Thermostability of α-mannosidase in plasma from cystic fibrosis patients and carriers

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We investigated the thermostability at 41°C of human plasma α-mannosidase in two cystic fibrosis patients, one carrier, and two healthy adults. We could not confirm the reported differences between these groups of subjects.

Human disease *Cystic fibrosis* *Pathogenetic mechanism* *Lysosomal enzyme* *α-Mannosidase*

1. INTRODUCTION

The molecular defect in cystic fibrosis (CF), the most common lethal inherited disease of whites [1], is unknown. One of the many metabolic abnormalities observed in the disease [2,3] seems to affect lysosomal enzymes. One of them, an acid α-mannosidase, was reported to show a decreased thermostability in patients and, to a lesser degree, in carriers, suggesting a close relation to the basic defect [4]. Because of the potential interest of these investigations for understanding the pathogenesis of CF, and since our laboratory routinely deals with lysosomal enzyme deficiencies, we repeated the α-mannosidase thermolability studies in plasma from patients cared for at our own institution.

2. SUBJECTS AND METHODS

Patient no.1 was a 2-month-old boy suffering from meconium ileus and pneumonias. After his death a few weeks after the study, the diagnosis of CF was confirmed by histological examination of the pancreas. Patient no.2 was a 12-year-old boy with typical severe pulmonary changes and pancreatic insufficiency. [NaCl] in sweat was elevated to levels diagnostic for CF in both patients on several occasions. The mother of patient no.1 and both controls were apparently healthy.

Blood sampling, preparation of plasma, heat inactivation, and measurement of α-mannosidase activity with a fluorogenic substrate were done exactly as in [4], following all the precautions mentioned. Experience with some delicate aspects of the procedures was gathered in preliminary experiments, until reproducible results were obtained.

Abbreviation: CF, cystic fibrosis

3. RESULTS

Residual activity of plasma α-mannosidase after various lengths of incubation at 41°C is shown in table 1. A difference that would distinguish patients or carriers from controls was not detectable.

4. DISCUSSION

Our inability to confirm an abnormal thermolability of α-mannosidase activity in plasma of CF-patients adds to a long list of observations in CF that have been difficult to reproduce in other laboratories [2,3]. Contradicting results of α-mannosidase thermostability in different laboratories can be explained in the following ways:

(i) Technical details have not been standardized sufficiently; e.g., some types of plastic tubes

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Table 1

Heat-inactivation at 41°C of α-mannosidase in plasma from two CF-patients, one carrier and two controls

Subjects	Time of inactivation (min)					
	40	80	120	160	200	240
Patient no.1 (age 2 months)	83	79	79	72	65	69
Patient no.2 (age 12 years)	100	98	98	84	85	85
Mother of patient no.1	86	80	75	67	69	66
Control no.1 (adult)	77	80	76	73	71	58
Control no.2 (adult)	72	76	76	66	69	60

Residual enzyme activity in % of activity without heat-inactivation

appear to be toxic (P. Hösli, personal communication);

(ii) α-Mannosidase thermolability is not very closely related to the basic defect in CF;

(iii) Genetic heterogeneity of CF is possible.

Since investigation of lysosomal abnormalities may yield potentially valuable information for understanding CF, we suggest that workers willing to reproduce studies of the kind referred to in this paper address directly to the laboratory where the original work was done.

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REFERENCES

[1] Wood, R.E., Boat, T.F. and Doershuk, C.F. (1976) Am. Rev. Respir. Dis. 113, 833–878.

[2] Davis, P.B. and Di Sant'Agnese, P.A. (1980) Pediatr. Res. 14, 83–87.

[3] Littlefield, J.W. (1981) New Engl. J. Med. 304, 44–45.

[4] Hösli, P. and Vogt, E. (1979) FEBS Lett. 104, 271–274.