

Brief Communication

Normal Adult with Absent HEX A: Immunoreactive HEX A Is Present

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SUMMARY

Fibroblasts from a normal adult with absent hexosaminidase A (HEX A) activity were demonstrated to possess immunoreactive HEX A as measured by G_{M2} β -D-N-acetylgalactosaminidase activity which precipitated with specific anti-HEX A antibodies. Possible explanations for the molecular defect are presented.

INTRODUCTION

We have studied an unusual variant (D. S.), a normal adult male lacking HEX A activity using synthetic substrates and half-normal activity using ganglioside G_{M2} as substrate [1]. Pedigree studies indicate that D. S. is an allelic compound, HEX A 2-5 (in the American Journal of Human Genetics nomenclature [2]), where HEX A 2 is the Tay-Sachs disease (TSD) (or similar) mutation, and HEX A 5 is a mutation which leads to the production of HEX A which retains activity for ganglioside G_{M2} but not for synthetic substrates.

The correctness of this proposal depends upon the demonstration that the enzyme with G_{M2} β -D-N-acetylgalactosaminidase activity in D. S.'s fibroblasts is indeed HEX A. To this end, we have carried out studies with antibodies specific to HEX A and have demonstrated that the G_{M2} β -D-N-acetylgalactosaminidase activity in D. S. has the physicochemical and immunochemical characteristics of normal HEX A.

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MATERIALS AND METHODS

Subject D. S. was described in an earlier report [1]. His fibroblasts, obtained by skin biopsy, were cultured as described previously [3]. Total hexosaminidase activities were estimated with 4-methylumbelliferyl- β -D-N-acetylglucosaminide (4MU-GlcNAc) [3]. Ganglioside G_{M2} β -D-N-acetylgalactosaminidase activity was assayed as described [4]. The batchwise method of Dance et al. [5] employing DEAE ion-exchange, was used to isolate HEX A and B from fibroblasts, and each was free from one another as determined by electrophoresis [6]. Control fibroblasts were cultured and assayed in the same manner as those from D. S.

Antibodies specific exclusively for HEX A were obtained by adsorption of the IgG fraction of goat anti-human placental HEX A on Sepharose-immobilized HEX B. Antibodies to HEX B were also prepared. Details of the antigen purification and the characterization of the different antibody preparations were described previously [7, 8].

Immunoprecipitation of HEX A and B from fibroblast supernates or separated HEX A and B fractions were carried out as previously described for β -galactosidase [9]. In brief, this involved incubation (17 hr) of antibody with enzyme, centrifugation at 14,000 g for 30 min, and assay of the supernate with the appropriate substrate.

RESULTS

The addition of specific anti-HEX A to HEX A or B separated from normal fibroblasts by batchwise ion-exchange chromatography resulted in precipitation of HEX A but not HEX B (fig. 1). Conditions were selected to give a similar number of enzyme units of each prior to immunoprecipitation. G_{M2} β -D-N-acetylgalactosaminidase activity closely paralleled HEX A activity during immunoprecipitation.

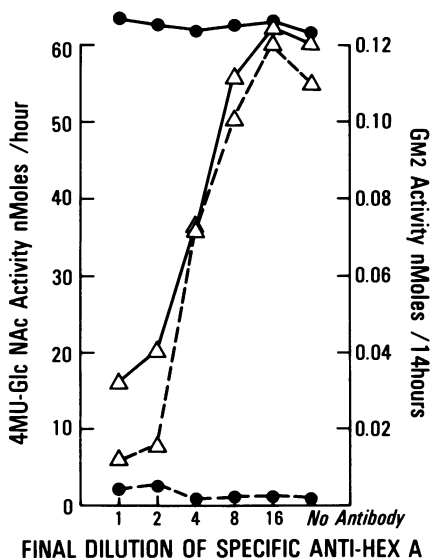


FIG. 1.—Immunoprecipitation of normal fibroblast HEX A and B with specific anti-HEX A. HEX A (Δ) and HEX B (\bullet) were isolated by batchwise ion-exchange chromatography from normal fibroblasts and subjected to immunoprecipitation with increasing dilutions of anti-HEX A. 4MU-GlcNAc (solid lines) and G_{M2} β -D-N-acetylgalactosaminidase activities (dashed lines) were measured after 30 min and 17 hr, respectively, and the supernatant activity was expressed as indicated. Note that HEX B has little G_{M2} β -D-N-acetylgalactosaminidase activity, and G_{M2} β -D-N-acetylgalactosaminidase activity parallels HEX A activity.

Traces of G_{M2} β -D-*N*-acetylgalactosaminidase activity were associated with HEX B, but did not exceed 1% of that of HEX A. Under the conditions employed, the ratio of activity for cleaving 4MU-GlcNAc to G_{M2} was 7,000 to 1. This ratio is higher than the 1,000 to 1 found previously [4] and is apparently due to the long incubation time used here (14 hr) giving a non-linear assay. When specific anti-HEX A was incubated with either HEX A or B, and assays were carried out prior to centrifugation, there was no inhibition or stimulation of 4MU-GlcNAc or G_{M2} cleaving activity.

When unfractionated fibroblast supernates from patient D. S. were treated with specific anti-HEX A, G_{M2} β -D-*N*-acetylgalactosaminidase activity was quantitatively immunoprecipitated, but 4MU-GlcNAc activity was not (fig. 2). Treatment of the supernate by batchwise column chromatography revealed that D. S.'s G_{M2} β -D-*N*-acetylgalactosaminidase activity bound and eluted from the column with the HEX A fraction, while the 4MU-GlcNAc activity eluted with the HEX B fraction. Anti-HEX B also quantitatively immunoprecipitated D. S.'s G_{M2} β -D-*N*-acetylgalactosaminidase activity (not shown).

DISCUSSION

These results demonstrate that in normal human fibroblasts G_{M2} β -D-*N*-acetylgalactosaminidase activity is associated with an enzyme having the charge characteristics and immunoreactivity of normal HEX A. The reaction with anti-HEX A (or anti-HEX B) does not impair the rate of G_{M2} cleavage by HEX A, which is in agreement with previous results with synthetic substrates [7]. This may indicate indirectly that the catalytic site is shallow and probably is not a part of an antigenic determinant. These results are consistent with the proposal [6] that HEX A specifically cleaves ganglioside G_{M2} and HEX B does not, but both isozymes cleave 4MU-GlcNAc.

A similar phenomenon, namely the apparent absence of HEX A activity (toward 4MU-GlcNAc) without any apparent related pathological manifestations had been described by Navon et al. [10]. It was later reported, however, that these variants exhibit half-normal G_{M2} -cleaving activity [11], and that HEX A in low but significant

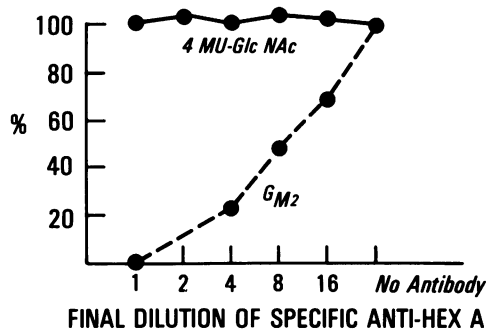


FIG. 2.—Immunoprecipitation of D. S.'s fibroblast supernate with specific anti-HEX A. 4MU-GlcNAc activity (solid line) and G_{M2} β -D-*N*-acetylgalactosaminidase activity (dashed line) were measured in the supernate after immunoprecipitation with increasing dilutions of anti-HEX A, and activities were expressed as % of original supernate. Note that G_{M2} β -D-*N*-acetylgalactosaminidase activity is precipitated, but 4MU-GlcNAc activity is not.

concentration (about 6% of the total hexosaminidase activity) could be demonstrated in tissues and cultured cells of the variant individuals [12].

In order to explain the molecular defect in D. S.'s HEX A, the functional roles of the different subunits of the human hexosaminidases must be considered. It has been proposed that three forms of the enzyme: HEX B, HEX S, and HEX A, differ in their subunit composition [13–15], the first two being homoligomers of beta and of alpha chains ($\beta_2\beta_2$ and $\alpha_2\alpha_2$, respectively), and the latter having both alpha and beta chains ($\alpha_2\beta_2$). All three isozymes have similar kinetic properties with respect to the hydrolysis of synthetic substrates [13, 15]. Thus, both types of subunits, when arranged as tetramers, possess similar catalytic site(s) for synthetic substrates. Their similarity is further corroborated by the low, but highly significant antigenic cross reactivity between HEX B and S [15], implicating certain homology of alpha and beta chains. With respect to the G_{M2} -cleaving site, two possibilities can be considered: (1) the α_2 subunit of HEX A contains the G_{M2} -cleaving site (and perhaps that for the other substrates as well), whereas the β_2 subunit bears a catalytic site which has activity toward the non- G_{M2} substrates, or (2) the α_2 subunit of HEX A does not function directly in the hydrolysis of G_{M2} , but rather serves as a "specifier" for G_{M2} with the actual catalytic site being on the β_2 subunit [16].

In either case, it is proposed that the interaction of subunit pairs in the HEX A tetramer has a profound effect upon the activity and substrate specificity of the holoenzyme.

In the case of D. S.'s HEX A, it seems unlikely that the defect resides in the catalytic site contributed by the β_2 chains, since his HEX B has normal physicochemical and enzymatic properties. Similarly, a major structural alteration in the catalytic or specifier properties of the α_2 chain is unlikely, since G_{M2} β -D-N-acetylgalactosaminidase activity of D. S.'s HEX A is retained. It is conceivable, as mentioned above, that subunit interactions between α_2 and β_2 pairs may have a profound effect on the enzymatic activity, and that a mutation of either subunit (in D. S., the alpha chain, more likely) at sites which determine subunit interaction, may result in abolishment of the 4MU-GlcNAc hydrolyzing capability without altering the α_2 subunit-directed, or catalyzed cleavage of G_{M2} .

Direct evidence to support or disprove these hypotheses requires structural analysis of pure mutant enzyme which is not yet possible due to the very small amounts of enzymes available.

REFERENCES

1. O'BRIEN JS, TENNANT L, VEATH ML, SCOTT CR, BUCKNALL WE: Characterization of unusual hexosaminidase A (HEX A) deficient human mutants. *Am J Hum Genet* 30:602–608, 1978
2. O'BRIEN JS: Suggestions for a nomenclature for the G_{M2} gangliosidoses making certain (possibly unwarrantable) assumptions. *Am J Hum Genet* 30:672–675, 1978
3. LEROY JG, HO MW, MACBRINN MC, ZIELKE K, JACOB J, O'BRIEN JS: I-cell disease: biochemical studies. *Pediatr Res* 6:752–757, 1972
4. O'BRIEN JS, NORDEN AGW, MILLER AL, FROST RG, KELLY TE: Ganglioside G_{M2} N-acetyl- β -D-galactosaminidase and asialo G_{M2} (G_{A2}) N-acetyl- β -D-galactosaminidase: studies in human skin fibroblasts. *Clin Genet* 11:171–183, 1977

5. DANCE N, PRICE RG, ROBINSON D: Differential assay of human hexosaminidases A and B. *Biochim Biophys Acta* 222:662–664, 1970
6. OKADA S, O'BRIEN JS: Tay-Sachs disease: generalized absence of a beta-D-N-acetylhexosaminidase component. *Science* 165:698–700, 1969
7. GEIGER B, NAVON R, BEN-YOSEPH Y, ARNON R: Specific determination of N-acetyl- β -D-hexosaminidase isozymes A and B by radioimmunoassay and radialimmunodiffusion. *Eur J Biochem* 56:311–318, 1975
8. GEIGER B, ARNON R: Hexosaminidase A and B from human placenta, in *Methods in Enzymology*, edited by GINSBURG V, New York, Academic Press, 1978 (in press)
9. O'BRIEN JS, NORDEN AGW: Nature of the mutation in adult β -galactosidase deficient patients. *Am J Hum Genet* 29:184–190, 1977
10. NAVON R, PADEH B, ADAM A: Apparent deficiency of hexosaminidase A in healthy members of a family with Tay-Sachs disease. *Am J Hum Genet* 25:287–293, 1973
11. TALLMAN JF, BRADY RO, NAVON R, PADEH B: Ganglioside catabolism in hexosaminidase A-deficient adults. *Nature* 252:254–255, 1974
12. NAVON R, GEIGER B, BEN-YOSEPH Y, RATTAZZI MC: Low levels of β hexosaminidase A in healthy individuals with apparent deficiency of this enzyme. *Am J Hum Genet* 28:339–349, 1976
13. GEIGER B, ARNON R: Chemical characterization and subunit structure of human N-acetylhexosaminidases A and B. *Biochemistry* 15:3484–3488, 1976
14. LEE JS, YOSHIDA A: Purification and chemical characterization of human hexosaminidases A and B. *Biochem J* 271:145–153, 1976
15. GEIGER B, ARNON R, SANDHOFF K: Immunochemical and biochemical investigations of hexosaminidase S. *Am J Hum Genet* 29:508–522, 1977
16. ROBINSON D, CARROLL M, STIRLING JL: Identification of a possible subunit of hexosaminidase A and B. *Nature* 243:415–416, 1973