# Genetic Evidence for Transmembrane Acetylation by Lysosomes

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**Acetyl-CoA:α-glucosaminide *N*-acetyltransferase is a lysosomal-membrane enzyme deficient in a genetic disorder, Sanfilippo disease type C. The enzyme catalyzes the transfer of an acetyl group from cytoplasmic acetyl–coenzyme A (acetyl-CoA) to terminal α-glucosamine residues of heparan sulfate within the organelle. Previous kinetic experiments indicated that the enzyme carries out a transmembrane acetylation via a ping-pong mechanism; the reaction can therefore be dissected into two half reactions—acetylation of the enzyme, and transfer of the acetyl group to glucosamine. Cells derived from patients were found to differ in their ability to perform each half reaction. Five cell lines (derived from three families) were able to catalyze acetylation of the lysosomal membrane and to carry out acetyl-CoA/CoA exchange, whereas a sixth cell line was devoid of this activity.**

AN ENZYME DEFICIENCY DISEASE can result from a mutation expressed at any step in the formation of a functional enzyme. One way to understand the normal enzymatic process and the maturational pathway necessary for its expression is to classify different patients by the type of mutation and the nature of the enzymatic phenotype.

Four distinct enzymatic deficiencies result in the inability to remove α-D-glucosamine sulfate from heparan sulfate. The resultant accumulation of heparan sulfate constitutes a distinct mucopolysaccharide storage disorder, Sanfilippo syndrome (mucopolysaccharidosis III) (*1*). Biochemical characteristics of the syndrome include intralysosomal storage of heparan sulfate–like fragments in all organs, and excretion of these fragments in the urine. Clinically the diseases are characterized by severe, progressive mental retardation; behavioral problems; relatively mild skeletal deformities; and death, usually in the late teens (*1*). The four separate enzymatic deficiencies comprising the Sanfilippo syndrome are classified as Sanfilippo A, resulting from mutations in the enzyme *N*-sulfoglucosaminide sulfamidase (*2*); Sanfilippo B, resulting from mutations in the enzyme α-*N*-acetylglucosaminidase (*3, 4*); Sanfilippo C, resulting from mutations in the enzyme acetyl-CoA:α-glucosaminide *N*-acetyltransferase (*5*); and Sanfilippo D, resulting from mutations in the enzyme *N*-acetylglucosamine-6-sulfate sulfatase (*6*). The Sanfilippo syndrome is uncommon, and the deficiency of acetyl-CoA:α-glucosaminide *N*-acetyltransferase (*N*-acetyltransferase) in the type C disease is particularly rare. In the Netherlands, where the disease may be more frequent than elsewhere, the incidence of the Sanfilippo syndrome is one in 24,000 births with approximately 25% representing type C (*1*).

The enzyme deficient in Sanfilippo C is a lysosomal membrane protein that catalyzes transfer of the acetyl group from–acetyl-coenzyme A (acetyl-CoA) to terminal α-glucosamine residues of heparan sulfate. It has not been purified, and consequently, classification of the mutants structurally and immunologically is not yet possible. However, a preliminary classification of Sanfilippo C mutants can be made on the basis of kinetic characterization of the *N*-acetyltransferase. The enzyme works by a Di-Iso Ping-Pong Bi-Bi mechanism, undergoing two enzyme isomerizations during the course of the transfer reaction (*7*). We have proposed a mechanism whereby the enzyme would be acetylated at an active-site histidine on the cytoplasmic side of the lysosomal membrane. The acetyl-enzyme intermediate would undergo a conformational change resulting in movement of the acetyl group into the lysosome. Once inside the lysosome, the acetylhistidine intermediate would be protonated, glucosamine would bind to a separate site on the inside of the lysosome, and the acetyl group would be transferred to form *N*-acetylglucosamine. A second conformational change would then occur, bringing the unacetylated enzyme back to the cytoplasmic side of the lysosome (*7, 8*).

This enzyme mechanism can be divided into two half reactions that represent (i) acetylation of the enzyme and (ii) subsequent transfer of the acetyl group from the enzyme to glucosamine. We found that fibroblasts from Sanfilippo C patients (*9*) can be classified into two groups—one group is

Table 1. Lysosomal enzyme activities of human fibroblast cell lines. Normal, Sanfilippo C fibroblasts, and heterozygote fibroblasts (*9*) were maintained as described (*13*). The mitochondria and lysosome pellets were isolated from confluent 175-cm$^2$ flasks of cells (*13*), and were assayed for the lysosomal enzymes β-hexosaminidase (*10*), acid phosphatase (*11*), β-glucosidase (*12*), and *N*-acetyltransferase (*7*). Duplicate assays were done and agreed to within 10%.

| Cell line | Enzyme activities* | | | |
|---|---|---|---|---|
| | β-Hexosaminidase | Acid phosphatase | β-Glucosidase | *N*-Acetyl-transferase |
| Normal | | | | |
| 01 | 363 | 107 | 56.6 | 337 |
| 02 | 298 | 99 | 23.0 | 197 |
| 103 | 118 | 33 | 5.1 | 155 |
| 104 | 254 | 99 | 8.6 | 152 |
| 105 | 242 | 91 | 14.4 | 198 |
| IMR-90 | 256 | 83 | 10.8 | 222 |
| WI-38 | 284 | 132 | 21.8 | 203 |
| Mean ± SD | 259 ± 74 | 91 ± 33 | 20.0 ± 17.4 | 208 ± 62 |
| Sanfilippo C | | | | |
| 61 | 148 | 25 | 5.0 | 1.3 |
| 92 | 268 | 149 | 16.1 | 0.8 |
| 93 | 279 | 165 | 16.7 | 1.7 |
| 96 | 233 | 182 | 14.1 | 2.2 |
| 97 | 284 | 165 | 18.3 | 6.3 |
| 100 | 244 | 190 | 18.7 | 0.5 |
| Mean ± SD | 243 ± 51 | 149 ± 66 | 14.8 ± 5.1 | 2.2 ± 2.2 |
| Heterozygous | | | | |
| 94 | 296 | 107 | 18.9 | 58 |
| 95 | 228 | 165 | 12.6 | 77 |
| 98 | 240 | 157 | 18.9 | 52 |
| 99 | 199 | 174 | 19.9 | 73 |
| 101 | 301 | 215 | 24.1 | 87 |
| 102 | 224 | 215 | 26.2 | 78 |
| Mean ± SD | 248 ± 41 | 174 ± 41 | 20.1 ± 4.8 | 72 ± 13 |

*Units of activity for β-hexosaminidase, acid phosphatase, and β-glucosidase are nmol/min/mg. Units for *N*-acetyltransferase are pmol/min/mg.

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Table 2. Enzyme activities of purified lysosomal membranes. Lysosomal membranes were purified from 12 confluent 175-cm$^2$ flasks of each cell line as described (*14*), except volumes were reduced proportionally to allow for a smaller amount of starting material. Approximately 75 to 100 μg of membrane protein could be isolated from 12 flasks. The purified membranes were assayed for *N*-acetyltransferase (*7*) and β-glucosidase (*12*). Duplicate assays were done, and agreed to within 10%. ND, not determined.

| Cell line | Enzyme activity (nmol/min/mg) | |
|---|---|---|
| | *N*-Acetyl-transferase | β-Gluco-sidase |
| Normal | | |
| 01 | 2.08 | ND |
| 02 | 5.08 | 34.2 |
| 105 | 1.90 | 38.2 |
| Sanfilippo C | | |
| 61 | 0.007 | 29.7 |
| 92 | 0.002 | 31.8 |
| 96 | 0.007 | 28.4 |
| 97 | 0.013 | 21.1 |
| 100 | 0.008 | 27.7 |
| Heterozygous | | |
| 95 | 0.99 | 23.2 |
| 98 | 1.14 | 26.3 |
| 101 | 1.41 | 34.0 |
| Rat liver | 3.90 | 34.6 |

able to acetylate the enzyme and the other is not.

Lysosomal enzymes β-hexosaminidase (*10*), acid phosphatase (*11*), β-glucosidase (*12*), and *N*-acetyltransferase (*7*) were assayed in isolated mitochondria and lysosome pellets (*13*) from seven normal individuals, six Sanfilippo C patients, and six cell lines derived from individuals who are heterozygous for Sanfilippo C (*9*). All cell lines had comparable activities of acid phosphatase, β-hexosaminidase, and β-glucosidase (Table 1); however, as expected, a difference in *N*-acetyltransferase activity was observed. Normal cell lines had *N*-acetyltransferase activities ranging from 152 to 337 pmol/min/mg, with an average activity of 208 pmol/min/mg. Sanfilippo C cell lines had 100-fold less measurable *N*-acetyltransferase activity (2.2 pmol/min/mg) than the normals. Sanfilippo C heterozygotes had an average *N*-acetyltransferase activity of 72 pmol/min/mg; approximately half as much activity as the normals. Thus, it appears likely that Sanfilippo C heterozygotes can also be recognized by their *N*-acetyltransferase activity.

In order to measure the half reactions, lysosomal membranes were purified from several of the cell lines by a modification of the procedure of Ohsumi *et al.* (*14*). All the purified membranes contained comparable activities of a peripheral lysosomal membrane enzyme, β-glucosidase (*15*) (Table 2).

The ability of acetyl-CoA to acetylate mutant *N*-acetyltransferases was examined by measuring exchange of label between [$^3$H]CoA and acetyl-CoA. Purified lysosomal membranes were incubated with unlabeled acetyl-CoA and [$^3$H]CoA at 37°C for 1 hour. Formation of acetyl-[$^3$H]CoA was measured by means of reversed-phase high-performance liquid chromatography (HPLC) (*7*). Normal and heterozygote lysosomal membranes were able to incorporate [$^3$H]CoA label into acetyl-[$^3$H]CoA (Table 3). Five of the Sanfilippo C mutants were also able to form acetyl-[$^3$H]CoA. Only mutant 61 was unable to carry out the exchange.

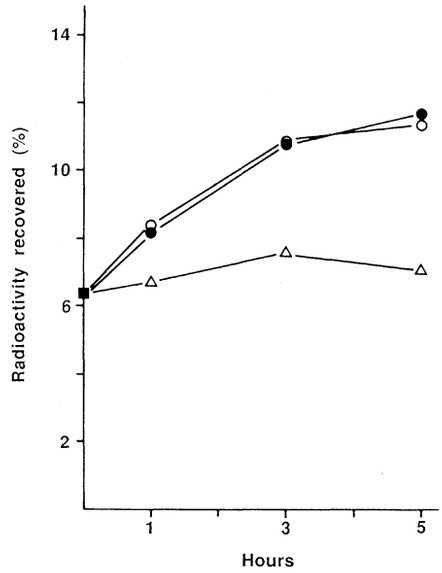


Fig. 1. Time course of the exchange activity. Purified lysosomal membranes (20 μg) were mixed with [$^3$H]CoA and acetyl-CoA at *p*H 7.0 and incubated at 37°C. At various times the membranes were pelleted and the supernatants were assayed for acetyl-[$^3$H]CoA (*7*). (●) Cell line 105, normal; (○) cell line 96, Sanfilippo C; and (△) cell line 61, Sanfilippo C.

To confirm that the mutant cell line 61 was unable to incorporate [$^3$H]CoA into acetyl-[$^3$H]CoA, a time course experiment was performed (Fig. 1). Over time, the amount of acetyl-[$^3$H]CoA in the incubation mixture increased when lysosomal membranes from both the normal cell line 105 and another of the Sanfilippo C mutants, cell line 96, were incubated with [$^3$H]CoA and unlabeled acetyl-CoA. However, the amount of labeled acetyl-CoA in the incubation mixture from cell line 61 remained near background over the entire time period (Fig. 1).

Since three of four Sanfilippo C mutants were able to acetylate the enzyme, mutant enzymes were assayed for their ability to transfer the acetyl group from the enzyme to glucosamine. Purified lysosomal membranes were incubated with [$^3$H]acetyl-CoA to form the acetylated enzyme intermediate. After incubation, lysosomal membranes were centrifuged, and the pellet was washed twice and assayed for radioactivity. As predicted from the exchange results, three of four Sanfilippo C lysosomal membranes tested (92, 96, and 100) were able to form an acetylated intermediate (Table 3). Amounts of acetylation ranged from 20 to 50% of normal. Only the lysosomal membranes from mutant cell line 61 were unable

Table 3. Activity of purified lysosomal membranes. Exchange activity–lysosomal membranes (20 μg) were mixed with [$^3$H]CoA and acetyl-CoA at *p*H 7.0, and exchange activity was measured by reversed-phase HPLC (*7*). Acetylation and transfer activity–lysosomal membranes (20 μg) were incubated with [$^3$H]acetyl-CoA, and assayed for the formation of the acetyl intermediate (*7*). Washed, acetylated membranes were incubated with 3 m*M* glucosamine, and formation of [$^3$H]*N*-acetylglucosamine (Glc N[$^3$H]Ac) was measured (*7*). The control for membrane acetylation was 1 m*M* *p*-chloromercuribenzoate–treated rat liver lysosomal membranes, which are unable to be acetylated (*16*). The control for transfer of the label to glucosamine was [$^3$H]-acetylated membranes incubated in the absence of 3 m*M* glucosamine. The reported values are the corrected values. ND, not determined.

| Cell line | Exchange activity pmol acetyl-[$^3$H]CoA/min | Acetylation pmol [$^3$H]acetyl/mg | Transfer pmol GlcN[$^3$H]Ac/mg |
|---|---|---|---|
| Normal | | | |
| 01 | ND | 0.5 | 1.01 |
| 02 | 2.5 | 1.1 | 1.41 |
| 105 | 2.2 | ND | ND |
| Sanfilippo C | | | |
| 61 | <0.2* | <0.01* | <0.01* |
| 92 | 1.4 | 0.4 | <0.01* |
| 96 | 1.5 | 0.1 | 0.04 |
| 100 | 2.1 | 0.2 | <0.01* |
| Heterozygous | | | |
| 95 | 1.6 | 0.2 | 0.91 |
| 98 | ND | 0.7 | 1.01 |
| 101 | ND | 0.3 | 0.93 |
| Rat liver | ND | 1.5 | 1.01 |

*Limit of detection.

to form this intermediate. Both normals, the three heterozygotes, and purified rat liver lysosomal membranes formed acetylated intermediates as expected.

The [$^3$H]-acetylated lysosomal membranes were incubated with 3 m*M* glucosamine for 15 minutes at room temperature. The membranes were spun down and the supernatant was applied to a silica gel thin-layer chromatography plate to separate the [$^3$H]*N*-acetylglucosamine from [$^3$H]-acetate (*7*). Even though the lysosomal membranes from the Sanfilippo C cell lines 92, 96, and 100 could form the acetylated intermediate, they were unable to transfer the acetyl group to glucosamine (Table 3), although cell line 96 appeared to have a very small amount of residual activity. In contrast, acetylated normal, heterozygote, and rat liver lysosomal membranes were all able to form *N*-acetylglucosamine.

Based on the half reaction studies of the mutant cell lines, we can now differentiate two classes of Sanfilippo C mutants. One class, represented by five out of six Sanfilippo C cell lines tested, has the ability to carry out acetyl-CoA/CoA exchange and to form the putative acetyl intermediate, but is unable to transfer the bound acetyl group to glucosamine. (The five lines represent three families, all of whom come from the Netherlands, and thus may have the same mutation.) The second class, composed of, at present, only one mutant cell line, is unable to catalyze acetyl-CoA/CoA exchange or form the acetylated intermediate.

The analysis of the two classes of mutants is the first direct evidence for the proposed transmembrane acetylation mechanism of *N*-acetyltransferase. Since no acetyl-CoA/CoA exchange is observed in the second mutant class, the exchange activity may be entirely due to the *N*-acetyltransferase and not to some other lysosomal membrane protein. The partial enzyme activity observed with the first class of mutants verifies that *N*-acetyltransferase works via a ping-pong mechanism, and that an acetylated enzyme intermediate is formed.

Acetylation of terminal α-linked glucosamine residues inside the lysosome is a required step in the degradation of heparan sulfate. Although acetyl-CoA is the acetyl donor in this reaction, it is unlikely that this cofactor could exist stably in the acidic and hydrolytic environment of the lysosome. *N*-Acetyltransferase provides a means for the cell to utilize cytoplasmically derived acetyl-CoA in this reaction without transporting the intact molecule across the lysosomal membrane. Vectorial transfer of the acetyl group through the lysosomal membrane appears to be a unique solution to a complex enzymatic and compartmental problem.


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# Detection of AIDS Virus in Macrophages in Brain Tissue from AIDS Patients with Encephalopathy


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**One of the common neurological complications in patients with the acquired immune deficiency syndrome (AIDS) is a subacute encephalopathy with progressive dementia. By using the techniques of cocultivation for virus isolation, in situ hybridization, immunocytochemistry, and transmission electron microscopy, the identity of an important cell type that supports replication of the AIDS retrovirus in brain tissue was determined in two affected individuals. These cells were mononucleated and multinucleated macrophages that actively synthesized viral RNA and produced progeny virions in the brains of the patients. Infected brain macrophages may serve as a reservoir for virus and as a vehicle for viral dissemination in the infected host.**


THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is associated with a variety of clinical disorders involving the peripheral and central nervous systems (CNS) (*1–4*). Premortem neurological findings are detectable in about one-third of AIDS patients, while neuropathological changes are present in over three-fourths of autopsied subjects (*4, 5*). From 17 to 60% of neurologically impaired AIDS patients develop an encephalitis, but only a minority of those cases can be attributed to a recognized opportunistic infection [for example, progressive multifocal leukoencephalopathy (PML) and herpes simplex] (*2, 3, 5*). Of those with encephalitis, the majority develop a subacute encephalopathy with progressive dementia and cerebral atrophy. Recent findings suggest that this form of encephalopathy may be a manifestation of


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