氏　　名	LEONARD CHAVAS	
学位（専攻分野）	博士（理学）	
学位記番号	総研大甲第899号	
学位授与の日付	平成１７年９月３０日	
学位授与の要件	高エネルギー加速器科学研究科　物質構造科学専攻 学位規則第６条第１項該当	
学位論文題目	Structural and Biochemical Studies on Glycoconjugate Alteration and Vesicle Transport	
論文審査委員	主　査　助教授	小林　克己
	教授	松下　正
	教授	河田　洋
	助教授	加藤　龍一
	助教授	若槻　壮市
	所長	宮城　妙子（宮城県立がんセンター研究所）

STRUCTURAL AND BIOCHEMICAL STUDIES ON GLYCOCONJUGATES ALTERATION AND VESICLE TRANSPORT

Structure Determination of Human Cytoplasmic Neu2

To date, 13 mammalian sialidases have been cloned, including four from humans. So far, only some bacterial and viral neuraminidase's structures are known, nothing from human or mammals. In this study, the crystal structure of the human cytosolic sialidase Neu2 was determined at the atomic level, either in an apo form or in complex with diverse inhibitors of substrate of reaction.

The core of the enzyme folds as a six-bladed β-propeller with 26 β-strands and five α-helices, typical of viral and bacterial sialidases. Neu2 adopts an irregular six-bladed structure caused by β-bulges of the third strand, as well as longer β-strands compared to the "classical" propeller foldings.

In the Neu2-DANA complex structure, 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA) lies in a half-chair conformation interacting with 10 amino acids of the active site. Interaction between Neu2 and the inhibitor DANA shows similarities with bacterial and viral counterparts but also exhibits some differences in the active site arrangement and dynamic nature of the loops containing residues responsible for catalysis and substrate recognition. An acidic crevice at the center of the β-propeller forms an activating core for the enzymatic catalysis and the basic residues at the mouth of the crevice coordinate substrates.

Two loops are disordered on the top side of the apo form Neu2: one contains Glu^{111} important for the substrate binding, and the other Asp^{46} for the catalysis. Soaking the apo form crystals with monosaccharide such as galactose, glucose, or maltose orders the former loop into α2, illustrating a plausible two steps model for a dynamic in the substrate recognition.

In the Neu2 structures, two Asp-boxes **STDHGRTW** (residues 129-136) and **SHDHGRTW** (residues 199-206) are easily identified (where boldface letters indicate residues structurally important for Asp-boxes). A third Asp-box **STNDGLDF** (residues 248-255) was found to have similar folding property comparing with the two firsts. The sequence of the third Asp-box in Neu2 extends the consensus sequence of Asp-boxes as **Ser**-X-(**Asp/Gln**)-X-**Gly**-X-(**Thr/Asp**)-Φ, where Φ stands for aromatic residues. A new screening may therefore give further insights into the function of these structural elements.

Finally, different crystal forms of the sialidase have been refined in order to facilitate inhibitors screenings. The influenza virus neuraminidase plays a critical role in the life cycle of the virus and has been the focus of new drug developments. Drug design of new agents against the viral sialidase comes from the fact that whereas diverse kinds of influenza virus neuraminidases were identified, their catalytic site is completely conserved among all influenza subtypes in terms of amino acids organization. To date, no data exist on whether the human sialidases can interact as well with viral and/or bacterial targeting drugs. To answer this question, complex structures of Neu2 with overall six different influenza virus inhibitors have been solved, illustrating the inhibitors lying in the human sialidase active site coordinated in a similar way. By comparing the recognition mechanism of each inhibitor, in Neu2 or in influenza virus neuraminidase, new drug design studies might be started, in order to preferentially recognize either human or viral sialidases.

Biochemical and Crystallographic Studies on Rab27a/b and Their Effectors

The integrity of eukaryotic cells strongly depends on membrane traffic, which is tightly linked to the regulation by a number of protein families such as Ras, Rho, Rab and Ran. Those proteins bind to various types of effector proteins, and execute versatile functions. More precisely, the Rab27 subfamily, consisting of Rab27a and Rab27b, is directly implicated in the transport of lysosome-related organelles, such as melanosomes in melanocytes or lytic granules in cytotoxic T-lymphocytes. In order to clarify the regulation process of such transport phenomena, biochemical and crystallization experiments have been carried out on both Rab27a/b, alone and in complex with two effectors Slac2-a and Slp4-a.

The individual GTPases, Rab27a and Rab27b, were purified for suitable crystallization experiments, both in their active (GTP-bound) or inactive (GDP-bound) forms. Extensive crystallization screenings have been performed, resulting in the apparition of some protein diffracting crystals in diverse conditions for Rab27b in its inactive form. The crystals grew in a tetragonal space group but did not diffract at higher resolution than 4.2 Å. Seleno-methionine substituted Rab27b protein was then purified and crystallized, but the crystal's diffraction limit did not improve. Further refinements in terms of protein deletion mutants and crystallization are now under way in order to obtain highly diffracting crystals.

The complexes between the GTPases and their effectors, respectively Slac2-a and Slp4-a, could be generated and purified, and have been subjects of intensive crystallization trials with the help of a crystallization robot. Unfortunately, to date no protein crystals suitable for X-ray structure determination have been obtained.

論文の審査結果の要旨

シアル酸分解酵素（シアリダーゼ、ノイラミダーゼ）は、ウィルスから哺乳類まで生物界に普遍的に存在することが知られている。その中でもヒト由来シアリダーゼの単離は比較的最近で、その生物学的機能の全容は明らかにされていないものの、癌、糖尿病、神経疾患において顕著に発現が変化することや、ウィルス由来の酵素との比較から特に感染症との関連などにおいて興味が持たれている。本研究では、現在４種類単離されているヒト由来シアリダーゼのうちの１つ Neu2 タンパク質に着目し、その放射光Ｘ線結晶構造の研究を行った。申請者はヒト Neu2 タンパク質の単独での結晶化を行いその立体構造を決定した。これは哺乳類由来のシアリダーゼの初めての立体構造の決定である。また、エフェクター分子および阻害剤存在下での複合体の立体構造決定も行い、特に阻害剤については６種類の異なるものについての構造を得た。Neu2 タンパク質の基本的な骨格は既知のウィルス由来のものと似ており、活性に必要な残基も空間的に保存されていた。一方、各種阻害剤との複合体の構造解析から、阻害剤の認識機能はヒトとウィルスで一部異なるところがあることが明らかになった。インフルエンザウィルスの本酵素はインフルエンザの治療薬のターゲットであり、薬剤分子がウィルス酵素の活性中心に結合することで酵素活性を失活させ、感染細胞からのウィルス粒子の遊離を阻害する。興味深いことに抗インフルエンザウィルス薬である阻害剤の１つはヒト Neu2 タンパク質にも結合することが示され、これらの知見から今後の副作用のない薬の開発に重要な知見を与えた。

低分子量 G タンパク質（細胞内シグナル伝達を担う GTP 結合タンパク質ファミリー）の１つで細胞内輸送の制御に関わる Rab27 タンパク質は、糖尿病やメラノドーシスなどの病気との関与が近年強く示されている。申請者はその構造と機能の理解を図るために、マウス由来の Rab27 タンパク質について、立体構造解析をめざして精力的に研究を行った。現在、低分解能ながら回折能のある結晶を得ており、今後の単体での構造決定および複合体での構造決定に向けた礎を築くことができた。