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活動報告

# 平成１４年度後期構造生物学坂部プロジェクトの活動

運営委員会委員長
坂 部 知 平

## Ⅰ．ＳＢＳＰ用第一実験ステーションＢＬ６Ｂ

### １．利用状況

平成１４年度後期のビームタイムは平成１５年１月１６日（木）午前９時に開始され２月２７日（木）午前９時に終了した。予約状況を表１に示す。前回と同様bonus日(入射器のマシンスタディ)を予備日とし、それ以外の予備日は取らなかった。bonus timeは１週間前迄に急を要する要求がなければキャンセルを行った。昼夜を問わず利用された。

R-AXIS Ⅳ++の導入により、検出器が複数になったため中期と同様後期開始時点では予約表に検出器の種類を示す記号を入れた。即ち、R-AXIS Ⅳ++を（R）、従来のLarge IPを（P）とした。しかし、終盤になって（P）に予約している人に問い合わせたところ、全員（R）で良いことが判明したので、表１に示されているように、最終的には全て（R）とした。

### ２．ビームラインアシスタント

今期はビームタイムが短く、しかも途中Single bunchで２分されている。通常前半より後半のビームタイム利用が多いので、今期は１人の方に後半を担当して頂いた。
今期のビームラインアシスタントの名簿を掲載すると共に氏に感謝する。

| 氏　　名 | 所　　属 | 期　　間 |
|---|---|---|
| 紙谷　康則 | 広島大学・片柳克夫研 | 2月１０日 ～ 3月1日 |

### ３．装置の状況

#### １）ＢＬ６Ｂビームライン

1月18日、He流量不足によるアラームが鳴った他は全く順調に稼動した。

#### ２）測定装置

R-AXISX Ⅳ++は極めて良好に稼動した。

最初に述べたように、大型IPの希望は無かった。

ビームタイムが短期であったため３×６ＣＣＤ検出器のテスト実験は行わなかった。

#### ３）低温吹付け装置

先回のビームタイム中低温吹付け装置が故障し、急遽、㈱リガクに窒素ガス抽出型試料吹付低温装置（cat.no.2364B302）及びその付属品である空冷型循環送水装置（cat.no.4811D）を発注した。当初の納入予定は３月であったが、今期開始以前に納入され、順調に稼動した。

#### ５）波長変更

波長変更は可能になったが、今期は波長変更の要求は無く、常時波長1.0ÅのＸ線が用いられた。

表１．平成 14 年度後期 BL6B ビームタイム使用状況

LAST UPDATE: 2/25/2003 11:39
USER NAME  _a:administer _c:industry _d:non-industry -:free
R:RaxisIV++, P:Big Imaging Plate, O:Other Detectors

| | | day: am:9.00-pm:9.00 | night: pm9.00-am:9.00(the next day) |
|---|---|---|---|
| 1/16 | THU R | bonus_time (day) | bonus_time (night) |
| 1/17 | FRI R | Fujisawa_Pharm._c (day) | - (night) |
| 1/18 | SAT R | Nippon_Roche_c (day) | - (night) |
| 1/19 | SUN R | - (day) | - (night) |
| 1/20 | MON R | machine_study (day) | machine_study (night) |
| 1/21 | TUE R | setting_test (day) | setting_test (night) |
| 1/22 | WED R | Kyowa_Hakko_Kogyo_c (day) | setting_test (night) |
| 1/23 | THU R | - (day) | - (night) |
| 1/24 | FRI R | Fujisawa_Pharm._c (day) | - (night) |
| 1/25 | SAT R | - (day) | - (night) |
| 1/26 | SUN R | - (day) | - (night) |
| 1/27 | MON R | machine_study (day) | machine_study (night) |
| 1/28 | TUE R | - (day) | - (night) |
| 1/29 | WED R | Yamanouchi_Pharm._c (day) | - (night) |
| 1/30 | THU R | - (day) | - (night) |
| 1/31 | FRI R | Banyu_Pharm._c (day) | - (night) |
| 2/ 1 | SAT R | - (day) | - (night) |
| 2/ 2 | SUN R | - (day) | - (night) |
| 2/ 3 | MON R | machine_study (day) | machine_study (night) |
| 2/ 4 | TUE R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/ 5 | WED R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/ 6 | THU R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/ 7 | FRI R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/ 8 | SAT R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/ 9 | SUN R | 3GeV_single-bunch (day) | 3GeV_single-bunch (night) |
| 2/10 | MON R | machine_study (day) | machine_study (night) |
| 2/11 | TUE R | Sankyo_Co._Ltd_c (day) | Sankyo_Co._Ltd_c (night) |
| 2/12 | WED R | Yamanouchi_Pharm._c (day) | - (night) |
| 2/13 | THU R | Ajinomoto_Co._Inc_c (day) | Ajinomoto_Co._Inc_c (night) |
| 2/14 | FRI R | Eisai_Co._Ltd_c (day) | - (night) |
| 2/15 | SAT R | Nishiyama_Makoto_d (day) | Nishiyama_Makoto_d (night) |
| 2/16 | SUN R | - (day) | - (night) |
| 2/17 | MON R | machine_study (day) | machine_study (night) |
| 2/18 | TUE R | - (day) | - (night) |
| 2/19 | WED R | Ajinomoto_Co._Inc_c (day) | Ajinomoto_Co._Inc_c (night) |
| 2/20 | THU R | Ajinomoto_Co._Inc_c (day) | Ajinomoto_Co._Inc_c (night) |
| 2/21 | FRI R | Sankyo_Co._Ltd_c (day) | Sankyo_Co._Ltd_c (night) |
| 2/22 | SAT R | co_users (day) | co_users (night) |
| 2/23 | SUN R | co_users (day) | co_users (night) |
| 2/24 | MON R | - (day) | - (night) |
| 2/25 | TUE R | - (day) | - (night) |
| 2/26 | WED R | Sankyo_Co._Ltd_c (day) | - (night) |
| 2/27 | THU R | BERI_c (day) | - (night) |

 top  home  rule  help  booking

Ⅱ．ＳＢＳＰ第２実験ステーションＢＬ６Ｃ

ＢＬ６Ｃは未だ完全な状態ではないが、データ転送に関するエラー以外殆ど解決した。

１．カメラ部２θラグ修理、及びエアリフターの改良後の可動状況

先号で記載した、カメラ部２θラグ修理、及びエアリフターの改良を行った結果、カセットの移動はスムーズになり、それ以来極めて良好に稼動している。

２．読み取り部クラッチの調整後の状況

殆どこの関係のトラブルはなくなった。しかしカセット２のみ読み取り後クラッチが抜けなくなり、自動データ収集がストップすることがある。これは潤滑油を塗ることで解決する。

３．データ転送に関するエラー

読み取りの最後で、読み取りデータ量が不足のサイン

Ch1 data size error(44000000/45000000).
Ch2 data size error(44000000/45000000).
Ch3 data size error(44000000/45000000).
Ch4 data size error(44000000/45000000).
Ch5 data size error(44000000/45000000).

が出てストップする。頻繁ではないが、日に２~３回起こる。

Ⅲ．コンピュータ関係

１．ネットワークとデータサーバの利用状況

ネットワークとサーバは大きなトラブルもなく稼働した。ビームタイムの始まる前にサーバの /homeおよび /save領域の整理を行った。/homeについては１ヶ月以前に作られたファイルは全て消去した。/save領域については多量に使用しているユーザにemailを送り移動/消去を依頼した。ほとんどのユーザは自主的に不要なファイルを消去しておられるが、一部に多量の測定データを/saveに入れたままで消去せずに帰られる場合が見受けられるので注意していただきたい。1月から２月の間にサーバに書き込まれた測定データの総量を図１に示す。

２．データサーバのバックアップ用にPC LINUX サーバを導入

BL6Cのテストデータなど再処理のために保存しておくデータのためにPC LINUX サーバを１台導入した。ユーザの方々もデータ処理をもう一度SBSPで実行する必要のあるデータはこのサーバに保存ができる。

３．障害等のレポート

・メール/ホームページが見られない現象が発生。
　　対応）DNSのデーモンが動いているようにみえるが、DNSがひけない。
　　Killしてデーモンを動かすとメール/ホームページが見られる事を確認。
・ alphaにおいてコンピュータダウン。　Jan 22 11:55:38
　　対応) 起動し直し、動作確認。
・Phaser740にてイメージユニットのエラーの為交換。
・ファイルサーバ（sb00a）--sbsp6c1間のデータ伝送速度が遅い。
　　対応） sbsp6c1のポートを自動認識させ、Fullへ設定したら速度が戻った事を確認。
・sbsp6c1のRAID組直しを行い、OSを入れ直す。

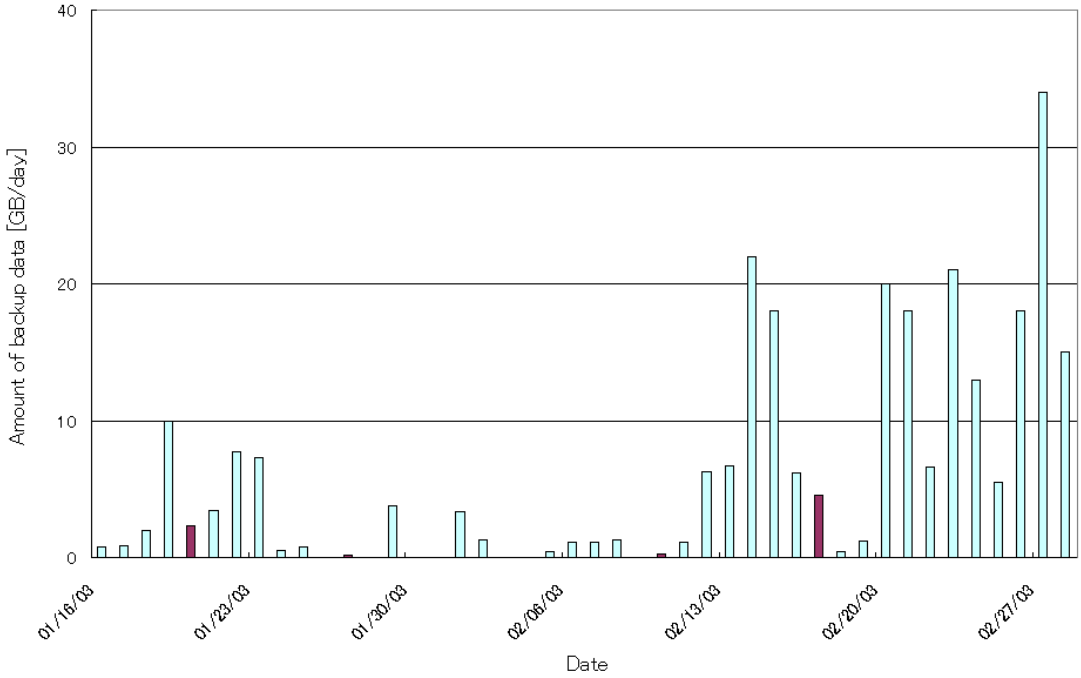


図１．１月～２月期におけるデータサーバ利用状況

Ⅳ．各種委員会報告

１．編集委員会

第２３回編集委員会が２００３年６月４日開催された。出席者は幾田まり、栗原宏之、坂部貴和子、坂部知平（五十音順）の４名であった。

構造生物Vol.9, No.1の原稿の最終チェックならびに印刷等のスケジュール確認が行なわれた。続いて、次号（Vol.9, No.2）の内容についての検討が行なわれ、執筆をお願いする方々および依頼担当者を決定した。

Ⅴ．業績紹介

１．栗原宏之（山之内製薬）、片山直子（山之内製薬）

**Target-induced Conformational Adaptation of Calmodulin Revealed by the Crystal Structure of a Complex with Nematode $Ca^{2+}$/Calmodulin-dependent Kinase Kinase Peptide**

*J. Mol. Biol.* **312**, 59-68 (2001)

Hirofumi Kurokawa[1,2], Masanori Osawa[3], Hiroyuki Kurihara[3], Naoko Katayama[3], Hiroshi Tokumitsu[4], Mark B. Swindells[5], Masatsune Kainosho[2] and Mitsuhiko Ikura[1*]

[1]Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Ontario M5G2M9, Canada
[2]Department of Chemistry Faculty of Science, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji Tokyo 192-0397, Japan
[3]Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd. Tsukuba 305-8585, Japan

[4]Department of Chemistry Kagawa Medical University Kagawa 761-0793, Japan
[5]Inpharmatica, London W1P2AX, UK

Summary

Calmodulin (CaM) is a ubiquitous calcium ($Ca^{2+}$) sensor which binds and regulates protein serine/threonine kinases along with many other proteins in a $Ca^{2+}$-dependent manner. For this multi-functionality, con-formational plasticity is essential; however, the nature and magnitude of CaM's plasticity still remains largely undetermined. Here, we present the 1.8 Å resolution crystal structure of $Ca^{2+}$/CaM, complexed with the 27-residue synthetic peptide corresponding to the CaM-binding domain of the nematode *Caenorhabditis elegans* $Ca^{+}$/CaM-dependent kinase kinase (CaMKK). The peptide bound in this crystal structure is a homologue of the previously NMR-derived complex with rat CaMKK, but benefits from improved structural resolution. Careful comparison of the present structure to previous crystal structures of CaM complexed with unrelated peptides derived from myosin light chain kinase and CaM kinase II, allow a quantitative analysis of the differences in the relative orientation of the N and C-terminal domains of CaM, defined as a screw axis rotation angle ranging from 156 ° to 196 °. The principal differences in CaM interaction with various peptides are associated with the N-terminal domain of CaM. Unlike the C-terminal domain, which remains unchanged internally, the N-terminal domain of CaM displays significant differences in the EF-hand helix orientation between this and other CaM structures. Three hydrogen bonds between CaM and the peptide (E87-R336, E87-T339 and K75-T339) along with two salt bridges (E11-R349 and E114-K334) are the most probable determinants for the binding direction of the CaMKK peptide to CaM.

２．栗原宏之（山之内製薬）、片山直子（山之内製薬）

**Coumarin and Chromen-4-one Analogues as Tautomerase Inhibitors of Macrophage Migration Inhibitory Factor: Discovery and X-ray Crystallography**

*J. Med. Chem.* **44**, 540-547 (2001)

Masaya Orita, Satoshi Yamamoto, Naoko Katayama, Motonori Aoki, Kazuhisa Takayama, Yoko Yamagiwa, Norio Seki, Hiroshi Suzuki, Hiroyuki Kurihara, Hitoshi Sakashita, Makoto Takeuchi, Shigeo Fujita, Toshimitsu Yamada, and Akihiro Tanaka,

Yamanouchi Pharmaceutical Company Ltd., 21 Miyukigaoka, Tsukuba Science City 305-8585, Japan

Summary

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine released from T-cells and macrophages. Although a detailed understanding of the biological functions of MIF has not yet been clarified, it is known that MIF catalyzes the tautomerization of a nonphysiological molecule, d-dopachrome. Using a structure-based computer-assisted search of two databases of commercially available compounds, we have found 14 novel tautomerase inhibitors of MIF whose $K_i$ values are in the range of 0.038-7.4 μM. We also have determined the crystal structure of MIF complexed with the hit compound **1**. It showed that the hit compound is located in the active site of MIF containing the N-terminal proline which plays an important role in the tautomerase reaction and forms several hydrogen bonds and undergoes hydrophobic interactions. A crystallographic study also revealed that there is a hydrophobic surface which consists of Pro-33, Tyr-36, Trp-108, and Phe-113 at the rim

of the active site of MIF, and molecular modeling studies indicated that several more potent hit compounds have the aromatic rings which can interact with this hydrophobic surface. To our knowledge, our compounds are the most potent tautomerase inhibitors of MIF. One of these small, drug-like molecules has been cocrystallized with MIF and binds to the active site for tautomerase activity. Molecular modeling also suggests that the other hit compounds can bind in a similar fashion.

３．幾田まり（万有製薬）、鎌田健司　(万有製薬)

**Crystallographic Approach to Identification of Cyclin-dependent Kinase 4 (CDK4)-specific Inhibitors by Using CDK4 Mimic CDK2 Protein**

*J.B.C.*, **276**. 27548-27554 (2001)

Mari Ikuta, Kenji Kamata, Kazuhiro Fukasawa, Teruki Honma, Takumitsu Machida, Hiroshi Hirai, Ikuko Suzuki-Takahashi, Takashi Hayama, and Susumu Nishimura

The Banyu Tsukuba Research Institute / Merck Resarch Laboratories, Okubo 3, Tsukuba, Ibaraki 300-2611, Japan

Summary

Genetic alteration of one or more components of the $p16^{INK4A}$-CDK4,6/cyclin D-retinoblastoma pathway is found in more than half of all human cancers. Therefore, CDK4 is an attractive target for the development of a novel anticancer agent. However, it is difficult to make CDK4-specific inhibitors that do not possess activity for other kinases, especially CDK2, because the CDK family has high structural homology. The three-dimensional structure of CDK2, particularly that bound with the inhibitor, has provided useful information for the synthesis of CDK2-specific inhibitors. The same approach used to make CDK4-specific inhibitors was hindered by the failure to obtain a crystal structure of CDK4. To overcome this problem, we synthesized a CDK4 mimic CDK2 protein in which the ATP binding pocket of CDK2 was replaced with that of CDK4. This CDK4 mimic CDK2 was crystallized both in the free and inhibitor-bound form. The structural information thus obtained was found to be useful for synthesis of a CDK4-specific inhibitor that does not have substantial CDK2 activity. Namely, the data suggest that CDK4 has additional space that will accommodate a large substituent such as the CDK4 selective inhibitor. Inhibitors designed to bind into this large cavity should be selective for CDK4 without having substantial CDK2 activity. This design principle was confirmed in the x-ray crystal structure of the CDK4 mimic CDK2 with a new CDK4 selective inhibitor bound.

４．曽我部智（日本ロシュ、現在武田薬品工業）、深海隆明（日本ロシュ、現在中外製薬）

**Crystal Structures of Candida albicans *N*-Myristoyltransferase with Two Distinct Inhibitors**

*Chemistry & Biology*, **9**, 1119-1128, 2002）

Satoshi Sogabe,[1,3] Miyako Masubuchi,[1] Kiyoaki Sakata,[1] Takaaki A. Fukami,[1] Kenji Morikami,[1] Yasuhiko Shiratori,[1] Hirosato Ebiike,[1] Kenichi Kawasaki,[1] Yuko Aoki,[1] Nobuo Shimma,[1] Allan D'Arcy,[2,4] Fritz K. Winkler,[2,5] David W. Banner,[2] and Tatsuo Ohtsuka[1]

**[1]Nippon Roche Research Center 200 Kajiwara, Kamakura, Kanagawa 247-8530 Japan**
**[2]Pharmaceutical Research F. Hoffmann-La Roche Ltd. CH-4070 Basel Switzerland**

Summary

Myristoyl-CoA:protein *N*-myristoyltransferase (Nmt) is a monomeric enzyme that catalyzes the transfer of the fatty acid myristate from myristoyl-CoA to the N-terminal glycine residue of a variety of eukaryotic and viral proteins. Genetic and biochemical studies have established that Nmt is an attractive target for antifungal drugs. We present here crystal structures of *C. albicans* Nmt complexed with two classes of inhibitor competitive for peptide substrates. One is a peptidic inhibitor designed from the peptide substrate; the other is a nonpeptidic inhibitor having a benzofuran core. Both inhibitors are bound into the same binding groove, generated by some structural rearrangements of the enzyme, with the peptidic inhibitor showing a substrate-like binding mode and the nonpeptidic inhibitor binding differently. Further, site-directed mutagenesis for C. *albicans* Nmt has been utilized in order to define explicitly which amino acids are critical for inhibitor binding. The results suggest that the enzyme has some degree of flexibility for substrate binding and provide valuable information for inhibitor design.

５．木下誉富（藤沢薬品工業）

**The structure of human recombinant aldose reductase complexed with the potent inhibitor zenarestat**

*Acta Cryst.,* D**58**, 622-626 (2002)

Takayoshi Kinoshita,[a] Hiroshi Miyake,[a] Takashi Fujii,[a] Shoji Takakura[b] and Toshio Goto[b]

[a]Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd, 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan, and [b]Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co. Ltd, Kashima 2-1-6, Yodogawa-ku, Osaka 532-8514, Japan

Summary

The crystal structure of the complex of human recombinant aldose reductase (AR) with zenarestat, one of its potent inhibitors, has been solved at 2.5 Å resolution. Zenarestat fits neatly in the hydrophobic active site and induces unique and dramatic conformational changes. For example, the benzene ring of zenarestat occupies a gap in the side chains of Leu300 and Trp111 that interact directly and forms a CH-π interaction in the native holoenzyme. As a result, the benzene ring of the inhibitor and these side chains form a CH-π-π interaction. Such structural information is key to understanding the mode of action of this class of inhibitors and for rational design of better therapeutics.

6. 柏木立己（味の素）、石川弘紀（味の素）、鈴木榮一郎（味の素）

**Crystal Structure of Microbial Transglutaminase from *Streptoverticillium mobaraense***

*J.B.C.*, **277**, 44252-44260 (2002)

Tatsuki Kashiwagi, Kei-ichi Yokoyama, Kohki Ishikawa, Kunio Ono, Daisuke Ejima, Hiroshi Matsui, and Ei-ichiro Suzuki*
The Central Research Laboratories, Ajinomoto Company Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan

Summary

The crystal structure of a microbial transglutaminase from *Streptoverticillium mobaraense* has been determined at 2.4 Å resolution. The protein folds into a plate--like shape, and has one deep cleft at the edge of the molecule. Its overall structure is completely different from that of the factor XIII-like transglutaminase, which possesses a cysteine protease-like catalytic triad. The catalytic residue, $Cys^{64}$, exists at the bottom of the cleft. $Asp^{255}$ resides at the position nearest to $Cys^{64}$ and is also adjacent to $His^{274}$. Interestingly, $Cys^{64}$, $Asp^{255}$, and $His^{274}$ superimpose well on the catalytic triad "Cys-His-Asp" of the factor XIII-like transglutaminase, in this order. The secondary structure frameworks around these residues are also similar to each other. These results imply that both transglutaminases are related by convergent evolution; however, the microbial transglutaminase has developed a novel catalytic mechanism specialized for the cross-linking reaction. The structure accounts well for the catalytic mechanism, in which $Asp^{255}$ is considered to be enzymatically essential, as well as for the causes of the higher reaction rate, the broader substrate specificity, and the lower deamidation activity of this enzyme.

７．石川弘紀（味の素）、鈴木榮一郎（味の素）

**Enhancement of nucleoside phosphorylation activity in an acid phosphatase**

Protein Engineering, **15 ,** 539-543 (2002)

Kohki Ishikawa, Yasuhiro Mihara[1], Nobuhisa Shimba, Naoko Ohtsu, Hisashi Kawasaki[1], Ei-ichiro Suzuki[2] and Yasuhisa Asano[3]

Central Research Laboratories and [1]Fermentation and Biotechnology Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho Kawasaki-ku, Kawasaki 210-8681 and [3]Biotechnolory Research Center, Faculty of Engineering, Toyama Prefectural University, 5180 Kurosawa, Kosugi, Toyama 939-0398, Japan

*Summary*

*Escherichia blattae* non-specific acid phosphatase (EBNSAP) possesses a pyrophosphate-nucleoside phosphotransferase activity, which is C-5'-position selective. Current mutational and structural data were used to generate a mutant EB-NSAP for a potential industrial application as an effective and economical protein catalyst in synthesizing nucleotides from nucleosides. First, Gly74 and Ile153 were replaced by Asp and Thr, respectively, since the corresponding replacements in the homologous enzyme from *Morganella morganii* reduced the $K_m$ value for inosine and thus increased the productivity of 5'-IMP. We determined the crystal structure of G74D/I153T, which has a reduced $K_m$ value for inosine, as expected. The tertiary structure of G74D/I153T was virtually identical to that of the wild-type. In addition, neither of the introduced side chains of Asp74 and Thr153 is directly involved in the interaction with inosine in a hypothetical binding mode of inosine to EB-NSAP, although both residues are situated

near a potential inosine-binding site. These findings suggested that a slight structural change caused by an amino acid replacement around the potential inosinebinding site could significantly reduce the $K_m$ value. Prompted by this hypothesis, we designed several mutations and introduced them to G74D/I153T, to decrease the $K_m$ value further. This strategy produced a S72F/G74D/I153T mutant with a 5.4-fold lower $K_m$ value and a 2.7-fold higher $V_{max}$ value as compared to the wild-type EB-NSAP.

８．伊藤秀一郎（三共）、畠忠（三共）

**Humanization of the Mouse Anti-Fas Antibody HFE7A and Crystal Structure of the Humanized HFE7A Fab Fragment**

*Biol. Pharm. Bull.* **25**, *1537-1545 (2002)*

Hideyuki HARUYAMA,[a] Shuichiro ITO,*[a] Kenji MIYADAI,[b] Tohru TAKAHASHI,[a] Reimi KAWAIDA,[a] Tomoko TAKAYAMA,[a] Hiroyuki HANZAWA,[a] Tadashi HATA,[a] Junko YAMAGUCHI,[a] Hiroko YOSHIDA-KATO,[a] Kimihisa ICHIKAWA,[a] Jun OHSUMI,[a] Shin YONEHARA,[c] and Nobufusa SERIZAWA[a]

*[a]Biomedical Research Laboratories, Sankyo Co., Ltd.; 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan: [b]Lead Discovery Research Laboratories, Sankyo Co., Ltd.; 389-4 Aza-ohtsurugi, Shimokawa, Izumi-machi, Iwaki, Fukushima 971-8183, Japan: and [c]Institute for Virus Research, Kyoto University; Kyoto 606-8507, Japan.*
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Summary

Binding of Fas ligand to Fas induces apoptosis. The Fas-Fas ligand system plays important roles in many biological processes, including the elimination of autoreactive lymphoid cells. We have previously obtained the mouse anti-Fas antibody HFE7A (m-HFE7A), which specifically induces apoptosis in inflammatory cells. In order to apply m-HFE7A for human therapy, we performed antibody humanization of m-HFE7A by grafting the mouse complementarity-determining regions (CDRs) to a human antibody. Five versions of humanized HFE7A (h-HFE7A) demonstrated the same antigen-binding affinity and same competition-binding activity against Fas as the chimeric HFE7A. Furthermore, these h-HFE7As induced the same degree of apoptosis in WR19L12a cells that express human Fas on their surface as chimeric HFE7A does. To further probe the structural basis for antibody humanization, we determined the three-dimensional structure of the h-HFE7A antigen-binding fragment (Fab) by X-ray crystallography and compared it with the crystal structure of the parent m-HFE7A Fab previously determined. The main-chain conformation in each h-HFE7A CDR is almost identical to that in m-HFE7A with root mean square (rms) deviations of 0.14-0.77 Å. However, a significant segmental shift was observed in the CDR-L1 loop. Together with the high temperature factors of the CDR-L1 residues, both the loops are flexible, suggesting that the CDR-L1 loop would undergo conformational change upon binding to the antigen. Our results indicate that the humanization of m-HFE7A succeeded in maintaining the main-chain conformation as well as the flexibility of the CDR loop.