Acta derm.-venereol. 49: 139–141, 1969

ACID HYDROLASES IN HUMAN SKIN

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The presence of lysosomes in human skin is becoming established (1). Evidence has been presented of: (i) the occurrence of acid phosphatase activity within membrane-bound structures as seen in the electron microscope (8), (ii) the presence of activity of acid hydrolases in a sedimentable fraction with high specific activity and structure-linked latency (2) and (iii) the presence of lysosome-like granulae as seen in the electron microscope (8, 9).

A few enzymes wholly or partly associated with the lysosomes in the liver have earlier been found in human skin, e.g. acid phosphatase, β-glucuronidase, cathepsin and arylsulphatase (1, 2, 8, 10). In this communication evidence is presented of several other acid hydrolytic enzyme activities in human skin.

Material and Methods

The sampling and homogenization of tissue and the procedures for assay of β-galactosidase, β-glucuronidase, β-acetyl-glucosaminidase, α-mannosidase, α-fucosidase, β-glucosidase, β-xylosidase and acid phosphatase were as described in previous separate communications (5, 6, 7). For the assay of the hydrolytic and transferase activities of α-glucosidase the methods of Hers and Van Hoof (3) were used. For some enzyme activities longer incubation and/or more concentrated homogenates than those described had to be used, since the enzyme activities were lower than in other tissues. Protein was assayed according to Lowry *et al.* (4). The following buffers were used: glycine-HCl, 1 M, (pH 2.0–2.5), acetate, 1 M, (pH 3.0–5.5), imidazol, 0.2 M, (pH 6.0–8.0).

For the separation of subcellular particles a method worked out for liver (7) was used.

Results

Several acid hydrolytic enzyme activities could be demonstrated in human skin (table 1). The pH optimum was found to be pH 4.4–5.4 (table 1, fig. 1 A–B). In the ultracentrifugal experiments the highest relative specific activity of acid phosphatase was found in the supernatant fraction (fig. 2). Possibly, also the lysosomal fraction had a relative specific activity higher than one. No definite interpretation can be made of these findings. Possibly, acid phosphatase is—at least to some extent—localized to very fragile particles in the lysosomal fraction. All attempts failed, however, to isolate with gentle methods such acid phosphatase-containing particles better than in the experiment described in fig. 2.

Discussion

The presence of activity of β-galactosidase in normal human skin and a deficiency of this activity in patients with gargoylism was recently suggested (5). Two other acid hydrolases, β-glucuronidase and β-acetyl-glucosaminidase, had normal or increased activities in skin from gargoylism patients (5). These findings indicate that acid hydrolytic enzyme activities are changed in skin in one storage disease. There would, consequently, seem to be some possibility

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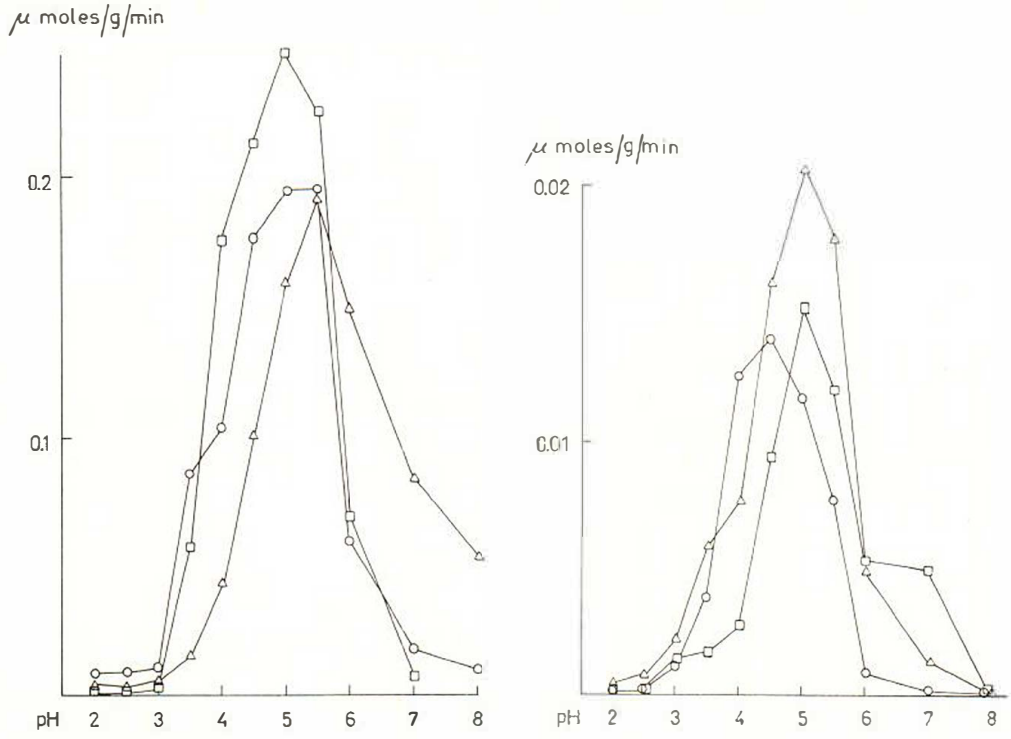

Fig. 1. pH-dependency of activities of acid hydrolases.

All figures given in μmoles substrate split/g wet weight/min.

A. ○—○ α-fucosidase
△—△ α-mannosidase
□—□ β-acetylglucosaminidase

B. ○—○ β-galactosidase
△—△ β-glucosidase
□—□ β-xylosidase (scale enlarged ten times).

Table 1. *Activity of acid hydrolases in human skin. When more than one assay was made, mean values are given*

	[a] μmoles substrate split/g protein/min. [b] μmoles substrate split/g wet weight/min. [c] % incorporation of ^{14}C — maltose/g wet weight/min.	pH-optimum
1. β-galactosidase,[d] n=11, Mean ± S.D.	[a] 0.390 ± 0.196	4.4
2. β-glucosidase, n=2	[b] 0.0168	5.0
3. α-glucosidase (maltose), n=1	[b] 0.0693	—
4. α-glucosidase (maltotransferase), n=1	[c] 0.0216	—
5. β-glucuronidase,[d] n=11, Mean ± S.D.	[a] 0.178 ± 0.140	—
6. β-acetylglucosaminidase,[d] n=11, Mean ± S.D.	[a] 2.65 ± 1.31	5.0
7. acid phosphatase, n=1	[a] 7.71	—
8. α-mannosidase, n=2	[b] 0.125	5.4
9. α-fucosidase, n=2	[b] 0.458	5.2
10. β-xylosidase, n=2	[a] 0.230	5.0

[d] From Öckerman (5)

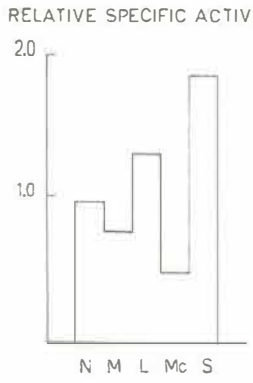

Fig. 2. Subcellular localization of acid hydrolases. A homogenate 1/10 in ice-cold 0.25 M sucrose was prepared from fresh human skin obtained by biopsy at operation for cholelithiasis. The homogenate was centrifuged at 0° in a Spinco model L ultracentrifuge as described in METHODS. Before analysis the sediments were suspended in water. N=nuclear fraction. M=mitochondrial fraction. L=lysosomal fraction. Mc=microsomal fraction. S=supernatant fraction. Relative specific activity (7)= $\frac{\text{percentage activity}}{\text{percentage protein}}$.

to use assay of these enzymes in the study of gargoylism and, possibly, other storage diseases. This was the motive for the present study of acid hydrolases in normal human skin. Several of the enzyme activities found here have not earlier been demonstrated in human or other mammalian skin.

SUMMARY

Normal human skin was used for the assay of several acid hydrolytic enzyme activities. The existence could be shown of activity of β-galactosidase, β-glucosidase, α-glucosidase (both hydrolytic and transferase activity), β-glucuronidase, β-acetyl-glucosaminidase, acid phosphatase, α-mannosidase, α-fucosidase and β-xylosidase. The pH-optimum was found to be between pH 4.4 and 5.4 in the six activities for which it was measured. Acid phosphatase had its highest specific activity in the supernatant and in a sedimentable subcellular particle fraction.

Acknowledgements

The skilful technical assistance of Miss S. Olsson and Miss M. Sjöland is gratefully acknowledged. Financial support was given by The swedish Medical Research Council (Grant No B68-13X-2307-01), the University of Lund and Förstamajblommans Riksförbund.

REFERENCES

1. Bagatell, F. K. and Dimitrow, K.: Lysosomes and acantholysis in human skin. *Acta derm.-venereol. 47:* 181, 1967.
2. Dicken, C. H. and Decker, R. H.: Biochemical evidence for the presence of lysosomes in the epidermis. *J. Invest. Derm. 47:* 426, 1966.
3. Hers, H. G. and Van Hoof, F.: Enzymes of glycogen degradation in biopsy material. *Methods in Enzymology 8:* 525, 1966.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin-phenol reagent. *J. Biol. Chem. 193:* 265, 1951.
5. Öckerman, P. A.: Acid hydrolases in skin and plasma in gargoylism. Deficiency of β-galactosidase in skin. *Clin. Chim. Acta 20:* 1, 1968.
6. Öckerman, P. A.: Identity of β-glucosidase, β-xylosidase and one of the β-galactosidase activities in human liver when assayed with 4-methylumbelliferyl-β-D-glycosides. Studies in cases of Gaucher's disease. *Biochim. Biophys. Acta 165:* 59, 1968.
7. Öckerman, P. A.: Glucose-6-phosphatase in human endometrium. *Acta Obst. & Gyn. Scand.,* in press.
8. Olsson, R. and Nordquist, R.: Ultramicroscopic localization of acid phosphatase in human epidermis. *J. Invest. Derm. 46:* 431, 1966.
9. Prose, P. H., Sedlis, E. and Bigelow, M.: The demonstration of lysosomes in the diseased skin of infants with infantile eczema. *J. Invest. Derm. 45:* 488, 1965.
10. Záruba, F., Uher, J., Koldovsky, O. and Heringová, A.: The activity of β-glucuronidase in human fetal skin. *Acta derm.-venereol. 47:* 123, 1967.