NOEV: *N*-octyl-4-epi-β-valienamine, NOV: *N*-octyl-β-valienamine, DGN: 1-deoxygalactonojirimycin, DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, LAH: lithium aluminum hydride, KO: knockout, Tg: transgenic

1. Introduction

Lysosome is one of the cellular organelles where various high molecular endogenous or exogenous compounds are systematically digested under the acidic condition (De Duve and Wattiaux, 1966). This physiological catalytic process is disturbed if mutations occur in one of the genes coding for the hydrolytic enzymes in the lysosome. Celllular dysfunctioin caused by an excessive storage of substrates ensues, and a genetic metabolic disease (lysosomal disease) develops clinically in humans and other animals with neurological and other somatic manifestations. This concept was first proposed for glycogen storage disease type I (Hers, 1965).

Since the mid-1960s, attempts were made to the development of therapy for patients with lysosomal diseases. Theorectically enzyme replacement therapy was the most promising approach, and eventually shown to be effective for Gaucher disease patients, the most prevalent metabolic storage disorder of humans (Brady, 2006). This approach has been extended to other lysosomal diseases. However, the effect has not been confirmed to brain pathology in patients with neurological manifestations.

G_{M1}-gagliosidosis is one of the lysosomal diseases with storage of ganglioside G_{M1}, keratan sulfate, and glycoprotein-derived oligosaccharides, presenting clinically with progressive neurological deterioration mainly in infancy and childhood (Suzuki et al., 2008). This disease has been our major target of research for more than 40 years. We analyzed correlation of phenotypic manifestations with storage compounds (Suzuki et al., 1971), enzyme activities (Suzuki et al., 1978; Suzuki et al., 1979), and enzyme molecules (Ko et al., 1983; Nanba et al., 1988). Finally we moved to molecular pathology of β-galactosidase (Oshima et al., 1988).

In parallel with with these experiments, in the early 1990s, we started molecular analyses of two genetically distinct human disease groups, β-galactosidosis (β-galactosidase deficiency disorders) caused by β-galactosidase gene mutations (Suzuki et al., 2008) and Fabry disease caused by α-galactosidase A gene mutations (Eng et al., 2008). A paradoxical phenomenon

was found that low molecular weight competitive inhibitors could serve as chemical chaperones to induce expression of catalytic activities of mutant enzymes after stabilization and successful intracellular transport to the lysosome in the cells. We reported this enhancement first in Fabry disease (Okumiya et al., 1995a; Fan et al., 1999a), and then in G_{M1}-gangliosidosis (Matsuda et al., 2003) and Gaucher disease (Lin et al., 2004) by using commercially available reagent 1-deoxygalactonojirimycin, or newly synthesized organic compounds as chemical chaperones.

Subsequently we developed new valienamine derivatives, *N*-octyl-4-epi-β-valienamine (NOEV) and *N*-octyl-β-valienamine (NOV) as chemical chaperones for mutant β-galactosidase and β-glucosidase proteins, respectively, to restore the enzyme activity in somatic cells from patients with G_{M1}-gangliosidosis and Gaucher disease (Matsuda et al., 2003; Lin et al., 2004; Iwasaki et al., 2006). We hope that this phenomenon will be applied to development of novel molecular therapeutic approach to lysosomal diseases, particularly with severe brain damage, in the near future. In this article we summarize our experimental results of chaperone effect and chaperone therapy mainly on NOEV for G_{M1}-gangliosidosis.

2. Competitive inhibitors of lysosomal enzymes

A. Carbasugar glycosidase inhibitors and related bioactive compounds

Carbasugars, previously known as pseudosugars, are a family of sugar mimics currently attracting interest among researchers in glycobiology and chemistry fields (Suami and Ogawa, 1990; Ogawa, 1998a, 2004a). The first example of carba-α-talopyranose was synthesized and called "pseudosugars." (McCasland et al., 1966) Later, naturally occurring bioactive carbasugar, 5a-carba-α-D-galactopyranose, was discovered (Miller et al., 1973). Carbasugars are (hydroxymethyl)-branched-chain cyclitols. They are topologically similar to normal sugars particularly in the arrangement of the hydroxyl and hydroxymethyl groups, but have the oxygen atom of the pyranose or furanose ring replaced by methylene. Humans cannot differentiate carbaglucose from true glucose by their taste (Suami et al., 1983). Furthermore

carbahexopyranoses exist in structurally stable α- and β-anomer forms which are not interconvertible. Therefore, chemical modification at C-1 positions may be possible, providing biologically interesting compounds. Potent glycosidase inhibitors NOEV **1** and NOV **2** (Fig 1A) are synthetic 5,5a-unsaturated 5a-carba-β-galacto and glucopyranosylamine derivatives, respectively.

In 1970, agrochemical antibiotic validamycin A **3** and homologues were discovered (Iwasa et al., 1970) and have so far been utilized to control sheath bright disease of rice plant (Fig. 1B). During their structural elucidation, three components were isolated: valienamine **5**, validamine **6** (Horii et al., 1971), and valiolamine **7** (Kameda and Horii, 1972). These compounds are strong α-glucosidase inhibitors themselves. In 1976, α-amylase inhibitor acarbose **4** composed of **5** was discovered (Schmidt et al., 1977) and has been clinically used to control diabetes.

B. Structural modification of valienamine

The enzyme inhibitory potency of **4** has been attributed to the core structure of methyl acarviosin **10,** a strong α-glucosidase inhibitor, and considered to be an analogue of transition-state structure postulated for enzymatic hydrolysis of maltose (Junge et al., 1984) (Fig. 1C). The potency of **3** has been attributed to strong trehalase inhibition *in vitro* by validoxylamine A **9** that mimicks the transition-state structure for hydrolysis of trehalose with trehalase. The valienamine moiety of **9** and **10** holds the pyranoid oxonium ion structures and binds more firmly to the corresponding active sites of enzymes via an imminium ion. Thus, they are competitive inhibitors, explaining a structural correlation between inhibitors and substrates. This knowledge opened up the possibility for development of therapeutically useful carbasugar derivatives. Thus, chemical modification of three components **5-7** was stimulated by successful medical application of **4** (Fig 1B), leading to a finding of semisynthetic voglibose **8**, *N*-(1,3-dihydroxyprop-2-yl)valiolamine that is fully compatible to **4** (Horii et al., 1986; Fukase and Horii, 1992).

One of the authors (SO) became interested in chemical modification of **5** and **6**, and designed some stereoisomers by analogy of the structural relationship between enzyme inhibitors and substrates. However, both the C-1 epimers of **5** and **6** unexpectedly lacked activity against β-glucosidase. Then valienamine-type glycosidase inhibitors **11-13** with β-*gluco*, α-*manno*, and β-*galacto* configurations, expected to be specific inhibitors of the corresponding hydrolases, were synthesized (Ogawa et al., 1982; Ogawa et al., 1992) (Fig. 1D). However, notable inhibitory activity could not be observed for any of them. Bioassay of the activity was carried out routinely using commercially available glycosidases. Although simple chemical modification of these amines, such as N-alkylation, might have improved their potency, the activity of intact amines should be a reliable hallmark for further development of its related compounds. Undoubtedly, biochemical features of **5** and **6** have been important models for further successful development.

C. Glycocerebrosidase inhibitors: carbaglycosylceramides

Some glycosylamides are significant immunomodulators (Lockhoff, 1991). We prepared some carbasugar analogues, such as compound **14**, as bioactive glycosylamides (Tsunoda and Ogawa, 1994) (Fig. 1E). This result suggested that the carbohydrate moiety of glycolipids could possibly be replaced to give rise to biologically active carbohydrate mimics.

Then a carbasugar mimic **15** of glucosylceramide was found to be a moderate inhibitor of glucocerebrosidase (Tsunoda and Ogawa, 1995a). Further attempts were made to prepare valienamine analogues **16** and **17**, carbaglucosyl and carbagalactosylceramides (Tsunoda et al., 1995b), which exhibited potent and specific inhibitory activity against the corresponding gluco- (IC_{50}, 0.3 μM; bovine liver) and galacto-cerebrosidases (IC_{50}, 2.7 μM; bovine liver).

D. *N*-Alkyl valienamines, potent β-galactosidase inhibitors

Complex ceramide chains were synthetically inaccessible. We therefore began to modify the structure by introducing a simple substitution of ceramide chain. Replacement with a

simple aliphatic chain resulted in an increase of inhibitory activity (Ogawa et al., 1996) (Fig. 1D). Table 1 shows enzyme inhibitory activity of *N*-alkyl derivatives **1, 2, 11a–d**, and **13a–c** [R=$(CH_2)_nCH_3$]toward some glycosidases (Ogawa, 2004a; Ogawa et al., 2004b). *N*-Octyl derivatives **1** and **2** were most promising for medical application (Ogawa et al., 2002). Biochemical role of *N*-octyl portion was predicted also by computer-assisted simulation studies (Sakakibara, unpublished data).

E. Preparative studies on valienamine type inhibitors

For further development of carbasugar chemistry, simple synthetic precursors are required. Diels-Alder *endo*-adduct **18**(+), (–) of furan and acrylic acid has been useful for this purpose. Preparative intermediates reported in previous studies (Ogawa, 2004a) could also be provided as optically pure forms from common hexopyranoses (Arjona et al., 2007) providing a convenient link of carba to true sugars. Optical resolution of racemic **18** could be readily conducted through fractional crystallization of the diastereomeric salt with optically active α-phenylethylamines (Ogawa et al., 1985), or enantioselective hydrolysis of the racemic 2-chloroethyl epoxy esters by means of pig liver esterase (Sugai et al, unpublished data).

Typical routes to valienamines related to NOEV **1** and NOV **2** were briefly described (Ogawa et al., 1998b) (Fig. 2A). Optically resolved *endo*-adduct **18**(–) with L-stereochemistry was converted into 1,6-dibromo-1,6-dideoxy-β-carbaglucose **20** through the triol **19**. Elimination of **20** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the conjugate alkadiene **21**, which was transformed into 1,4-dibromides **22**α,β. The primary bromo group was first replaced with an acetate ion and then the secondary one with azide anion to give α,β-mixture of the azide precursors **23**α,β. The azido function was reduced with H_2S or Ph_3P to give the free bases **5** and **11**.

Effective N-alkylation of **11** is important for provision of active compounds. Compound **23**β was protected and reduced (→**24**). The free amine **24** was treated with a series of acid chlorides to give the amides (**25**). Reduction of **25** with lithium aluminum hydride (LAH)

(→**26**), followed by deprotection, afforded the *N*-alkyl valienamines (→NOV **2. 11a-d**) in good yields (Ogawa et al., 1996).

NOEV **1** is the 4-epimer of **2**. Preparation of **1** was first conducted cumbersomely by Walden inversion at C-4 of **2** through oxidation of 4-OH, followed by selective reduction (Ogawa et al., 2002). Alternatively, **18**(+) was converted into the bromo acetate (**27**), which was cleaved with HBr to give the tribromide (**28**) (Fig. 2B). Compound **28** was treated with methoxide and the resulting anhydride was opened and then acetylated to give the dibromide (**29**). Dehydrobromination (→**30**) followed by protection gave the alkadiene **31**. Treatment with bromine gave the 1,4-dibromide (**32**), which was similarly converted into the bromide **33**. Thus **33** was similarly transformed into the β-valienamine **13** with β-*galacto* configuration. Modification of the β-galactose-type valienamine will be achieved by direct displacement of bromide **33** with alkyl amine (→**34**). The substitution reaction selectively occurs as expected by neighboring assistance of the 2-acetoxyl or through direct S_N2 fashion to afford, after deprotection, *N*-alkyl-4-epi-β-valienamines (**13a-c**) including NOEV (Ogawa et al., 2004b).

Transition-state type glycosidase inhibitor valienamines have thus been recognized as desirable carbohydrate mimics for designing new glycosidase inhibitors. Current technical difficulty is comparative inaccessibility to the carbahexose skeletons. Recently, we identified some routes to carbasugars through chemical transformation of (–)-*vibo*-quercitol derived from *myo*-inositol by biogenesis (Fig. 2C) (Ogawa et al., 2005; Ogawa and Kanto, 2007a). This route establishes a link between naturally abundant cyclitols and chiral carbasugars.

F. Future aspect

Carbaglycosylamines are chemically stable (hydroxymethyl)aminocylitols and expected to play roles as non-hydrolyzable mimics of glycopyranosylamines (Ogawa and Kanto, 2007a; Ogawa et al., 2007b). Diverse modification of the biochemical and topologic nature of carbaglycosylamines may be achieved by substitution at the anomeric position, unsaturation at C-5 and C-5a, and hydroxylation at C-5 and/or C-5a, leading to improvement of biological

function. As shown by the inhibitory activity of the *N*-alkyl derivatives **11a–d** and **13a–c** of β-valienamines, the inclusion of hydrophobic *N*-alkyl chains is likely to contribute to improvement of its potential significance. Furthermore, additional modification of their physicochemical nature is advisable for the purpose of generating strong binding to active sites of enzyme or peptide molecules.

3. β-Galactosidosis: Genetic human disorders caused by β-galactosidase gene mutations.

Lysosomal β-galactosidase (EC 3.2.1.23), encoded by a gene *GLB1* (3p21.33), catalyzes hydrolysis of ganglioside G_{M1} and related glycoconjugates such as oligosaccharides derived from glycoproteins and keratin sulfate in human somatic cells. Allelic mutations of the gene result in excessive storage of the substrates in various cells and tissues.

G_{M1}-gangliosidosis (OMIM 230500) is expressed clinically as generalized neurosomatic disease in children (infantile form, juvenile form), and rarely in adults (adult form), caused by widespread abnormal storage of ganglioside G_{M1}, mucopolysaccharide keratin sulfate and glycoprotein-derived oligosaccharides in the central nervous system, skeletal system, and other tissues and visceral organs . Specific gene mutations are known for each clinical form (Yoshida et al., 1991). Morquio B disease (OMIM 253010) is another clinical phenotype presenting with generalized skeletal dysplasia without neurological involvement . Again specific gene mutations different from those in G_{M1}-gangliosidosis have been identified (Oshima et al., 1991). More than 100 gene mutations are collected, and successful gene diagnosis is well established using restriction enzymes specific to individual mutations (Suzuki et al., 2008).

At present only symptomatic therapy is available for the brain lesion in human G_{M1}-gangliosidosis patients. Enzyme replacement therapy is currently in use for clinical practice for Gaucher disease, Fabry disease and other lysosomal diseases. However, the beneficial effect has not been confirmed for the brain damage, although general somatic signs and symptoms are clearly improved by continuous enzyme replacement therapy (Schiffmann

and Brady, 2002). Secretion of feline β-galactosidase was reported in the transfected cell culture system, but the effect on the central nervous system was not shown (Samoylova et al., 2008).

After several years of basic investigations mainly for mutant α-galactosidase A in Fabry disease, we proposed chemical chaperone therapy for brain pathology in G_{M1}-gangliosidosis, using an *in vitro* enzyme inhibitor *N*-octyl-4-epi-β-valienamine (NOEV) (**1**; Fig 1A), a chemical compound newly produced by organic synthesis described above (Ogawa et al., 2002), as a potent stabilizer of mutant β-galactosidase in somatic cells from patients with this disorder (Matsuda et al., 2003).

4. Genetic metabolic diseases: molecular pathology and an approach to possible molecular therapy

Molecular pathology of inherited metabolic diseases can be classified into the following three major conditions (Suzuki, 2006).

(a) Biosynthetic defect of the protein in question. Mutant enzyme is not synthesized, and accordingly rescue of the protein is not possible.

(b) Defect of biological activity. In spite of normal biosynthesis, the protein does not maintain biological activity because of its structural abnormality. There is no possibility to restore the biological activity of this molecule.

(c) Unstable mutant protein with normal or near-normal biological activity under appropriate environmental conditions. The mutant protein has normal biological function in its mature form. However, it is unstable and rapidly degraded immediately after biosynthesis. The protein function is expected to be restored if the molecule is somehow stabilized and transported to the cellular compartment where it is expected to exhibit biological activity; the lysosome in the case of lysosomal enzyme.

We tested these possibilities first in Fabry disease, and found some mutant enzyme

proteins were unstable at neutral pH in the endoplasmic reticulum/Golgi apparatus, and rapidly degraded because of inappropriate molecular folding (Ishii et al., 1993). Addition of galactose in the culture medium of lymphoblasts from Fabry patients and COS-1 cells expressing mutant enzyme proteins surprisingly induced a high expression of α-galactosidase A activity in cultured fibroblasts from Fabry patients (Okumiya et al., 1995a).. However, a high concentration of galactose was necessary for treatment of these enzyme-deficient cells in culture. We concluded that a long-term treatment with galactose at this high dose was not realistic, although a short-term human experiment was reported on the beneficial cardiac function in a case of Fabry disease (Frustaci et al., 2001). Accordingly we searched for other compounds that could enhance the enzyme activity in mutant cells.
1-Deoxygalactonojirimycin (DGJ) was found to be effective for stabilization and high expression of the enzyme activity (Fan et al., 1999a; Fan et al., 1999b). DGJ showed the chaperone effect mainly on mutant α-galactosidae A. Its activity toward mutant β-galactosidase was 50-fold lower in a culture system experiment (Tominaga et al., 2001).

After subsequent extensive molecular analysis we reached the following conclusion (Suzuki, 2006). A substrate analogue inhibitor binds to the misfolded mutant lysosomal protein as a kind of molecular chaperone (chemical chaperone), to achieve normal molecular folding at the endoplasmic reticulum/Golgi compartment in somatic cells, resulting in formation of a stable molecular complex at neutral pH. The protein-chaperone complex is safely transported to the lysosome, where it dissociates under the acidic conditions, the mutant enzyme remains stabilized in its normal folding, and its catalytic function is expressed (Fig 3).

5. Therapeutic approach to brain pathology by chemical chaperones

A. New chaperones: valienamine derivatives

We had particular interest in primary neuronopathic lysosomal diseases. Accordingly after studies on galactose and DGJ for α-galactosidase A, we started an extensive search for

specific compounds for β-galactosidase. No commercially available compound was found as bioactive chaperone. Fortunately we came across two synthetic valienamine derivatives: β-galactosidase inhibitor *N*-octyl-4-epi-β-valienamine (NOEV) and β-glucosidase inhibitor *N*-octyl-β-valienamine (NOV) (**1** and **2**; Fig 1A). NOV was the first compound synthesized as a glucocerebrosidase inhibitor (Ogawa et al., 1996), and subsequently NOEV was synthesized by epimerization of NOV (Ogawa et al., 2004b). They are specific competitive inhibitors of β-galactosidase and β-glucosidase, respectively, In our laboratory NOEV studies moved faster than NOV simply because of accumulation of more experimental data and clinical materials for β-galactosidase and G_{M1}-gangliosidosis.

NOEV is a potent inhibitor of lysosomal β-galactosidase *in vitro*. It is stable and soluble in methanol or DMSO. The hydrochloride salt is freely soluble in water. Molecular weight is 287.40. IC_{50} is 0.125 μM toward human β-galactosidase (Matsuda et al., 2003).

NOEV is 50-fold more active than DGJ in chaperone effect on mutant human β-galactosidase in G_{M1}-gangliosidosis. Our calculations suggest that at least 10% of normal enzyme activity is necessary for washout of the storage substrate in lysosomal diseases (Fig 4). The age of onset in patients expressing enzyme activity above this level is theoretically beyond the human life span. The same calculation was reported on some other lysosomal diseases (Conzelmann and Sandhoff, 1983).We anticipate that the effective NOEV concentrations in human cells and animal tissues are much lower than the IC_{50} calculated *in vitro*, based on the results of tissue concentration after oral NOEV administration in experimental model mice (Suzuki et al., 2007). In fact, NOEV is effective at the IC_{50} concentration in the culture medium for enhancement of mutant enzyme activity (Iwasaki et al., 2006). We hope it will be clinically used as a specific enzyme enhancer without exerting inhibitory effect in the cells.

B. Chaperone experiments on culture cells

About one-third of the cultured fibroblast strains from G_{M1}-gangliosidosis patients

responded to NOEV; mainly from juvenile form and some infantile form patients. The effect is mutation-specific (Iwasaki et al., 2006). The R457Q mutant responded to NOEV maximally at 0.2 μM, and the R201C or R201H mutant at 2 μM. The mouse fibroblasts expressing mutant human β-galactosidase showed essentially the same results (Tominaga et al., 2001). Molecular interaction between the chaperone and mutant protein depends on the structuractural modification of the mutant enzyme protein (Sakakibara et al, unpublished data). Addition of ganglioside mixture in the culture medium increased intracellular G_{M1} in the R201C cells causing juvenile G_{M1}-gangliosidosis (Matsuda et al., 2003). This storage was almost completely prevented by NOEV.

C. Chaperone experiments on genetically engineered G_{M1}-gangliosidosis model mice

For animal studies we developed a knockout (KO) mouse strain with complete deficiency of β-galactosidase (Matsuda et al., 1997), and then a transgenic (Tg) strain based on KO, expressing human R201C mutation (4% of the enzyme activity in the brain from wild-type mice) (Matsuda et al., 2003). Both showed neurological deterioration with some difference in severity. Life span was 7-10 months for KO and 12-18 months for Tg. Neuropathology corresponded to the clinical severity (Matsuda et al., 2003). Short-term oral NOEV administration resulted in significant enhancement of the enzyme activity in all the R201C mouse tissues examined, including the brain (Matsuda et al., 2003). Immunohistochemstry revealed an increase in β-galactosidase activity and decrease in G_{M1} and G_{A1} storage.

Oral NOEV treatment for the R201C Tg mouse showed an increase of the NOEV content in the brain in parallel with β-galactosidase activity, and G_{M1} storage decreased (Suzuki et al., 2007). NOEV disappeared rapidly, within a few days after withdrawal. In this study we tried a new scoring system for neurological assessment (Ichinomiya et al., 2007) (Table 2). Treatment at the very early clinical stage (2 months) resulted in a positive clinical effect within a few months, although complete arrest or prevention of disease progression was not achieved under this experimental condition (Table 3). The latency before the clinical effect

was longer if the therapy was started in the late symptomatic stage (6 months). We concluded that NOEV treatment at the early stage of disease is mandatory for prevention of the brain damage.

This result indicated the following sequence of events in the mouse brain (Suzuki, 2006). After oral administration, NOEV goes directly into the bloodstream without intestinal digestion, is delivered to the brain through the blood-brain barrier, and restore the mutant β-galactosidase activity, resulting in substrate digestion and clinical improvement. No specific adverse effects have been observed for at least 6 months of continuous oral administration. For achievement of clinical drug development, however, we need to study further possible adverse effects and to establish the optinal dose and frequency of administration in order to achieve the best clinical effect.

6. Molecular mechanisms of chaperone effect in lysosomal disease

As described above, β-galactosidase gene mutations result in excessive accumulation of substrates and various clinical phenotypes: G_{M1}-gangliosidosis and Morquio B disease. Single base substitutions do not necessarily lead to a complete loss of enzyme function. However, the enzyme activity is not always expressed even if the potential catalytic function is not completely lost, simply because of intracellular instability of the mutant enzyme molecule due to inappropriate or incorrect protein folding. Molecular pathology of this type occurs at least in one-third of the patients with β-galactosidase deficiency (Iwasaki et al., 2006). Chemical chaperone corrects the molecular abnormality of this type, and assists intracellular transport to the lysosome, finally releasing the mutant enzyme as a stable bioactive protein (Fig 4).

We postulated that enzyme-chaperone binding would become less strong under the acidic condition in the lysosome. Then the mutant enzyme molecule is released and stable catalytic activity appears. However, the precise mechanism of this NOEV effect is unknown at present. We therefore started computational analysis for prediction of molecular interactions between the β-galactosidase protein and the chaperone compound NOEV.

First, the three-dimensional structure of human enzyme was predicted employing a homology modeling method 3D-JURY (Ginalski et al., 2003; Kajan and Rychlewski, 2007), because the structure of this enzyme is not yet available. *Penicillium sp.* β-galactosidase was used as the template structure for homology modeling, and the predicted structure of human β-galactosidase has been obtained as shown in Fig 5A.

Second, plausible conformation of β-galactosidase-NOEV complex was determined in support of AUTODOCK4 (Morris et al., 1998). The conformation was subjected to further structural optimization. The result of the complex structure was successfully computed by AUTODOCK4 (Fig 5B).

Third, the binding free energy of the two molecules in the complex was calculated by using AMBER9 (Case et al., 2005). The computed binding free energy was -20.08 (kcal/mol) at pH 7.

Fourth, we calculated the effect of low pH in the lysosome on the binding affinity between the β-galactosidase and NOEV molecules. The low pH effect was represented as protonation of charged residues estimated by PROPKA (Li et al., 2005). The computed binding free energy at pH 5 was -18.06 (kcal/mol); higher than that at pH 7. This result indicates that affinity between β-galactosidase and NOEV is weakened at pH 5 compared with that at pH 7. Consequently, we concluded that (1) the enzyme-NOEV complex has lower free energy than the unbound enzyme, and (2) protonation of an active site residue causes free energy change consistent with the chemical chaperone hypothesis.

7. Conclusion

This new therapeutic strategy (chaperone therapy) is in principle applicable to all lysosomal diseases, if a specific compound is developed for each enzyme in question. We have already confirmed the effect in Fabry disease, G_{M1}-gangliosidosis, and Gaucher disease. Other related diseases also are currently studied by other investigators (Sawkar et al., 2002; Tropak et al., 2004). Theoretically this principle can be applied to all other lysosomal diseases.

Furthermore, there may well be other genetic diseases to be considered, if molecular pathology in somatic cells has been clarified in detail. We hope studies in this direction will disclose a new aspect of molecular therapy for inherited metabolic diseases with central nervous system involvement in the near future.

Acknowledgments

This research was supported by grants from the Ministry of Education, Culture, Science, Sports, and Technology of Japan (13680918, 14207106), and the Ministry of Health, Labour and Welfare of Japan (H10-No-006, H14-Kokoro-017, H17-Kokoro-019). We thank all collaborators contributing to this research project for the past 16 years.

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