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Homology modeling of human sialidase enzymes NEU1, NEU3 and NEU4 based on the crystal structure of NEU2: Hints for the design of selective NEU3 inhibitors

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Abstract

Four types of human sialidases have been cloned and characterized at the molecular level. They are classified according to their major intracellular location as intralysomal (NEU1), cytosolic (NEU2), plasma membrane (NEU3) and lysosomal or mitochondrial membrane (NEU4) associated sialidases. These human isoforms are distinct from each other in their enzymatic properties as well as their substrate specificity. Altered expression of sialidases has been correlated with malignant transformation of cells and different sialidases have been known to behave differently during carcinogenesis. Particularly, increased expression of NEU3 has been implicated in the survival of various cancer cells and also in the development of insulin resistance. In the present study, we have modeled three-dimensional structures of NEU1, NEU3 and NEU4 based on the crystal structure of NEU2 using the homology modeling program MODELER. The best model in each enzyme case was chosen on the basis of various standard protein analysis programs. Predicted structures and the experimental protein–ligand complex of NEU2 were compared to identify similarities and differences among the active sites. The molecular electrostatic potential (MEP) was calculated for the predicted models to identify the differences in charge distribution around the active site and its vicinity. The primary objective of the present work is to identify the structural differences between the different isoforms of human sialidases, namely NEU1, NEU2, NEU3 and NEU4, thus providing a better insight into the differences in the active sites of these enzymes. This can in turn guide us in the better understanding and rationale of the differential substrate recognition and activity, thereby aiding in the structure-based design of selective NEU3 inhibitors.

Keywords: Homology modeling; Comparative modeling; Sialidase (neuraminidase); Structure-based inhibitor design; Molecular electrostatic potential (MEP); Active site modeling

1. Introduction

Sialidases (E.C.3.2.1.18) also known as neuraminidases belong to a group of glycohydrolytic enzymes, which remove sialic acid residues from a variety of sialoglycoconjugates. They are widely distributed among the different classes of organisms such as viruses, bacteria, protozoa and vertebrates [1]. Sialidases are thought to be involved in various biological processes like infection, proliferation, differentiation, catabolism, signal transduction, antigenic properties and inter–intra cell interactions [2].

Several mammalian sialidases have been cloned and characterized at the molecular level. In humans, four types of sialidases are known and have been classified based on their subcellular localization as intra lysosomal (NEU1) [3,4], cytosolic (NEU2) [5,6], plasma membrane (NEU3) [7,8] and lysosomal or mitochondrial membrane (NEU4) [9]. A comparison of human sialidases with respect to their location, substrate specificity, function and the changes they undergo in cancer cells has been described elsewhere in literature [10]. In addition to subcellular localization, these sialidases also differ

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in substrate preferences, pH required for optimum activity and also in immunological properties. Despite their different locations and substrate specificities, all human sialidases have highly conserved active site residues, the F/YRIV/P motif in the N-terminal part and the Asp boxes (consensus S/TXD(X)GXTW/F present as three to five repeat in the protein) similar to their viral and bacterial counter parts [11]. These results indicate a monophyletic origin of the sialidases and thus giving the basis for molecular modeling studies to elucidate the structure–function relationships between the family members. The comparative biochemistry and molecular biology of human sialidases has been reviewed excellently elsewhere [2].

Lysosomal sialidase (NEU1) possesses narrow substrate specificity for oligosaccharides, glycopeptides and a synthetic substrate 4MU-Neu5Ac (4-methylumbelliferyl-*N*-acetylneuraminic acid) [12] and is involved in lysosomal catabolism of sialoglycoconjugates by collaborating with lysosomal proteases or endoglycosidases [13]. Lysosomal storage diseases like sialidosis and galactosialidosis caused by NEU1 deficiency, interferes with the pathways for degradation of sialylated glycoconjugates [3,14]. In addition to this NEU1 has been proposed to be involved in cellular signaling during immune responses as well as monocytes differentiation [15,16]. Cytosolic sialidase (NEU2) is active against oligosaccharides, glycopeptides and gangliosides [17]. In mammals, it has been implicated in myotube formation [18]. The exact mechanism of myoblast differentiation through its natural substrate remains obscure but it is claimed to decrease the GM3 ganglioside content, associated with the cytoskeleton [19]. Plasma membrane sialidase (NEU3) hydroyzes gangliosides specifically (except GM1 and GM2) in presence of non-ionic surfactant (Triton X-100) [8,20,21]. Since gangliosides are abundant on the plasma membrane and are known modulators of several surface events like cell differentiation, cell proliferation and signal transduction, NEU3 is expected to be involved in cell surface functions by modulating gangliosides [22]. NEU3 involvement in neural differentiation has also been described in literature [23]. Lysosomal or mitochondrial membrane sialidase (NEU4) has broad specificity, active against all major sialoglycoconjugates and has been implicated in the catabolism of glycolipids [11,24]. A recent report reveals the possibility of NEU4 being involved in apoptosis pathway at the mitochondrial level by regulating ganglioside GD3 [24]. Further functions of NEU4 are yet to be explored.

Further, there is also some correlation between sialidase expression and the alteration in sialyation levels during malignant transformation of cells [10,22]. In murine, overexpression of lysosomal sialidase in cancer cells showed suppression of metastasis and tumor progression as well as increased sensitivity to apoptosis. Cytosolic sialidase overexpression in melanoma and colonadenocarcinoma cells of mouse has been inversely correlated with their invasiveness and metastatic potential, which is associated with a decrease in sialyl Lewis X and GM3 as well. Although it has been reported that NEU3 is not associated with metastatic potential and invasiveness, certain human cancer cells showed increased expression of NEU3 [25]. This increased expression resulted in inhibition of apoptosis accompanied by increased expression of Bcl2, followed by decreased expression of caspase. Inhibitory role of NEU3 in apoptosis is proposed to be mediated by accumulation of a possible sialidase product lactosyl ceramide (Lac-Cer), which induces Bcl2 expression or by rapid degradation of GD3 [25]. In addition, NEU3 mediated gangliosides depletion results in the activation of integrin-induced kinase/Akt, followed by deactivation of caspase-9 in SCCl2 cells [26]. A recent review discusses distinct aspects of NEU3 inhibition and its relevance in the cure of cancer [22]. NEU3 is also found to be involved in insulin signaling in two ways [27]. First is the negative regulation of insulin signaling by associating with the Grb2 protein and second is the suppression of insulin receptor (IR) phosphorylation through the modulation of gangliosides. Taken together, these anomalous functions of NEU3 are different from the possible regulatory functions of other human sialidases. So selective NEU3 inhibition may be a useful approach in cancer and diabetes therapy or in any case would be a valuable tool for exploring differential functions of human sialidases. The sialic acid transition-state analogue, DANA (2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid) is available as a weak inhibitor of sialidase enzymes, but it is not selective for NEU3. Structure-based approach in this regard may provide hints on how to exploit the non-selective inhibitor substituents that extend out of the active site pocket or 'de novo design' for isoform-selective inhibitor design. Structure homology and sequence alignment methods have been very useful in structure-based design approaches and can tackle the challenge of selectivity. Moreover, the success story of the structure-based drug design of viral sialidase inhibitors for flu fever through computational analysis giving the impetus for our efforts in this subject.

In the present study, we report homology models of NEU1, NEU3 and NEU4 using the crystal structure of NEU2. Using homology models, we have studied active site and its vicinity of human sialidases, which can be exploited for the design of selective NEU3 inhibitors.

2. Methods

All computations and simulations were carried out on an Intel P4 based Microsoft windows 2000 workstation using Discovery Studio Modeling 1.1 Package (Accelrys) [28].

2.1. Template search

The amino acid sequences of NEU1 (accession no. Q99519, entry name: NEUR1_HUMAN), NEU3 (accession no. Q9UQ49, entry name: NEUR3_HUMAN) and NEU4 (accession no. Q8WWR8, entry name: NEUR4_HUMAN) were obtained from Swiss-Prot database [29]. The Gapped-BLAST [30] through NCBI was used to identify homologous structures by searching the structural database of protein sequences in the protein data bank (PDB) [31] using *DS protein similarity* search module. The crystal structure of human NEU2 (PDB code: 1VCU, Chain B) was selected as a template for homology modeling of NEU1, NEU3 and NEU4 [32].