. 4) as indeed was the case with the other acid-

hydrolases measured in this instance. Irrespective of the true explanation for the very low normal values our data strongly suggest that when screening patients for Fucosidosis, serum α-L-fucosidase activities should be interpreted with caution.

Acknowledgements

We gratefully acknowledge the cooperation of the Blood Transfusion Service, Royal Infirmary of Edinburgh, of Dr G. Stark of the Royal Hospital for Sick Children, Edinburgh, and of Dr J. Watt of the Royal Dundee Liff Hospital. We thank the Science Research Council for the provision of a research studentship (Grant No. B/69/513) to P.R.

References

1 Ockerman, P.A. (1968) Clin. Chim. Acta 20, 1—6
2 Gordon, B.A. and Feleki, V. (1970) Clin. Biochem. 3, 193—202
3 Van Hoof, F. and Hers, H.G. (1968) Eur. J. Biochem. 7, 34—44
4 Zielke, K., Okada, S. and O'Brien, J.S. (1972) J. Lab. Clin. Med. 79, 164—169
5 Robinson, D. and Thorpe, R. (1973) Clin. Chim. Acta 47, 403—407
6 Robinson, D. and Thorpe, R. (1974) Clin. Chim. Acta 55, 65—69