Vol. 119, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1984 Pages 101-107

HEXOSAMINIDASE A DEFICIENT ADULTS: PRESENCE OF α CHAIN PRECURSOR IN CULTURED SKIN FIBROBLASTS

Amos Frisch*, Dana Baram** and Ruth Navon**

*Department of Biochemistry, Faculty of Life Sciences,
Tel-Aviv University
**Department of Human Genetics, Sackler Faculty of Medicine,
Tel-Aviv University and Sheba Medical Center,
Tel-Hashomer, Israel

Received January 4, 1984

Cultured skin fibroblasts from hexosaminidase A deficient adults synthesize the α and β chain precursors of β-hexosaminidase (EC 3.2.1.30) of the same molecular weight as that synthesized by normal fibroblasts. However, the amount of the α chain precursor is greatly reduced. The α chain precursor in secretions from these fibroblasts consists of 19% of the total β-hexosaminidase secreted compared to about 50% in normal cells. Attempts to increase the amount of detectable cellular α chain precursor by addition of protease inhibitors or by more extensive extraction methods have failed. Mature α chains were not detected. The presence of α chain precursor in fibroblasts from hexosaminidase A deficient adults can be used to distinguish between them and true Tay-Sachs disease homozygotes.

The lysosomal enzyme β-hexosaminidase (Hex) exists in human tissues as two isozymes: Hex A and Hex B. Hex A is a heteropolymer composed of two different polypeptide chains α and β while Hex B is a homopolymer composed of β chains only (1). The "natural history" of Hex in normal cultured fibroblasts was studied by Hasilik and Neufeld (2). They found that the α and β chains are synthesized as precursors of higher molecular weight (α= 67,000;β= 63,000) which are subsequently processed to the mature polypeptides (α= 54,000;β = 29,000 + smaller fragments).

In Tay-Sachs disease (TSD) the α chains are missing thus causing total deficiency of Hex A and accumulation of GM_2 gangliosides, which lead to degeneration of the nervous system and death in infancy (1). Deficiency of Hex A in adults was first reported in 1973 (3) and since then more cases were found (4 - 6). These Hex A deficient adults (HexADA) exhibit neurological

Abbreviations: Hex, β-hexosaminidase; TSD, Tay-Sachs disease, HexADA, hexosaminidase A deficient adult.

0006-291X/84 $1.50

symptoms which vary considerably between families as well as among members of the same family.

Elucidation of the molecular defect in HexADA should be of great value for antenatal diagnosis and for genetic counseling since it would enable a differentiation between them and classical TSD patients. The aim of the present study was to gain insight into the molecular defect in HexADA by following the biosynthesis and processing of the α and β chains of Hex in their cultured skin fibroblasts.

MATERIALS AND METHODS:

Cultured fibroblasts: Fibroblast cultures were established from 2 - 3 mm skin biopsies. The cells were maintained in Dulbecco's Modified Eagle Medium (GIBCO) supplemented with 12% fetal bovine serum and antibiotics.

Hex A activity: Hex A activity was measured in skin fibroblasts, serum and leucocytes by the heat inactivation method as previously described (7). Briefly, homogenates of cells were incubated for 3 hours at 50°C and Hex activity was measured before and after this heat treatment with the fluorogenic substrate 4-methylumbellifer 2-acetamido-2-deoxy-β-D-glucopyranoside (Koch-Light). Hex A activity was calculated as the percentage of heat-labile Hex activity (heat-labile activity x 100/total activity).

Biosynthesis and processing of Hex in cultured fibroblasts: Metabolic labeling of fibroblast cultures, immunoprecipitation of Hex, separation on gels and detection by fluorography were performed essentially according to Hasilik and Neufeld (2) substituting (^{3}H) leucine by (^{35}S) methionine with the following modifications: Prior to labeling the cells were rinsed twice with Dulbecco's Modified Eagle Medium (Biolab, Jerusalem) which contained low concentration of L-methionine (0.5µg/ml). The labeling medium for a 75 cm^2 flask contained 6 ml of the above low methionine medium, 0.3 ml dialysed fetal bovine serum (GIBCO) and 50 µCi L-(^{35}S) methionine (Amersham). The cells were pulse labeled for 3 hours and then chased for another 20 hours by addition of 75µg/ml of unlabeled L-methionine. Cells and media were collected at the end of the pulse (3 hours) or pulse/chase (23 hours). The Hex that was immunoprecipitated from homogenates made from one 75 cm^2 flask, was used for each slot on the polyacrylamide gel.

Goats' antibody against human placental Hex A was a generous gift from the laboratory of Dr. E.F. Neufeld.

RESULTS:

Hex A activity in HexADA: Nine HexADA from three unrelated Ashkenazi families were examined. Family A has been previously studied (3). Family B was referred through a neuromuscular clinic and family C was identified through the National Israeli Tay-Sachs Disease Screening Program. Hex A activity in serum, leucocytes and cultured skin fibroblasts of HexADA, as measured by the heat inactivation method, is very low and falls within the range of TSD homozygotes (Table I). Thus, the heat inactivation method cannot distinguish between true TSD homozygotes and HexADA. However, residual Hex A activity, significantly

Table I: Percent Hex A activity in HexADA serum, leucocytes and skin fibroblasts

	serum	leucocytes	skin fibroblasts
Family A			
Patient 1	0	10	9
2	12	0	2
3	0	8	1
4	8	5	8
Family B			
Patient 1	0	8	5
2	6	7	0
Family C			
Patient 1 (mother of patients 2&3)	3	2	7
2	0	1	11
3	6	7	10
TSD	0-13(n=31)	0-13(n=31)	0-13(n=13)
Heterozygotes for TSD	32-46(n=62)	37-54(n=91)	
Normal controls	48-64(n=77)	58-69(n=157)	

above the levels found in TSD homozygotes, has been detected in family A by more sensitive and direct methods (8).

Biosynthesis and processing of α and β chains of Hex in HexADA cultured skin fibroblasts: We studied the biosynthesis and processing of α and β chains in cultured HexADA skin fibroblasts from families A, B and C, as well as from TSD patients, obligate heterozygotes for TSD and normal controls. Fibroblasts of two HexADA from each of the three families were studied. Figure (1.3, 7) depicts the results from a representative one. As shown in Fig. 1.3, HexADA fibroblasts, unlike those of TSD patients, do synthesize the α chain precursor of the same apparent molecular weight (67,000) as that synthesized by normal cells. However, its quantity is smaller than that found in TSD heterozygotes. The β chain precursor appears normal in molecular weight and amount. Most HexADA fibroblasts chased for 23 hrs. did not contain the α chain either in precursor or in processed form (Fig. 1.7). However, in two cases, very small amounts of processed α chain were identified. It is worth noting that detection of processed α chain (Mr 54,000) is prone to artefacts from β chain intermediate (Mr 52,000). As a control, biosynthesis and processing of cathepsin D, another lysosomal enzyme, was studied in HexADA fibroblasts by

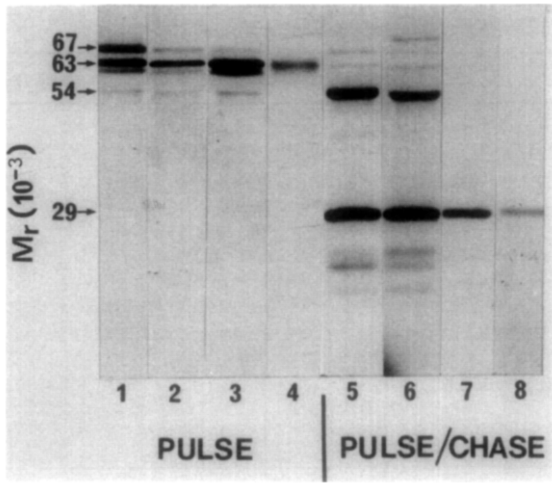

Fig. 1: Labeling of Hex from different cultured fibroblasts
Fibroblast cultures were labeled, Hex immunoprecipitated, separated on gel and visualized by fluorography as described under Materials and Methods.
Pulse: 1) normal control; 2) TSD heterozygote; 3) HexADA; 4) TSD homozygote.
Pulse/chase: 5) normal control; 6) TSD heterozygote; 7) HexADA; 8) TSD homozygote.

the same methods used for Hex. Biosynthesis and processing of cathepsin D in these cells was completely normal.

Quantitation of the α chain precursor in secretions of cultured HexADA fibroblasts: Fibroblasts from one HexADA and from control individuals were labeled and the Hex from all the cellular secretions was immunoprecipitated and separated by gel electrophoresis. Since fibroblast secretions are known to contain only the precursor forms of lysosomal enzymes (2), the bands corresponding to the α and β chains were excised and their radioactivity was measured directly. In the secretions from fibroblasts of three control individuals, the percentage of the α chain precursor $(\alpha \times 100)/(\alpha+\beta)$ was 47, 51 and 56 (average 51). In the HexADA, the α chain precursor in cellular secretions consisted of 19 percent (Table II).

Effects of protease inhibitors and extensive extraction methods on the amount of α chain precursor: The low amount of α chain in the HexADA cells might be due to its particular sensitivity to intracellular proteases. In an attempt to increase the amount of detectable α chain precursor a mixture of protease

Table II: Quantitation of the α and β chains in Hex secreted by different fibroblasts

Cell	C.P.M. in band α	C.P.M. in band β	$\frac{\alpha \times 100}{\alpha + \beta}$
Control I	1393	1553	47
Control II	1415	1093	56
Control III	1807	1738	51
HexADA	237	1024	19

Hex immunoprecipitated from secretions of different fibroblasts was separated on gel (see fig. 2). The bands corresponding to the α (Mr 76,000) and β (Mr 63,000) chain precursors were excised and their radioactivity measured as described (2).

inhibitors (leupeptin, antipain, pepstatin A, chymostatin (each 50 μg/ml) elastinal 30 μg/ml) was added to the medium 48 hrs. before labeling and throughout the pulse time. No increase in the amount of α chain precursor was observed (not shown). In another attempt, HexADA fibroblasts were extracted by a strong detergent (1% sodium dodecyl sulfate) according to the method of Proia and Neufeld (9) but the relative amount of the α chain precursor was not

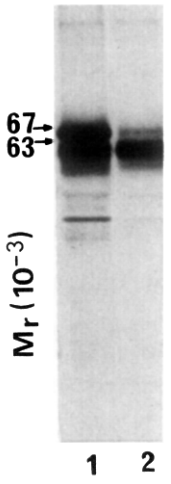

Fig. 2: Hex in secretions from different fibroblasts
Fibroblasts in 75 cm^2 flasks were continuously labeled for 22 hrs. Media containing the secretions were collected, concentrated and Hex was immunoprecipitated, separated on gel and visualized by fluorography as described under Materials and Methods.

1) Control secretions 2) HexADA secretions

increased (not shown). This excludes the possibility that α chain precursor of HexADA is tightly bound to cellular membranes as was found for non Jewish TSD (9).

DISCUSSION: The clinical manifestations in TSD and in HexADA are quite different. TSD patients die during infancy while HexADA reach adulthood and develop symptoms (mild to severe) only during the second decade of life. The routine heat inactivation method for measuring Hex A activity shows total lack of activity in fibroblasts from both TSD and HexADA patients and thus cannot be used to distinguish between them. Metabolic labeling together with immunological detection, the method used in the present work, shows that HexADA fibroblasts can synthesize the α chains, thus distinguishing between classical TSD and HexADA patients. Furthermore, the presence of α chains is a clear cut phenomenon which is less prone to artefacts than methods based on a difference in charge and heat stability of Hex A and Hex B. This method can now be used together with other methods (8) for diagnosis, genetic counseling and antenatal diagnosis of the HexADA. Indeed, one pregnancy at risk was identified recently and the fetus was diagnosed as having this HexADA variant.

The reduced amount of the α chain in the HexADA fibroblasts could result either from a particular sensitivity of the mutated protein to proteolysis and denaturation or from a defect in an earlier event such as translation or transcription. The fact that the amount of α chain precursor is greatly reduced even after a short pulse (1.5 hr.), and the inability of protease inhibitors to increase its amount, may indicate that the reduction in α chain quantity is an early event, occurring long before the α chain reaches the lysosome Cell-free translation of mRNA isolated from HexADA fibroblasts is currently studied and would indicate whether these cells produce normal amounts of translatable mRNA for the α chain.

ACKNOWLEDGMENT:

This study was supported by a grant #937 from the Chief Scientist's Office, the Ministry of Health, Israel.

REFERENCES:

1. O'Brien, J.S. (1983) in: The Metabolic Basis of Inherited Disease (Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S., eds.) pp. 945 - 969, McGraw Hill, New York.
2. Hasilik, A. and Neufeld, E.F. (1980) J. Biol. Chem. 255, 4937 - 4945.
3. Navon, R., Padeh, B., Adam, A. (1973) Am. J. Hum. Genet. 25, 287 - 293.
4. Rapin, I., Suzuki, K., Suzuki, K. and Valsamis, M. (1976) Arch. Neurol. 33, 120 - 130.
5. Willner, J.P., Grabowski, G.A., Gordon, R.E., Bender, A.N. and Desnick, R.J. (1981) Neurology 31, 787 - 798.
6. Argov, Z. and Navon, R. (1983) submitted.
7. Padeh, B., Navon, R. (1971) Isr. J. Med. Sci. 7, 259 - 263.
8. Navon, R., Geiger, B., Ben-Yoseph, Y. and Ratazzi, M.C. (1976) Am. J. Hum. Genet. 28, 339 - 349.
9. Proia, R.L. and Neufeld, E.F. (1982) Proc. Natl. Acad. Sci, USA 79, 6360 - 6364