FEBS Letters 346 (1994) 103–107

FEBS 13897

Minireview

Intracellular trafficking of glycosphingolipids: role of sphingolipid activator proteins in the topology of endocytosis and lysosomal digestion

K. Sandhoff*, A. Klein

Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany

Received 10 March 1994

Abstract

Glycosphingolipids (GSL) are components of the outer leaflet of the plasma membrane (PM) of vertebrate tissues. Our current knowledge of GSL metabolism and their intracellular traffic has been derived from metabolic studies but the exact mechanisms by which GSLs are transported from sites of synthesis (endoplasmic reticulum and Golgi) to the sites of residence (PM) and degradation (lysosomes) have not been clearly defined. It is now established that components of the PM reach the lysosomal compartment mainly by endocytic membrane flow. According to a new model, GSLs derived from the PM are thought to end up in intra-endosomal vesicles which could be delivered, by successive processes of membrane fission and fusion, along the endocytic pathway directly into the lumen of the lysosomes. Here the GSLs are degraded in a step-wise manner by exohydrolases. However, the catabolism of membrane-bound GSLs with short hydrophilic head groups needs the assistance of sphingolipid activator proteins (SAPs), which lift the GSLs from the plane of the membrane and present them for degradation to the lysosomal exohydrolases, which are usually water-soluble. The inherited deficiency of one of these enzymes or SAPs causes the lysosomal storage of their respective GSL substrates. In the case of the simultaneous deficiency of all 4 different SAPs the storage of all GSLs with short hydrophilic head groups occurs within multivesicular bodies and/or intra-lysosomal vesicles.

Key words: Glycosphingolipid; Intracellular traffic; Multi-vesicular body; Sphingolipid activator protein; Endocytosis

1. Topology of biosynthesis of glycosphingolipids

Glycosphingolipids (GSL) are amphiphilic components of the PM characteristic of vertebrate tissues [1–3]. On the cell surface, different GSL structures form cell type- and differentiation-specific patterns. These patterns change with viral transformation and oncogenesis (see [4,5] for reviews).

Our current knowledge of GSL biosynthesis and their intracellular traffic has been derived from metabolic studies. The mechanism by which GSLs are transported from sites of synthesis to other membranes has not been well defined. It was generally assumed [6,7] that GSL formation is coupled, as is the formation of glycoproteins, to a vesicular membrane flow from the endoplasmic reticulum (ER) through the cisternae of the Golgi complex to the PM (see [8] for review). However, the involvement of glycolipid binding and/or transfer proteins in the transport of GSL cannot be excluded. The role of several such proteins has not been identified [9,10].

Glycosphingolipid biosynthesis starts with the condensation of serine and palmitoyl-CoA, yielding 3-ketosphinganine [11]. This ketone-derivative is rapidly reduced to sphinganine by an NADPH-dependent reductase [12]. The introduction of the 4-*trans* double bond occurs after addition of an amide-linked fatty acid (see [13] for review). Thus sphingosine is not an intermediate of the de novo biosynthetic pathway for ceramide.

All enzymatic steps involved in the biosynthesis of ceramide appear to be located on the cytosolic face of the ER [14].

The next step in the biosynthesis of GSLs and sialylated GSLs (gangliosides), namely the glucosylation of ceramide, is localized on the cytosolic side of Golgi membranes [15,16]. The following step, i.e. the formation of lactosylceramide (LacCer), a common precursor of most glycosphingolipid families, has presumably a luminal topology.

The sequential addition of monosaccharide or sialic acid residues to the growing oligosaccharide chain, yielding ganglioside GM3 and successively more complex gangliosides, is catalyzed by membrane-bound glycosyltransferases which have been shown to be restricted to the luminal surface of the Golgi apparatus (see [8] for review). A transfer of glucosylceramide (GlcCer) from the cytosolic to the luminal side of the Golgi membranes is therefore required but its occurrence has not been experimentally proven.

Recent studies demonstrated that transfer of the same sugar residue to analogous glycolipid acceptors, differing only in the number of neuraminic acid residues bound to the inner galactose of the oligosaccharide chain, is

*Corresponding author. Fax: (49) (228) 737 778.

Abbreviations: GSL, glycosphingolipid; SAP, sphingolipid activator protein; PM, plasma membrane; ER, endoplasmic reticulum.

0014-5793/94/$7.00 © 1994 Federation of European Biochemical Societies. All rights reserved.
SSDI 0014-5793(94)00282-Z

catalyzed by one and the same glycosyltransferase in rat liver Golgi (see [13] for review).

2. Topology and mechanism of lysosomal glycolipid degradation role of sphingolipid activator proteins

Components and fragments of the PM reach the lysosomal compartment mainly by an endocytic membrane flow via the early and late endocytic reticulum [17]. During this vesicular flow, molecules in the membrane are subjected to a sorting process which directs some of the molecules to the lysosomal compartment, some others to the Golgi and yet others even back to the PM [18–21]. It remains, however, an open question whether components of the PM are included as components of the lysosomal membrane after successive steps of vesicle budding and fusion along the endocytotic pathway. We think that it is quite unlikely that the portion of the lysosomal membrane originating from the PM should be more or less selectively degraded by the lysosomal enzymes.

On the other hand, the observation of multivesicular bodies at the level of the early and late endosomal reticulum [21–24] suggests that parts of the endosomal membranes (possibly those enriched in components derived from the PM) bud off into the endosomal lumen and thus form intra-endosomal vesicles. These vesicles, enriched in PM components, could be delivered by successive processes of membrane fission and fusion along the endocytic pathways, directly into the lysosol for final degradation of their components (Fig. 1). Thus glycoconjugates, originating from the outer leaflet of the PM, would enter the lysosol seated on the outer leaflet of endocytic vesicles, facing the digestive juice of the lysosomes. This hypothesis [25] is supported by the accumulation of multivesicular storage bodies in cells, e.g. Kupffer cells and fibroblasts, of patients with a complete deficiency of the *sap*-precursor protein with a combined activator protein deficiency [26,27], as well as by the observation that the epidermal growth factor receptor derived from the PM and internalized into lysosomes of hepatocytes is not integrated into the lysosomal membrane [28]. This view [25] is in accordance with recent observations by van Deurs et al. [29] who found that spherical multivesicular bodies were the predominant endocytic compartments in HEp-2 cells and that multivesicular bodies matured within 60–90 min into lysosomes still containing internal vesicles.

It is not yet clear whether the early endosomes are gradually transformed to late endosomes and then to lysosomes. This would require the addition of several typical components for each organelle to the transforming and maturing compartment [30,31]. It is also possible that early endosomes, late endosomes and lysosomes already exist and that intra-endosomal carrier vesicles are

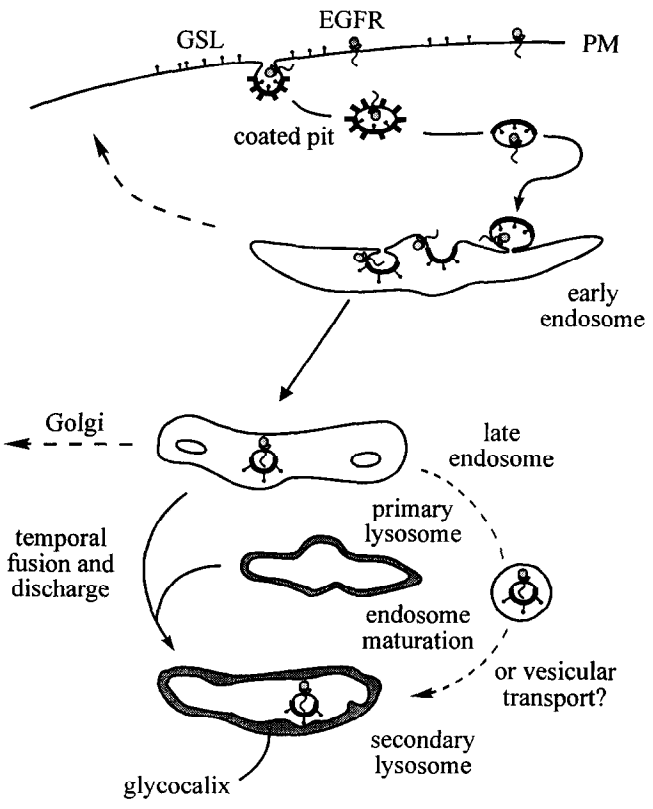

Fig. 1. A new model for the topology of endocytosis and lysosomal digestion of GSLs derived from the plasma membrane (modified from [25]). During endocytosis glycolipids of the plasma membrane are supposed to end up in intra-endosomal vesicles (multivesicular bodies) from where they are discharged into the lysosomal compartment. GSL, glycosphingolipid; PM, plasma membrane; EGFR, epidermal growth factor receptor; ↑, glycolipid; →, proposed pathway of endocytosis of GSL derived from the plasma membrane into the lysosomal compartment; - - ->, other intracellular routes for GSL derived from the plasma membrane.

transported successively from one compartment to another. Recently Mullock and Luzio [32] proved the association and fusion from endosomes with pre-existing lysosomes in a cell-free preparation from rat liver. Since the experimental conditions did not permit the maturation of endosomes to lysosomes, the results must indicate some vesicular transport between pre-existing compartments.

Finally, components of the PM reaching the lysosomes are digested by a mixture of hydrolases most active in the acidic environment of the lysosol. On the other hand, lysosomal membranes should be protected against premature digestion. This is accomplished by a massive glycocalix formed on the inner surface of the lysosomal membrane by the extended carbohydrate moiety of the LIMPs (lysosomal integral membrane proteins) and LAMPs (lysosomal associated membrane proteins) constituting the lysosomal membranes [33].

Degradation of GSL occurs by step-wise action of specific acid exohydrolases. Several of these enzymes

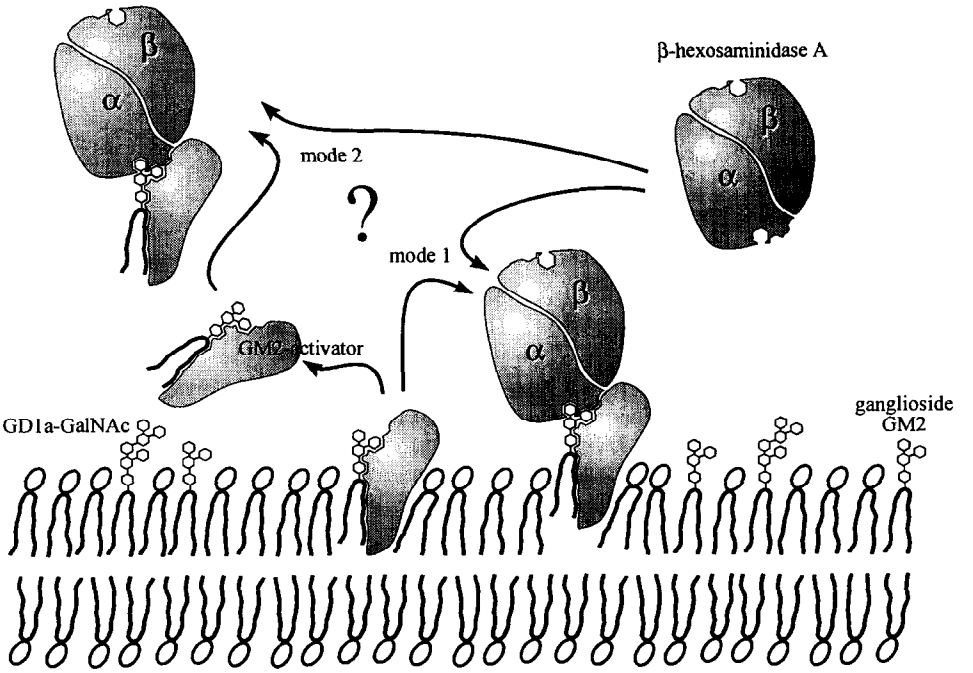

Fig. 2 Model for the GM2-activator stimulated degradation of ganglioside GM2 by human hexosaminidase A (modified from [25]). Water-soluble hexosaminidase A does not degrade membrane-bound ganglioside GM2, which has a short carbohydrate chain, in the absence of GM2-activator or appropriate detergents. However, it degrades ganglioside GD1a-GalNAc, which has an extended carbohydrate chain, and also analogues of ganglioside GM2, which contain a short acyl residue or no acyl residue (lysoganglioside GM2). They are less firmly bound to the lipid bilayer and more water-soluble than GM2. Ganglioside GM2 bound to a lipid-bilayer, e.g. of an intra-lysosomal vesicle (see Fig. 1) is hydrolyzed in the presence of the GM2-activator. The GM2-activator binds one ganglioside GM2 molecule and lifts it a few Å out of the membrane. This activator–lipid complex can be reached and recognized by water-soluble hexosaminidase A which cleaves the substrate (mode 1). It is, however, also possible that the water-soluble activator–lipid complex leaves the membrane and that the enzymatic reaction takes place in free solution (mode 2). The terminal GalNAc residue of membrane-incorporated ganglioside GD1a-GalNAc protrudes from the membrane far enough to be accessible to hexosaminidase A without an activator.

need the assistance of small glycoprotein cofactors, the so-called SAPs [25], when they attack membrane- or vesicle-bound GSL substrates with short oligosaccharide head groups.

Since the discovery of sulphatide activator protein [34], several other factors have been described but their identity, specificity and function have remained unclear. When sequence data became available it turned out that only two genes code for the five SAPs known [25]. One gene carries the genetic information for the GM2-activator and the other for the *sap*-precursor, which is processed to four homologuous proteins, including sulfatide activator protein (*sap*-B) and glucosylceramidase activator protein (*sap*-C).

A range of experimental data [35] suggests a mechanism of action for the GM2-activator. Hexosaminidase A is a water-soluble enzyme which acts on substrates of the membrane surface only if they extend far enough into the aqueous phase (Fig. 2). Like a razor blade or a lawn-mower the enzyme recognizes and cleaves all substrates (e.g. GD1a-GalNAc) which stick out far enough into the aqueous space. Those GSL substrates with oligosaccharide head groups too short to be reached by the water-soluble enzyme, however, cannot be degraded. Their degradation requires a second component, the GM2-activator, a specialized GSL-binding protein, which complexes the substrate (e.g. ganglioside GM2), lifting and even extracting it from the membrane and presenting it to the hexosaminidase A for degradation. While GM2-activator (a liftase) and hexosaminidase A (a lawn-mower) represent selective and precisely tuned machinery for the degradation of a few structurally similar membrane-bound sphingolipids, *sap*-B stimulates the degradation of many lipids by several enzymes from human, plant and even bacterial origin [36]. Thus *sap*-B seems to act as a kind of physiological detergent with broad specificity and solubilizes glycolipid substrates [37].

Unlike GM2-activator and *sap*-B, *sap*-C is reported to form complexes with membrane-associated enzymes and apparently activates them [38–41].

In summary, GSLs with short hydrophilic head groups are degraded by a two-component system: an enzyme and an activator protein. This seems to be much safer for the stability of short-chain GSLs outside the lysosomal compartment, e.g. on the cell surface.

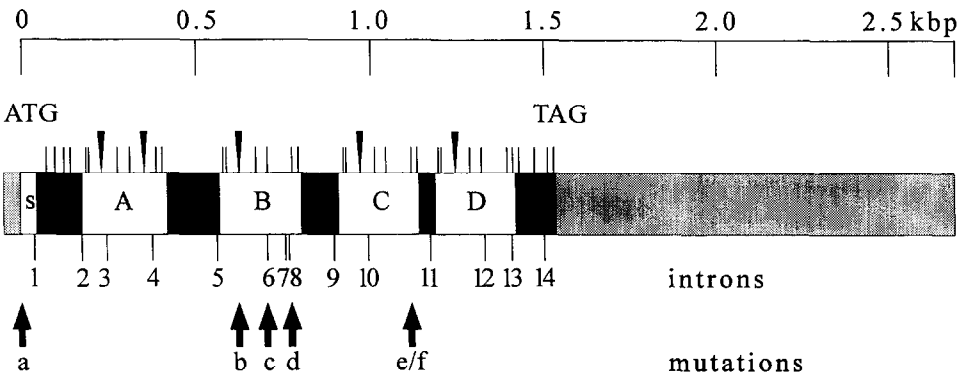

Fig. 3 Structure of the *sap*-precursor cDNA (according to [25], supplemented by a further mutation). The cDNA of *sap*-precursor codes for a sequence of 524 amino acids (or of 527 amino acids, see [51]) including a signal peptide of 16 amino acids (termed s, for the entry into the ER) [25,54]. The four domains on the precursor, termed saposins A–D by O'Brien et al. [46], correspond to the mature proteins found in human tissues: A, *sap*-A or saposin A; B, *sap*-B or saposin B or SAP-1 or sulfatide-activator; C, *sap*-C or SAP-2 or saposin C or glucosylceramidase activator protein; D, *sap*-D or saposin D or component C. The positions of cysteine residues are marked by vertical bars and those of the N-glycosylation sites by arrow heads. The positions of the 14 introns and of the known mutations leading to diseases are also given: (a) A1→T (Met^{1}→Leu) [27]; (b) C650→T (Thr^{217}→Ile) [47,48]; (c) 33 bp insertion after G777 (11 additional amino acids after Met^{259}) [49,50]; (d) G722→C (Cys^{241}→Ser) [51]; (e) G1154→T (Cys^{385}→Phe) [52]; (f) T1155→G (Cys^{385}→Gly) [53].

3. Pathobiochemistry of inherited enzyme and activator protein deficiencies

As already mentioned, the final degradation of sphingolipids occurs in the lysosome. Here they are degraded in a step-wise manner starting at the hydrophilic end of the molecule by exohydrolases. The inherited deficiency of one or the other of these enzymes causes the lysosomal storage of the respective substrates. The diseases resulting from these defects are rather heterogeneous from the biochemical as well as from the clinical point of view (see [42] for review).

The analysis of sphingolipid storage diseases without detectable hydrolase deficiency resulted in the identification of several point mutations in the GM2-activator gene (see [25] for review) and in the *sap*-precursor gene (Fig. 3). Interestingly, mutations affecting the *sap*-C domain (Gaucher factor) resulted in a variant form of Gaucher disease and mutations affecting the *sap*-B domain (sulfatide activator) resulted in variant forms of metachromatic leukodystrophy. On the other hand, a mutation in the start codon, ATG, of the *sap*-precursor resulted in a defect of the *sap*-precursor, SAPs A, B, C, D, and also in a simultaneous storage of ceramide and GSLs with short oligosaccharide head groups, such as glucosylceramide, lactosylceramide, galactosylceramide, sulphatide and ganglioside GM3 in the patients' tissue [26,43,44]. As detected by electron microscopy storage occurs predominantly within multivesicular bodies and/or within intra-lysosomal vesicles [44]. Obviously in the absence of the four SAPs, GSLs with short carbohydrate chains are not degraded. This also stabilizes the intralysosomal vesicles against degradation by lysosomal enzymes. This observation also suggests another distinct function that GSLs and other glycoconjugates may have on PMs; they may protect cell surfaces against premature digestion by extracellular hydrolases, e.g. phospholipases. Bianco reported that a molar fraction of 0.2 or more of ganglioside GM1 in the lipid monolayer completely protects the phospholipids in those monolayers against digestion by phospholipase A2 from pig pancreas [45].

Acknowledgements: We thank Dr. Gerhild van Echten-Deckert (Bonn, Germany), Prof. Harun Yusuf (Dhaka, Bangladesh) and Prof. Kunihiko Suzuki (Chapel Hill, USA) for helpful discussions. The work done in authors' laboratory was supported by the Deutsche Forschungsgemeinschaft (SFB 284).

References

[1] Svennerholm, L. (1984) in: Cellular and Pathological Aspects of Glycoconjugate Metabolism, vol. 126 (Dreyfus, H., Massarelli, R., Freysz, L. and Rebel, G. eds.) pp 21–44, INSERM, France.
[2] Ledeen, R.W. and Yu, R.K. (1982) Methods Enzymol. 83, 139–189.
[3] van Echten, G. and Sandhoff, K. (1989) J. Neurochem. 52, 207–214.
[4] Hakomori, S.I. (1984) Trends Biochem. Sci. 9, 453–458.
[5] Markwell, M.A.K., Svennerholm, L. and Paulson, J.C. (1981) Proc. Natl. Acad. Sci. USA 78, 5406–5410.
[6] Fishman, P.H. and Brady, R.O. (1976) Science 194, 906–915.
[7] Morré, D.J., Kartenbeck, J. and Franke, W.W. (1979) Biochim. Biophys. Acta 559, 71–152.
[8] Schwarzmann, G. and Sandhoff, K. (1990) Biochemistry 29, 10865–10871.
[9] Thompson, L.K., Horowitz, P.M., Bently, K.L., Thomas, D.D., Alderete, J.F. and Klebe, R.J. (1986) J. Biol. Chem. 261, 5209–5214.
[10] Tiemeyer, M., Yasuda, Y. and Schnaar, R.L. (1989) J. Biol. Chem. 264, 1671–1681.
[11] Mandon, E.C., van Echten, G., Birk, R., Schmidt, R.R. and Sandhoff, K. (1991) Eur. J. Biochem. 198, 667–674.
[12] Stoffel, W., Le Kim, D. and Sticht, G. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 664–670.

[13] van Echten, G. and Sandhoff, K. (1993) J. Biol. Chem. 268, 5341–5344.
[14] Mandon, E., Ehses, I., Rother, J., van Echten, G. and Sandhoff, K. (1992) J. Biol. Chem. 267, 11144–11148.
[15] Coste, H., Martel, M.-B. and Got, R. (1986) Biochim. Biophys. Acta 858, 6–12.
[16] Trinchera, M., Fabbri, M. and Ghidoni, R. (1991) J. Biol. Chem. 266, 20907–20912.
[17] Griffiths, G., Hoflack, B., Simons, K., Mellman, I. and Kornfeld, S. (1988) Cell 52, 329–341.
[18] Koval, M. and Pagano, R.E. (1989) J. Cell Biol. 108, 2169–2181.
[19] Koval, M. and Pagano, R.E. (1990) J. Cell Biol. 111, 429–442.
[20] Wessling-Resnick, M. and Braell, W.A. (1990) J. Biol. Chem. 265, 690–699.
[21] Kok, J.W., Babia, T. and Hoekstra, D. (1991) J. Cell Biol. 114, 231–239.
[22] Zachgo, S., Dobberstein, B. and Griffiths, G. (1992) J. Cell Sci. 103, 811–822.
[23] Hopkins, C.R., Gibson, A., Shipman, M. and Miller, K. (1990) Nature 346, 335–339.
[24] McKanna, J.A., Haigler, H.T. and Cohen, S. (1979) Proc. Natl. Acad. Sci. USA 76, 5689–5693.
[25] Fürst, W. and Sandhoff, K. (1992) Biochim. Biophys. Acta 1126, 1–16.
[26] Harzer, K., Paton, B.C., Poulos, A., Kustermann-Kuhn, B., Roggendorf, W., Grisar, T. and Popp, M. (1989) Eur. J. Pediatr. 149, 31–39.
[27] Schnabel, D., Schröder, M., Fürst, W., Klein, A., Hurwitz, R., Zenk, T., Weber, G., Harzer, K., Paton, B., Poulos, A., Suzuki, K. and Sandhoff, K. (1992) J. Biol. Chem. 267, 3312–3315.
[28] Renfrew, C.A. and Hubbard, A.L. (1991) J. Biol. Chem. 266, 21265–21273.
[29] van Deurs, B., Holm, P.K., Kayser, L., Sandvig, K. and Hansen, S.H. (1993) Eur. J. Cell Biol. 61, 208–224.
[30] Stoorvogel, W. (1993) Biochem. Soc. Trans. 21, 711–715.
[31] Murphy, R.F., Schmid, J. and Fuchs, R. (1993) Biochem. Soc. Trans. 21, 716–720.
[32] Mullock, B.M. and Luzio, J.P. (1993) Biochem. Soc. Trans. 21, 721–722.
[33] Carlsson, S.R. and Fukuda, M. (1990) J. Biol. Chem. 265, 20488–20495.
[34] Mehl, E. and Jatzkewitz, H. (1964) Hoppe-Seyler's Z. Physiol. Chem. 339, 260–276.
[35] Meier, E.M., Schwarzmann, G., Fürst, W. and Sandhoff, K. (1991) J. Biol. Chem. 266, 1879–1887.
[36] Li, S.-C., Sonnino, S., Tettamanti, G. and Li, Y.-T. (1988) J. Biol. Chem. 263, 6588–6591.
[37] Vogel, A., Schwarzmann, G. and Sandhoff, K. (1991) Eur. J. Biochem. 200, 591–597.
[38] Berent, S.L. and Radin, N.S. (1981) Biochim. Biophys. Acta 664, 572–582.
[39] Ho, M.W. and Light, N.D. (1973) Biochem. J. 136, 821–823.
[40] Ho, M.W. (1975) FEBS Lett. 53, 243–247.
[41] Ho, M.W. and Rigby, M. (1975) Biochim. Biophys. Acta 397, 267–273.
[42] Scriver, C., Beaudet, A.L., Sly, W.S. and Valle, D. (1989) The Metabolic Basis of Inherited disease, 6th edn., vol. II, David McGraw-Hill, New York.
[43] Paton, B.C., Schmid, B., Kustermann-Kuhn, B., Poulos, A. and Harzer, K. (1992) Biochem. J. 185, 481–488.
[44] Bradova, V., Smid, F., Ulrich-Bott, B., Roggendorf, W., Paton, B.C. and Harzer, K. (1993) Hum. Genet. 92, 143–152.
[45] Bianco, I.D., Fidelio, G.D. and Maggio, B. (1989) Biochem. J. 258, 95–99.
[46] O'Brien, J.S., Kretz, K.A., Dewji, N., Wenger, D.A., Esch, F. and Fluharty, A.L. (1988) Science 241, 1098–1101.
[47] Rafi, M.A., Zhang, X-L., De Gala, G. and Wenger, D.A. (1990) Biochem. Biophys. Res. Commun. 166, 1017–1023.
[48] Kretz, K.A., Carson, G.S., Morimoto, S., Kishimoto, Y., Fluharty, A.L. and O'Brien, J.S. (1990) Proc. Natl. Acad. Sci. USA 87, 2541–2544.
[49] Zhang, X-L., Rafi, M.A., De Gala, G. and Wenger, D.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1426–1430.
[50] Zhang, X-L., Rafi, M.A., De Gala, G. and Wenger, D.A. (1991) Hum. Genet. 87, 211–215.
[51] Holtschmidt, H., Sandhoff, K., Kwon, H.Y., Harzer, K., Nakano, T. and Suzuki, K. (1991) J. Biol. Chem. 266, 7556–7560.
[52] Schnabel, D., Schröder, M. and Sandhoff, K. (1991) FEBS Lett. 284, 57–59.
[53] Rafi, M.A., de Gala, G., Zhang, X. and Wenger, D.A. (1993) Somatic Cell Mol. Genet. 19, 1–7.
[54] Nakano, T., Sandhoff, K., Stümper, J., Christomanou, H. and Suzuki, K. (1989) J. Biochem. (Tokyo) 105, 152–154.