が FD3 および FD4 の C7 の位置と相互作用していることを意味している．V 型のアデニル酸シクラーゼを刺激する FD5 と FD6 の負の静電ポテンシャルは**第 1 図 - (g)**, **- (h)** にあるように C8 と C7 の裏側に大きく広がり，C6 の位置には広がりがない．したがって V 型アデニル酸シクラーゼの正の静電ポテンシャルは FD5 と FD6 の C7, C8 と相互作用をすることを意味する．

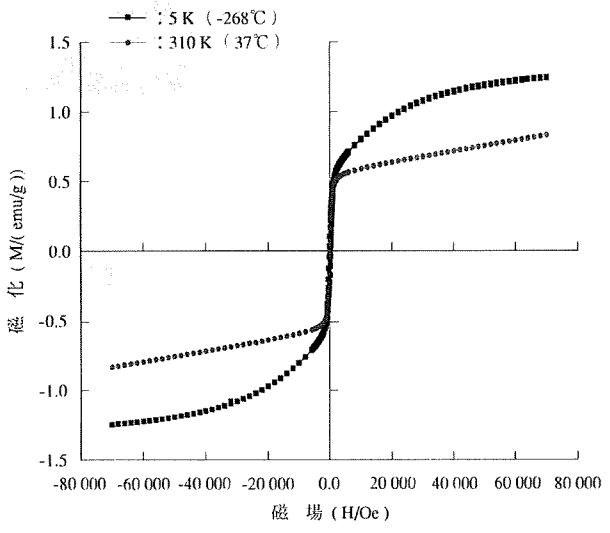
これらの結果，フォルスコリン誘導体の負の静電ポテンシャルは各サブタイプ型のアデニル酸シクラーゼの正の静電ポテンシャルと相互作用することでその特異性をもつことが分かった．本評価に費やした時間は 3 か月であり，従来法[3]の 5 年と比較してフォルスコリン誘導体の活性評価時間を大幅に短縮した．

3. 磁場誘導ドラッグ・デリバリ・システムに用いる有機磁性体の解析と実証実験[7]

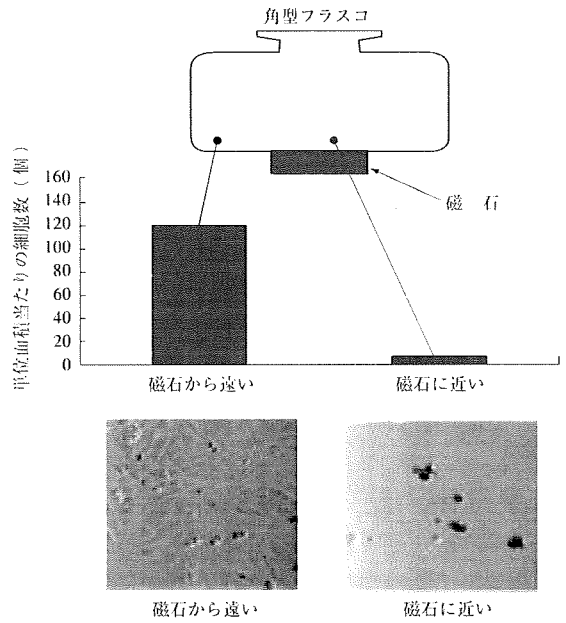
世の中に存在する物質は原子から構成され，その原子は原子核と電子からなる．その電子一つはこまのように回転しており，左回りの時は上が N 極，下が S 極（これを上向きスピン電子状態という），そして右回りの時は上が S 極，下が N 極（これを下向きのスピン電子状態という）となる．したがって電子 1 個単独で存在する場合は磁石としての機能をもつ．しかし，共有結合等のように，上向きのスピン電子と下向きのスピン電子の二つの電子がそろった場合は電子がもつ磁石の性質を打ち消し合うことになり磁石としての性質を失う．多くの有機化合物は，共有結合を中心に構成されているため，上向きのスピン電子と下向きのスピン電子はそろい，結果として電子の磁石としての性質は失われ，磁石にくっつかなくなる．たとえば，我々の身近にあるプラスチック材料などが磁石にくっつかないのはこの理由である．したがって，磁石にくっつくような有機化合物を設計するにはスピン電子の電子状態を制御する必要がある．具体的には，物理の言葉では同じ方向のスピン電子のみを局所的にそろえる．化学の言葉ではラジカル電子を分子中に安定に存在させることが有機磁性体の設計の指導原理である．我々は第一原理計算によって対を組まない電子（すなわち安定ラジカル電子）をコンピュータによって数える方法[8]を開発した．本方法を用いて有機磁性体の探索を行い，超伝導量子干渉素子 (SQUID：Superconducting Quantum Interface Devices) を，Quantum Design 社（アメリカ）の MPMS (Magnetic Property Measurement System) を用いて「磁場－磁化曲線」を測定したところ DNA 切断剤と呼ばれる抗がん剤の中に −268 ～ 37℃まで強磁性体の特徴であるヒステリシスループをもつ有機磁性体 (EI236)[9]を見いだした（**第 2 図**）．磁場誘導 DDS の可能性が出てきたので，EI236 を用いて次の生化学実験を行った．

ラットの筋肉のがん細胞である L6 細胞が 30%培養容器の培養面を覆う（コンフルエント）の状態になった時に EI236 の粉末を均等にふりかけた後，フラスコ横底面にネオジウム永久磁石を設置し 48 時間後に培地の状態を観察した．**第 3 図**の上側はラット L6 細胞の培地がある角型フラスコに棒磁石を接触させた状態を示している．次いで，48 時間後角型フラスコ底面の一端から他端までを撮影し，細胞数を算出した結果を**第 3 図**の中央に示す．**第 3 図**の中央における磁石に近いとは，角型フラスコ底面における磁石端面を示し，磁石から遠いとは，角型フラスコ底面において磁石端面と反対側にある領域を示す．**第 3 図**に示すように，磁石から近いでは EI236 が引き寄せられて EI236 の濃度が増し抗がん作用によって細胞数が遠位よりも極端に低いことが分かる．したがって，EI236 は磁石を用いて誘導が可能で，磁石近位の部分の薬剤濃度を高くすることでがん細胞の増殖を防ぐことが可能であることが分かった．

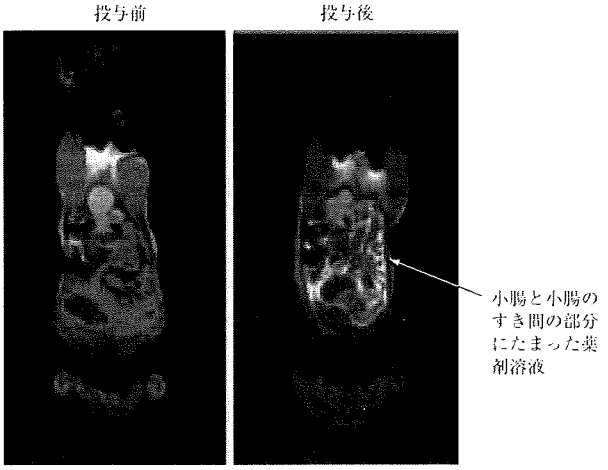
次に，薬剤を動物に投与して MRI で撮影を行った．**第 4 図**は，9 週齢のメスのラット（日本 SIC 製 ddy）用い，これに，磁性をもつ EI236 を溶液に溶解したもの（濃度 5 mg/m*l*）を皮下注射によって投与した後，脂肪，ガドリウム造影剤などで高信号が得られる撮影モードである T1



第 2 図 EI236 の「磁場－磁化」曲線測定結果
Fig. 2 Magnetic field versus magnetization curve of EI236



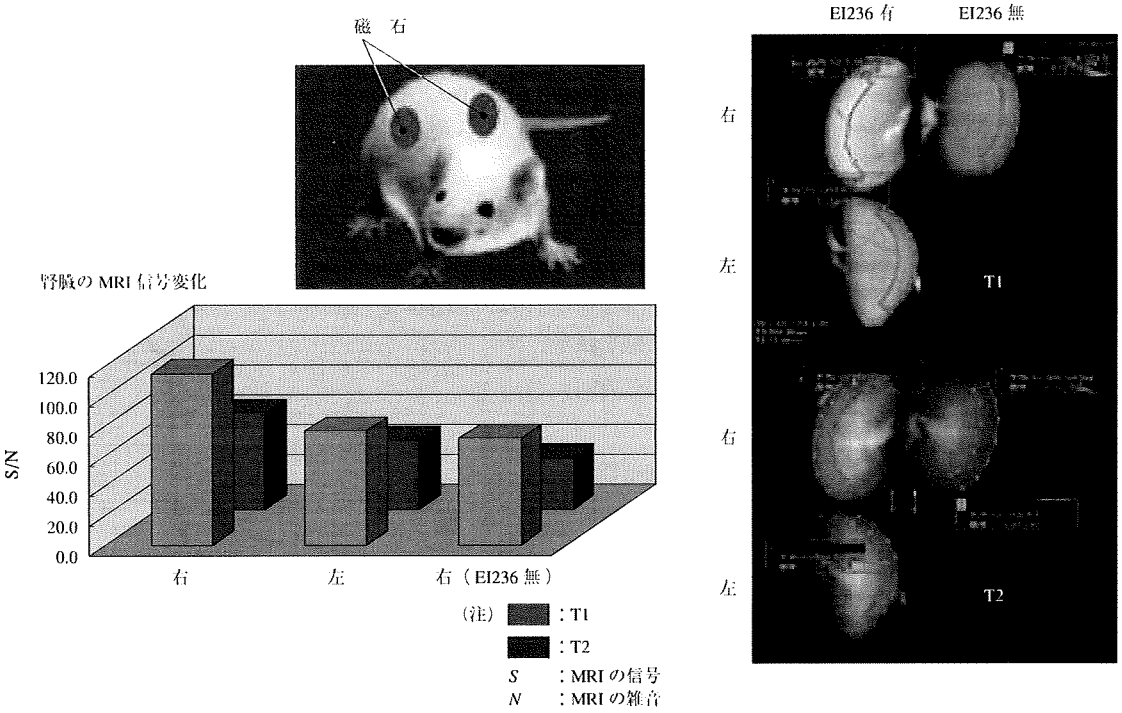
第 3 図　がん細胞（L6 細胞）の磁場誘導実験
Fig. 3　Magnetic target experiment of cancer cell (L6)



第 4 図　EI236 の MRI 造影効果確認試験
Fig. 4　MRI imaging of EI236
(left hand side: before administration,
right hand side: after administration)

強調の MRI 画像である．EI236 を投与すると投与前に比べて臓器と臓器のすき間や腹腔膜に沿って造影効果が見られた．矢印の部分は小腸と小腸の隙間の部分に溜まった有機磁性体であり，本 EI236 が体内で白く光る陽性造影剤であることが分かった．

さらに，磁場で EI236 の簡易磁場誘導実験を動物で行った．上記と同様のラットの下大静脈から濃度 5mg/m*l* で 0.2 m*l* 静注し 10 分後，ラットから腎臓を摘出し MRI 造影実験を行った．**第 5 図**に腎臓の MRI 造影剤実験結果をしめす．T1 強調画像で見たところ，右側の腎臓の T1 強調の MRI 信号は左側と比較して約 35％程度増加しており，ネオジウム永久磁石を当てた右側の腎臓に EI236 が集積していることが分かった．一方，血液，関節液などで高信号が得られる MRI 撮影モードである T2 強調画像で



第 5 図　EI236 の MRI 造影効果確認試験（腎臓の MRI 信号変化）
Fig. 5　MRI imaging of EI236 (MRI signal change of kidney)

は，MRI 信号の変化は見られなかった．

4. 結　　言

第一原理計算による有機機能材料分野の解析の一例として創薬分野への適用を行った．

自律神経調節薬（フォルスコリン誘導体）の活性評価については，フォルスコリン誘導体のアデニル酸シクラーゼのサブタイプ特異性が正の静電ポテンシャルではなく負の静電ポテンシャルにあることを見いだした．本方法を用いれば，アデニル酸シクラーゼの各サブタイプを選択的に刺激し活性化する薬剤，すなわち自律神経調節薬のスクリーニングが従来の 5 年から約 3 か月に短縮されることを明らかにした．

また磁場誘導ドラッグ・デリバリ・システムに用いる有機磁性体の解析と実証実験では，第一原理計算に基づき強磁性体を見いだす独自の評価法[8]を考案した．次にその評価法を用いることで EI236 を見いだし，実証実験の結果 EI236 は 37℃においても強磁性体的性質をもっていることを明らかにした．さらに生化学実験を行ったところ，EI236 は，磁場誘導 DDS が可能な抗がん剤として機能し，さらに MRI 造影剤としても使用の可能性があることが分かった．最近では，上記のような有機磁性薬の探索だけではなく，市販薬の側鎖を改変することによって磁性をもたせることにも成功している．

第一原理計算による材料解析技術は，コンピュータ解析の導入が最も進んでいる創薬分野にも適用可能であることを実証した．

― 謝　辞 ―

東北大学大学院理学研究科物理学専攻 電子物理学講座 ナノネットワーク固体物理研究室 谷垣勝己教授には「磁場－磁化」曲線の測定，横浜市立大学医学部循環制御医学研究室の皆さんには生化学実験についてご協力をいただいたことをここに記し，深く感謝します．

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新規磁性薬剤化合物の画像診断への応用

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研究目的：

エレクトロニクスの材料分野を中心として、有機磁性体の研究開発が進んでいる。かつて磁性体は無機化合物のみであると考えられていたが、80 年代より多数の有機磁性体化合物の発見や合成が進んでおり、実用化に向けた検討が進められている。我々は材料分野の研究に用いられてきた、物理学的な計算手法である第一原理解析法を駆使することにより、有機化合物に磁性を予測する方法を開発した。本手法を用いることにより、医療、とりわけＭＲＩを中心とした画像解析に有用であると考えられる有機化合物を中心に、磁性予測を行い、ＭＲ画像診断における「機能を有する造影剤」として開発し、新規画像診断技術として開発していくことが目的である。

方法および結果：

第一原理解析法はこれまで超伝導材料やエレクトロニクス材料の開発に用いられてきた手法であり、この手法の応用により、有機化合物に磁性を予測することが可能となった。この磁性予測技術を用いて、医薬材料化合物の磁性予測を行った。さらに解析精度を上げるために様々な計算手法を導入すると共に、実際の化合物合成を行い、その磁性を測定することにより、我々の磁性解析法を改善していった。これまで 3 年間の試行錯誤の結果から、単に磁性を予測するだけでなく、磁性を設計し、既存の非磁性体化合物を磁性化する手法を開発することが出来た。このことは既存の薬理化合物に対して磁性化設計を行い、新規磁性薬理化合物として再開発できることを意味する。

我々の同定した磁性有機化合物のひとつに、抗がん作用を有するものがある（ＥＩ２３６）。我々はこの化合物の抗がん剤としての薬理作用を検討すると共に、磁性特性を用いた磁場による誘導作用、交流磁場賦課による発熱作用を検討すると共に、磁性特性によるＭＲＩにおける造影効果を検討した。

ＥＩ２３６はマグネタイトに匹敵する磁性を有することが磁場磁化曲線から明らかとなった。極低温から 37 度にいたるまで、幅広い温度帯において強磁性を示すことがわかった。またＥＩ２３６は薬理効果としての抗腫瘍作用を持つことがわかった。悪性黒色腫細胞をはじめとして、様々ながん細胞に対して腫瘍増殖を抑制することが判明した。抗腫瘍効果はＤＮＡに直接作用し、がん細胞の細胞死を誘導するメカニズムが示唆されている。

またＥＩ２３６を培養がん細胞に添加し、磁石で誘導することによって、抗がん作用自体が誘導されることがわかった。このことは、ＥＩ２３６自身が磁場誘導され、抗がん作用が同時に誘導されたことを示す。同様の実験を、マウス尾部に発生させた悪性黒色腫において検討した。ＥＩ２３６を全身投与し、尾部に永久磁石を当てることによって、ＥＩ２３６を悪性黒色腫局所に誘導した。コントロール群、ＥＩ２３６全身投与群、ＥＩ２３６全身投与＋磁場誘導群の 3 群において治療効果の比較を行ったところ、磁場誘導群において顕著な腫瘍の縮小および退縮が認められた。

以上の性質を持つＥＩ２３６がMR 造影剤として機能するかを検証するために、ファントム実験および動物モデルにおいて造影効果を検討した。ファントム実験においては様々な環境設定を行い、同化合物の MR シグナルが濃度依存的に上昇することを確認した。さらに T1 および T2 などの測定の諸条件の検討を行った。これにより単なるファントムのみならず組織中においても濃度依存的にＭＲシグナルが上昇することがわかった。これらの検討結果を元に、前述の尾部腫瘍モデルにおいて、マウスに同化合物を経静脈的に全身投与した後に、永久磁石を尾部腫瘍に作用させたところ、マウス尾部に同化合物が集積し、MR シグナルの増強として造影できることを証明した。これらの結果から、ＥＩ２３６は磁場によって誘導可能な抗がん剤であり、MR の造影剤として機能することがわかった。

考察：

本研究プロジェクトの目的である有機磁性体のMR造影剤の開発に際して、ＥＩ２３６がファントム実験および動物実験によってMR造影剤として作用することが実証された。このことは、薬理学的な機能(抗がん効果)をもつ有機磁性体化合物が、薬理学的効果を発揮すると同時に、ＭＲにおける造影機能を示すことを証明したことになる。ファントム実験等から、濃度測定が可能であることがわかっており、これは抗がん剤の局所集積量を定量出来ることを意味する。抗がん剤の局所における定量は、これまで実現されたことは無く、ＭＲ画像診断としての応用のみならず、抗がん剤治療の開発にも大きな進歩をもたらす可能性がある。さらに、同様の薬剤開発によって今後の薬物治療と画像診断にも大きな影響を及ぼす可能性がある。

Isoform-Selective Regulation of Adenylyl Cyclase by Forskolin Derivatives: Prediction of Selectivity by Computer-Based Analysis

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Abstract: Adenylyl cyclase is a membrane-bound enzyme that catalyzes the conversion of ATP to cAMP upon various hormonal stimulations. Isoform-selectivity among forskolin derivatives that forskolin and its derivatives are a direct activator of adenylyl cyclase, can be predicted mostly by the distribution of the negative electrostatic potential of each derivative.

Keywords: Adenylyl cyclase, Isoform-selectivity, cAMP, Forskolin, Electrostatic potential, First principles calculations.

INTRODUCTION

Adenylyl cyclase (AC) is a membrane-bound enzyme that catalyzes the conversion of ATP to cAMP upon stimulation of numerous hormonal receptors, and thus plays an important role in regulating function of body organs [1]. It is known that there are at least nine isoforms (types 1-9) of AC that differ in tissue distribution and biochemical properties. Forskolin is a natural plant that has been used in traditional medicine in India [2]. Forskolin directly activates AC and thus because of this it acts like a β-adrenergic agonists, widely used drugs for acute heart failure. Forskolin increases ventricular contractility and induces vasodilatation in animals *in vivo*, and thus it was once considered for the treatment of acute heart failure as well. Despite such expectations, however, forskolin was never used as an alternative to β-adrenergic agonists in modern medicine because it has multiple side effects, most of which result from poor AC isoform-, and thus organ-selectivity [2].

More recently, a new forskolin derivative, or NKH477, has been developed , which lacks the side effects and is widely used in the treatment of acute heart failure in Japan [3]. This compound is known to possess high selectivity to the type 5 isoform [4], a dominant AC isoform in adult hearts, and thus selectively activates cardiac AC, leading to enhanced cardiac contractility. Accordingly, forskolin derivatives with increased AC isoform selectivity can be used in the treatment of diseases, in which catecholamine signal needs to be activated in an organ-dependent manner.

AC isoform selectivity of forskolin derivatives can be examined, at least in part, by utilizing information from crystallographic studies. Recent crystallographic studies have demonstrated the molecular mechanism of forskolin-mediated activation of AC; forskolin directly binds to AC at the opposite end of the site, to which ATP binds within the catalytic core [5]. Forskolin binds the two domains (C1 and C2) of AC by a combination of hydrophobic and hydrogen interactions, increasing the catalytic rate of this enzyme. Crystallographic studies also showed that there is a relatively large open space between the C6 and C7 residue positions of forskolin within its binding site of AC. In a previous study from our laboratory, we synthesized more than 200 new forskolin derivatives and have demonstrated that the modification at the positions of C6 and C7 indeed enhanced AC isoform selectivity [6]. For example, when the C6 position of forskolin was modified to an α-, β-unsaturated carbonyl group (6-(4-acrylbutyryl) forskolin), it selectively stimulated type 2 AC. The polar substitution at the C7 position as well as the attachment of C-C double bonds to the ring core of forskolin (the C5 and C6) increased type 3 AC selectivity. Mechanisms of interaction between such derivatives and AC isoforms were also confirmed by virtual docking computer analysis using results from crystallographic studies [6].

The above analysis was based on classical mechanics using empirical parameters that would reproduce *in vitro* pharmacological assays, which are very labor intensive and time consuming. In terms of accuracy of molecular interactions, particularly for the involvement of charge transfer and polarization, the classical molecular dynamics analysis commonly employed in the virtual docking study may not be always reliable. We were thus interested in applying a quantum mechanics based analysis, or first principles calculations, to the above process, which does not require intensive *in vitro* pharmacological assays. The first principles calculations have been used to determine the interaction between ligand and receptor by treating full atoms within the analyzing system. However, such a system has an extremely high degree of complexity because the system typically contains thousands of atoms of receptor proteins. In the current approach, however, we have analyzed forskolin derivatives themselves, and examined potential differences among six forskolin derivatives, which have been extensively examined in our laboratory for AC isoform selectivity [6]. We have employed both non-local and local analyses. Non-local analysis is not dependent on the position, such as binding energies, the first ionization

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energy, the electron affinity and the HOMO (Highest Occupied Molecular Orbital)-LUMO (Lowest Unoccupied Molecular Orbital) gaps, while local analysis includes dependency of the position, such as electrostatic potentials and distribution maps of the HOMO and the LUMO for analysis of the derivatives. We will demonstrate the mechanisms, by the use of quantum mechanics based analysis, that how the modification at the positions of C6 and C7 produces AC isoform selectivity, and the feasibility of applying this method to predicting AC isoform selectivity.

MATERIALS AND METHODS

1. Forskolin and its Derivatives

We have analyzed forskolin and its derivatives that have high AC isoform selectivity as shown in detail in a previous study [6] (Table **1**). All derivatives are modified at R6, R7 and/or R13 residues. Briefly, FD1 is 6-[N-(2-isothiocyanatoethyl)aminocarbonyl] forskolin; the relative potency of stimulation of FD1 *versus* forskolin was 219 % for type 2, 46 % for type 3, and 21 % for type 5 AC. FD2, 6-(4-acrylbutyryl) forskolin (117 % for type 2, 59 % for type 3, and 23 % for type 5 AC); FD3, 7-deacetyl-7-hydroxamylforskolin (108 % for type 2, 221 % for type 3, and 94 % for type 5 AC); FD4, 5,6-dehydroxy-7-deacetyl-7-nicotinoylforskolin (116 % for type 2, 307 % for type 3, and 77 % for type 5 AC); FD5, 6-[3-(dimethylamino) propiony]forskolin (NKH477) (109 % for type 2, 72 % for type 3, and 180% for type 5 AC); FD6, 6-[3-(dimethylamino) propionyl]-14 15-dihydroforskolin (51 % for type 2, 22 % for type 3, and 139 % for type 5 AC).

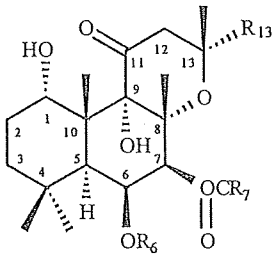
2. Methods of Calculation

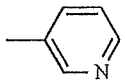
Calculations listed below have been performed using the PC cluster (Bestsystems, Tsukuba and Tokyo, Japan) as parallel computing of Advanced Applied Science Department in Ishikawajima-Harima Heavy Industries Co. Ltd. We have used software based upon Dmol^3 [7] (Accelrys, San Diego, CA, U.S.A.) by the use of Linux operating system of Redhat ES 4.0 (Redhat Inc., Raleigh, NC, U.S.A.).

3. Computational Details

We have calculated forskolin and its derivatives by linear combination of atomic orbital (LCAO) with spin polarized calculation. We have performed all electron calculation with double numerical basis-set including polarization function [7, 8] using the discrete variational method (DVM) [9-11]. A finite basis-set cutoff of 4.0 Å was used to reduce computational time without any significant loss in accuracy [12]. The local density approximation (LDA) [13] and the generalized-gradient approximation (GGA) by Perdew, Burke and Ernzerhof (PBE) [14] and Becke, Lee, Yang and Parr (BLYP) [15, 16] have been applied to obtain exchange-correlation energy functional. The GGA functionals depend

Table 1. The Chemical Structure of Forskolin and its Derivatives (6)



Compound	Position			Selectivity
	R_6	R_7	R_{13}	
Forskolin	H	CH_3	$CH{=}CH_2$	Non-selective
FD1	$CONHCH_2CH_2NCS$	CH_3	$CH{=}CH_2$	Type 2 AC
FD2	$COCH_2CH_2CH_2COCH{=}CH_2$	CH_3	$CH{=}CH_2$	Type 2 AC
FD3	H	NHOH	$CH{=}CH_2$	Type 3 AC
FD4	5,6-dehydoxy		$CH{=}CH_2$	Type 3 AC
FD5 (NKH477)	$COCH_2CH_2N(CH_3)_2$	CH_3	$CH{=}CH_2$	Type 5 AC
FD6	$COCH_2CH_2N(CH_3)_2$	CH_3	CH_2CH_3	Type 5 AC

The abbreviation of forskolin derivatives used in the text and the modification of their residues at R6, R7, and R13 are shown. FD1, 6-[N-(2-isothiocyanatoethyl) aminocarbonyl] forskolin; FD2, 6-(4-acrylbutyryl)forskolin; FD3, 7-deacetyl-7-hydroxamylforskolin; FD4, 5,6-dehydroxy-7-deacetyl-7-nicotinoylforskolin; FD5, 6-[3-(dimethylamino)propiony]forskolin (NKH477) ;FD6, 6-[3-(dimethylamino)propionyl]-14 15-dihydrofprskolin. The structure of forskolin and the position of each residue modified (R_6, R_7, and R_{13}) are indicated.

on both the local electron charge density and the magnitude of its gradient. The idea of the functionals is similar between PBE functional and BLYP functional although PBE functional is applied to the bulk system and BLYP functional to a molecule in general.

In order to evaluate the structures of forskolin and its derivatives, i.e. optimized structures, wavefunctions and eigenvalues, we utilized the following methods. A molecular structure was set according to the chemical formula of the forskolin and its derivatives, followed by dynamic optimization of the structure without empirical parameters. The structural and electrical optimizations were performed by the direct inversion in the iterative subspace (DIIS) [17, 18]. The derivatives were optimized by minimizing a total energy up to 2.7×10^{-4} eV and relaxed by minimizing a force acting on an atom up to 0.54 eV/nm. After determining the Kohn Sham equation that can approximate Schroedinger equation, we evaluated physical and chemical properties of these compounds through wavefunctions and eigenvalues.

The first ionization potential and the electron affinity of a molecule are calculated using density functional method [19]. The method can be expressed by total energy differences between two different states. There are,

$$IP = -(E_N - E_{N-1}) \quad (1)$$

and

$$EA = -(E_{N+1} - E_N) \quad (2)$$

where IP is the first ionization potential, EA the electron affinity, E_{N+1} the total energy of the molecule with the number of $N + 1$ electrons, E_N the total energy of the molecule with the number of N electrons and E_{N-1} the total energy of the molecule with the number of N-1 electrons. The binding energy is calculated as

$$E_b = E_{tot} - \sum_i E^i_{atom} \quad (3)$$

where E_b is the binding energy of the molecule, E_{tot} the total energy of the molecule and E^i_{atom} is the total energy of the i^{th} isolated atom [20-26].

RESULTS

We have analyzed forskolin and its derivatives (FD1 to FD6), of which AC isoform selectivity has been studied in detail [6]. These derivatives have been subdivided into three groups, FD1/2, FD3/4, and FD5/6, which selectivity stimulate type 2, type 3 and type 5 AC isoforms, respectively [6] (Table 1).

1. AC Stimulating Activity Analysis with Non-Local Study

We first employed non-local analysis, which does not depend upon the position within the molecule, but rather analyzes the dynamic properties of an entire molecule using multiple parameters. The analysis does not address properties with respect to the position, such as activities of a specific site in the molecule. We have determined LDA and the generalized-gradient approximation (GGA) with respect to the different approximation of exchange correlation functional. GGA was determined by both PBE functional and BLYP functional so that we could predict the binding energy of molecule precisely compared to that of LDA. It is known that BLYP functional gives a quite accurate description of hydrogen-bonded systems compared to PBE functional [27-30].

a. The First Ionization Energy, the Electron Affinity and Energy Gaps of the HOMO and the LUMO

Determination of the HOMO and the LUMO is based upon the frontier orbital theory, and is a method of calculating molecular orbitals [31-35]. According to Koopman's theorem, the energy level of the HOMO is directly related to the first ionization potential and the energy level of the LUMO, in contrast, is directly related to the electron affinity[36, 37]. The first ionization potential of the molecule is the energy required to remove one electron in a molecule. The electron affinity is the amount of energy absorbed when an electron is added to a neutral molecule to form an ion with a -1 charge ($-e$). It has a negative value if energy is released. When both the HOMO and the LUMO energies are related to the chemical interaction, those are called as radical interactions. For example, hard nucleophilies have a low energy HOMO; soft nucleophiles have a high energy HOMO; hard electrophiles have a high energy LUMO; and soft electrophilies have a low-energy LUMO. The HOMO-LUMO gap, i.e. the difference in energy between the HOMO and the LUMO, is an important stability index. The reason can be explained by the absolute hardness of a chemical species. The absolute hardness of a chemical potential $\mu[N, v]$ [38] is defined as,

$$\eta = \frac{1}{2}\left(\frac{\partial \mu}{\partial N}\right)_v = \frac{1}{2}\left(\frac{\partial^2 E}{\partial N^2}\right)_v \quad (4)$$

where η is the absolute hardness of a chemical species, E the total energy, N the number of electrons, v the external potential that is defined in density functional theory [19]. η measures the resistance to charge redistribution after the interactions. When hardness is small, it is easy for electrons to go to the others or come from the others. η is equivalent to the HOMO-LUMO gap through the finite difference approximation [19]. A large HOMO-LUMO gap implies high stability for the molecule because of its lower affinity in chemical interactions, and a small HOMO-LUMO gap implies low stability. The HOMO-LUMO gap has thus been used as an approximation to the excitation energy of the molecule.

We first thought that the isoform selectivity might be determined by the gap between the HOMO and the LUMO. We first calculated the first ionization energy and the electron affinity for each derivative using GGA (BLYP) and then the HOMO-LUMO gap (HOMO minus LUMO) by the methods of LDA and GGAs (BLYP and PBE).

Table 2 shows the first ionization potential and the electron affinity of forskolin and its derivatives with BLYP exchange correlation potential. Those energies were variable among derivatives. The first ionization potential varied from 6.379 (FD2) to 7.108 (FD4), and the electron affinity varied from 0.240 (forskolin) to 1.576 (FD2). When FD1 and FD2 were compared, the electron affinity of FD1 was lower than that of FD2, suggesting that FD1 can easily accepts electrons. The first ionization energy of FD1 was higher than

Table 2. The First Ionization Potential and the Electron Affinity (eV) of Forskolin and its Derivatives with BLYP Exchange-Correlation Functional

	Forskolin	FD1	FD2	FD3	FD4	FD5	FD6
Ionization potenitial (IP)	7.101	6.880	6.379	6.705	7.108	6.496	6.429
Electron affinity (EA)	0.240	0.910	1.576	1.526	1.057	0.336	0.585

that of FD2, suggesting that FD1 poorly gives up electrons. FD1 may interact with type 2 AC *via* acceptor like interactions, whereas FD2 may interact with type 2 AC *via* donor like interactions. Similarly, FD4 may interact with type 3 AC *via* acceptor like interactions, and FD3 may interact with type 3 AC *via* donor like interactions. When FD5 and FD6 were compared, the electron affinity of FD5 was slightly lower than that of FD6, and the first ionization energy of FD5 was slightly higher than that of FD6. Because both FD5 and FD6 contain a tertiary amine at R6, and the amine of FD5 and FD6 has a nucleophilic character due to a lone electron pair of nitrogen atom, FD5 and FD6 may have lower electron affinity.

We then compared the HOMO-LUMO gap of each derivative (Table 3). The gap was very different between FD2 and FD1, and the gap of FD2 was smallest among all between FD5 and FD6. Fig. (1) shows isosurfaces of the HOMO and the LUMO of FD5. The HOMO located at the R7 residue and the LUMO at the C9, the C10 and the C11 positions, and the modification at these residues is known to inactivate this derivative. Fig. (2) shows isosurfaces of FD6, which are very similar to those of FD5.

Putting together, the above findings suggest that AC isoform selectivity is less likely to be determined by the HOMO-LUMO gaps of these derivatives, at least determined by the first ionization energy and the electron affinity.

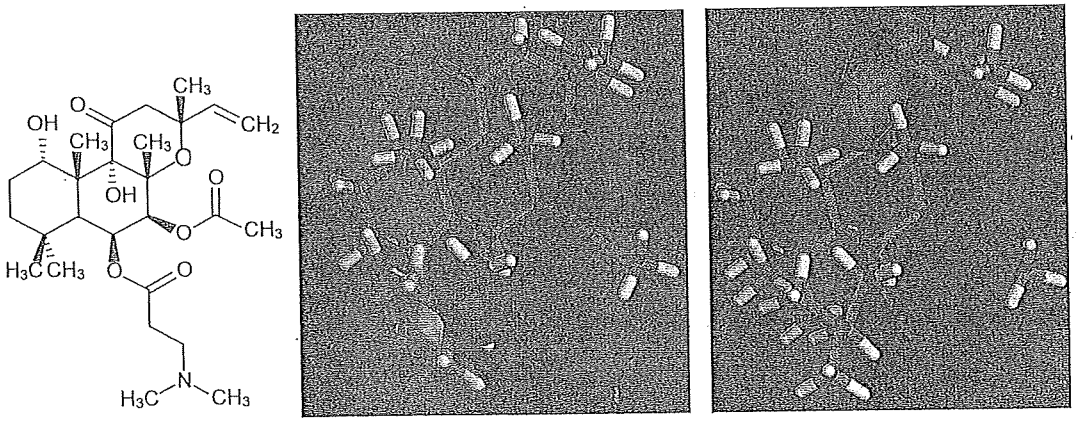
b. Binding Energies

We also analyzed the binding energy of each of forskolin derivatives because it was possible that the isoform selectivity among such derivatives might simply reflect differences in binding energy. We have also determined the

Table 3. The HOMO-LUMO Gaps (eV) of Forskolin and its Derivatives. In General, PBE Functional is Applied to the Bulk System and BLYP Functional to a Molecule

	Forskolin	FD1	FD2	FD3	FD4	FD5	FD6
LDA	2.914	2.962	1.521	3.129	2.855	3.194	2.910
GGA (BLYP)	3.147	3.224	1.628	3.412	3.043	2.801	2.908
GGA (PBE)	3.107	3.163	1.505	3.314	3.041	2.844	2.960

the derivatives, suggesting that FD2 is the most reactive compound. The gap was modestly different between FD3 and FD4 while the gap was similar between FD1 and forskolin as well as between FD5 and FD6. The distribution map of the HOMO and LUMO in space was very similar value of the water molecule as a reference, which is known to have a very low affinity. As shown in Table 4, these derivatives had relatively small (below –4.425 eV/atom) and very similar (-5.160 eV/atom ~ -5.444 eV/atom by LDA and –4.425 eV/atom ~ -4.855 eV/atom by GGA) values in the



(a) HOMO (b) LUMO

Fig. (1). Isosurface of HOMO (left) and LUMO (right) of FD5. The positive value is shown in blue and negative in yellow. Chemical structure of FD5 is also shown (left).

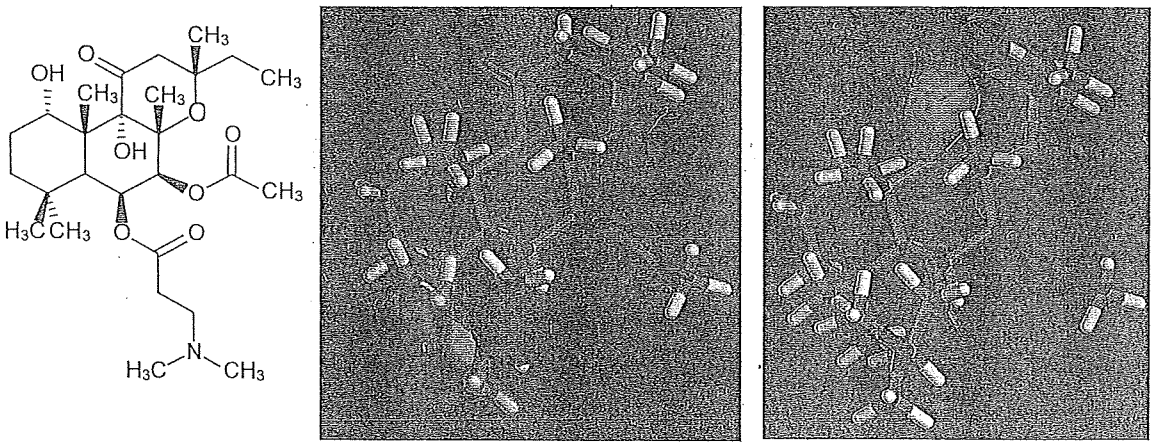


Fig. (2). Isosurface of HOMO (left) and LUMO (right) of FD6. The positive value is shown in blue and negative in yellow. Chemical structure of FD6 is also shown (left).

binding energy among the three groups (FD1/2, FD3/4, and FD5/6), indicating that the stability of these derivatives is similar at least in binding. Note that the value of the water molecule was relatively large (-3.817 eV/atom by LDA and -3.406 ~ -3.444 eV/atom by GGA) compared with those of the forskolin derivatives. These data suggest that the isoform selectivity is less likely due to the differences in binding energy.

Thus, the analysis using the HOMO and the LUMO has suggested the complexity of the mechanisms of generating AC isoform selectivity that molecular dynamics are not necessarily similar even between the derivatives with comparable isoform selectivity.

2. AC Stimulating Activity Analysis with Local Analysis

We next employed local analysis, which is suitable for looking at local positions of a molecule. The analysis addressed properties with respect to the position, such as activities of specific site in the molecule.

a. Isosurfaces of Electrostatic Potential

We have used the generalized-gradient approximation (GGA) throughout calculations on isosurfaces of electrostatic potential. Since all calculations on isosurfaces of electrostatic potential by the generalized gradient calculations (BLYP and PBE) were consistent with those of LDA, we used BLYP for calculating electrostatic potentials as described below. We calculated the electrostatic potential of each forskolin derivative because such potential might determine the affinity of each derivative to a specific AC isoform. The electrostatic interaction is represented by both the atomic charges and the positive point charges in space, such as within a grid surrounding the molecule, and can be either attractive or repulsive. For example, an electropositive part of a forskolin derivative seeks to dock to an electronegative part of an AC isoform.

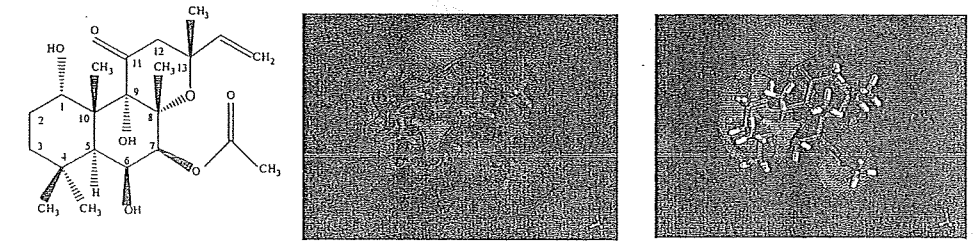
Isosurfaces of the electrostatic potential are summarized in Fig. (3). An isosurface is a three-dimensional analog of contour map. It can represent a surface of constant value of electrostatic potential within volume. Positive potential that takes higher value inside is shown in blue and the negative one that takes lower value in yellow. All isosurfaces are drawn at 5.880×10^{-4} eV in the positive and -5.880×10^{-4} eV in the negative.

Positive potentials were widely distributed over the molecule while the negative potentials were split into two regions in all derivatives. An example of forskolin is shown in Fig. (3a). The distribution of positive potentials of forskolin derivatives was mostly the same to that of forskolin, suggesting that such distribution is less likely to contribute to the isoform-selectivity. However, we found that the distribution of negative potentials was very different among them, but was conserved, importantly, between the derivatives with similar AC isoform selectivity as shown in Figs. (3b-h). FD1 and FD2, for example, which are selective to type 2 AC, had the negative potential spreading largely on the reverse side of the C6 and C7 positions but, to a smaller degree, on the front of the C7 position. The substituent of FD1 at R6 contains an isothiocyanate, which act as electrophilies with carbon atom as the electrophilic

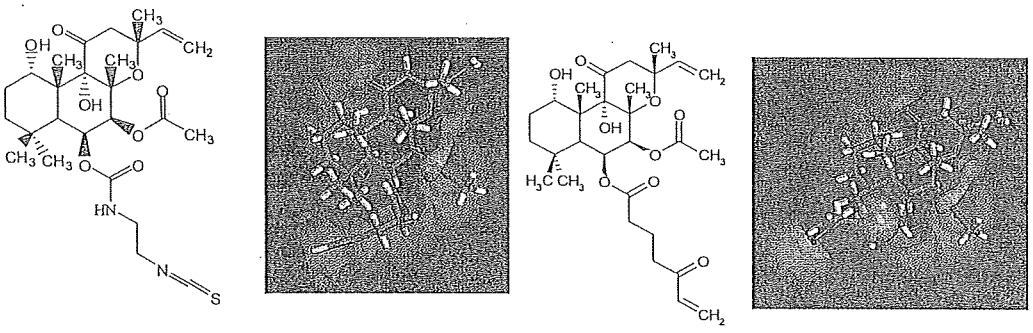
Table 4. The Binding Energies (eV/atom) of Forskolin, its Derivatives and Water. In General, PBE Functional is Applied to the Bulk System and BLYP Functional to a Molecule

	Forskolin	FD1	FD2	FD3	FD4	FD5	FD6	H_2O
LDA	-5.203	-5.265	-5.290	-5.191	-5.444	-5.201	-5.160	-3.817
GGA (BLYP)	-4.497	-4.518	-4.551	-4.425	-4.643	-4.472	-4.437	-3.406
GGA (PBE)	-4.662	-4.689	-4.718	-4.600	-4.855	-4.634	-4.597	-3.444

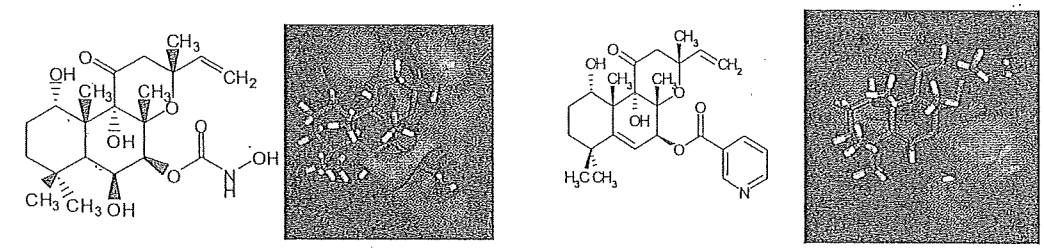
center. On the other hand, the substituent of FD2 at R6 contains an α/β unsaturated ketone, which has a polar character. The results show FD2 of the R6 substituent $COCH{=}CH_2$ out of $COCH_2CH_2COCH{=}CH_2$ had the same character of the substituent of FD1 at the R6. This implicated that the positive electrostatic potential of type 2 AC [27] interacts FD1 and FD2 mostly at the reverse side of the C6, C7 and R7 positions. FD3 and FD4 are selective to type 3 AC and its negative potentials were entirely wrapping the C7 position, implicating that the positive electrostatic potential of type 3 AC interacts with FD4 at the C7 position from any directions. FD5 and FD6 is selective to type 5 AC and its negative potentials spread widely on the reverse side of the C8, the C7 position while they were weak at the C6 position, implicating that the positive electrostatic potential of type 5 AC interacts mostly with the reverse side of the C8, the C7 positions, and the C7 position.



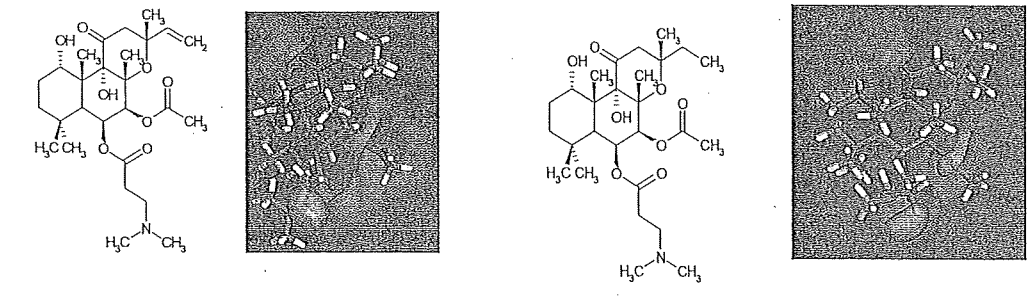
(a) Forskolin (positive potential and negative potential) (b) Forskolin (negative potential)



(c) FD1 (negative potential) (d) FD2 (negative potential)



(e) FD3 (negative potential) (f) FD4 (negative potential)



(g) FD5 (negative potential) (h) FD6 (negative potential)

Fig. (3). **Isosurface of electrostatic potential of forskolin and its derivatives.** The positive value is shown in blue and negative in yellow, and chemical structure of each derivative is also shown (left). (a) Forskolin (positive potential and negative potential), (b) Forskolin (negative potential), (c) FD1 (negative potential), (d) FD2 (negative potential), (e) FD3 (negative potential), (f) FD4 (negative potential), (g) FD5 (negative potential), (h) FD6 (negative potential).

The above findings suggest that the negative electrostatic potentials of these forskolin derivatives may interact with the positive electrostatic potential of each AC isoform, and that this interaction may play a potentially important role in producing isoform-selectivity among forskolin derivatives.

DISCUSSION

The isoform-selective stimulation of forskolin can be potentiated through specific modifications at the C6 and/or the C7 position of forskolin and that the combination of multiple modifications has additive effects in enhancing selectivity [6]. It is speculated that forskolin is a partial activator of AC and that the modification of forskolin at the C6 and the C7 positions may simply increase the maximal degree of AC activation. The affinity of our forskolin derivatives (FD1-6) for AC does not depend upon the structure at the C6 and C7 positions [6]. The hydrogen bonding with AC at the C1, the C7 and the C11 positions played an important role in the previous report [6]. Our analyses have revealed that the distribution of the negative electrostatic potential on the C6 and the C7 residues may play a role in type-specific regulation of AC by forskolin derivatives.

Analysis of forskolin derivatives using first principles calculations, which is based on quantum mechanic analysis, has revealed potential mechanisms for enhanced selectivity among forskolin derivatives. Most important, AC isoform-selectivity was best characterized by the distribution of the negative, but not positive, electrostatic potential of these derivatives, suggesting that the positive electrostatic potential of AC isoforms may play an important role in defining the interaction with these forskolin derivatives. In support of this concept, the negative electrostatic potential distributing over the C6 and C7 positions, of which modification is known to increase selectivity; was similar between the derivatives with comparable AC isoform selectivity, but very different among derivatives with distinct selectivity. Furthermore, presence of multiple mechanisms to generate AC isoform selectivity was suggested by the analysis using the first ionization potential and the electron affinity because these indexes were different even between the derivatives with comparable isoform selectivity. Acceptor like interactions plays an important role in AC isoform selectivity among types 2, 3 and 5 since FD1 which selectivity stimulates type 2 AC, FD4 which selectivity stimulates type 3 AC and together with FD5 which selectivity stimulate type 5 AC tend to have the lower electron affinity than the other counterpart.

Our study also demonstrated another potential advantage of employing the first principles calculations to analyze selectivity to enzyme isoforms. The first principles molecular dynamics has been used to determine the interaction, for example, between agonist and receptor by treating full atoms within the analyzing system. In the current study, we have focused our efforts on analyzing only agonists (forskolin derivatives) to examine if such analysis is sufficient to find properties that may correlate with AC isoform selectivity. The method of approach employed in our study was relatively simple because we did not have to analyze the AC protein that possesses a large number of atoms or utilized data from crystallographic studies. We do not know if the same approach can be applicable to other types of compounds that react with different effecter enzymes. However, our approach was indeed useful to predict AC isoform-selectivity, at least in part, among forskolin derivatives, and this may be used to design new forskolin derivatives at the time of molecule designing in future studies. Calculated properties like first ionization potential, electron affinity and HOMO-LUMO gaps could be useful indices if the mechanism of action of the forskolin and its derivatives could be related to a covalent or an ionic binding process. Our analysis also has a potential of further developing isoform-targeted, such as not only stimulators but also inhibitors without an extremely high degree of complexity calculations, such as full treatment of ligand and receptor. By the use of a similar approach, it may be possible to analyze the interaction between this enzyme isoform and other inhibitors, such as P-site inhibitors [39].

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ABBREVIATIONS

AC = adenylyl cyclase

FD1 = 6-[N-(2-isothiocyanatoethyl)aminocarbonyl] forskolin

FD2 = 6-(4-acrylbutyryl) forskolin

FD3 = 7-deacetyl-7-hydroxamylforskolin

FD4 = 5,6-dehydroxy-7-deacetyl-7-nicotinoylforskolin

FD5 = 6-[3-(dimethylamino)propiony]forskolin or NKH477

FD6 = 6-[3-(dimethylamino)propionyl]-14 15-dihydro-forskolin

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cAMP-Mediated Regulation of CYP Enzymes and Its Application in Chemotherapy

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Abstract: Certain anti-cancer prodrugs are subject to cytochrome P450 (CYP)-mediated metabolism and become more active. Because CYP activity may be regulated by phosphorylation *via* adenylyl cyclase/protein kinase A, selective adenylyl cyclase subtype activators may be utilized in future chemotherapy to regulate CYP activity as a switch in a tumor tissue-specific manner.

Key Words: Cytochrome P450, protein kinase A, adenylyl cyclase, phosphorylation, forskolin, anti-cancer drugs.

CYP ENZYMES AND DRUG METABOLISM

A variety of metabolizing enzymes present in the liver and other organs can catalyze the reactions that can convert various xenobiotics, which are ingested into the body, to harmless or less harmless compounds [1, 2]. Such reactions are mostly performed by the mixed function oxygenase system, which is called cytochrome P450 (CYP), a key metabolic enzyme family [3]. In human, the CYP superfamily comprises 57 genes arranged in 18 families and 42 subfamilies as well as 46 pseudogenes [3]. These genes encode for enzymes involved in the metabolism of drugs, foreign chemicals, fatty acids, and cholesterol. Additionally, they play important roles in steroid synthesis and metabolism, bile acid as well as vitamin D synthesis and metabolism. Similarly, many ingredients in foods, as well as a number of toxicants, allergens, and carcinogens also serve as substrates for CYP. Mutations in many CYP genes cause inborn errors of metabolism, which may lead to increased risk of cancer or other diseases. It is also important to note that certain xenobiotics, which themselves are not carcinogenic, may be transformed by endogenous CYP into ultimate carcinogens, suggesting that CYP can generate more harmful compounds, which can be used for anti-cancer therapy under specific conditions.

REGULATION OF CYP BY cAMP/PKA

The enzymatic activity of CYP may change in response to various external stimuli, and the mechanisms for such changes are not specific to CYP, but rather similar to those for other enzymes [4-10]. The total amount of CYP may be increased, usually *via* induction at the level of gene transcription, or the enzyme catalytic activity *per se* may be changed *via* post-translational modifications, such as phosphorylation. While the former regulation may require several hours to days, the latter takes only seconds to minutes and is readily reversible, which is a major advantage of regulating the enzyme activity *via* phosphorylation.

Indeed, it is well known that CYPs are subject to regulation *via* phosphorylation [11]. The phosphorylation of essential components of CYP monooxygenase system, i.e., CYP and CYP reductase, was originally demonstrated using the catalytic subunit of protein kinase A (PKA). Later studies demonstrated that CYPs were phosphorylated not only *in vitro* (using purified kinases and CYPs), but in intact cells, as well as in whole animals. Importantly, CYP phosphorylation is highly isoenzyme-selective [12]. An example is CYP2 family [13], such as CYPB1/2B2 and CYP2E1 [14], which contains the consensus amino acid sequence for PKA-mediated serine/threonine phosphorylation. Because the phosphorylation of CYPs is a very fast process that occurs usually in seconds to minutes, and the phosphorylated proteins are inactivated immediately, it was suggested that the phosphorylation mechanism acts as a rapid switch to regulate CYP activity without changing the total amount of this enzyme, leading to dynamic changes in the control of the toxic metabolites of carcinogens as well as for the control of effectiveness of anti-cancer drugs [12], which are important issues in chemotherapy.

CYP-GENE TRANSFER AND ANTICANCER THERAPY

CYPs, most notably 1A, 1B, 2C, 3A, 2D subfamily members, are expressed not only in the liver, but in many tumor cells. For example, CYP1B1 is readily detectable in tumors, such as lung, breast, liver, gastrointestinal tract, prostate, and bladder tumor cells. Certain anti-cancer drugs, especially those in the form of prodrugs, are subject to CYP-mediated metabolism, and include alkylating agents (cyclophosphamide (CPA), ifosphamide (IFA), dacarbazine, procarbazine) and fluoropyrimidine. Some may become more active through CYP-mediated metabolism, and this mechanism may be used to activate certain anti-cancer drugs in a tumor tissue specific manner. For example, 2-(4-aminophenyl)benzothiazoles may be activated in CYP1A1 inducible tumors. CYP3A-mediated activation of AQ4N, an anticancer prodrug, into cytotoxic

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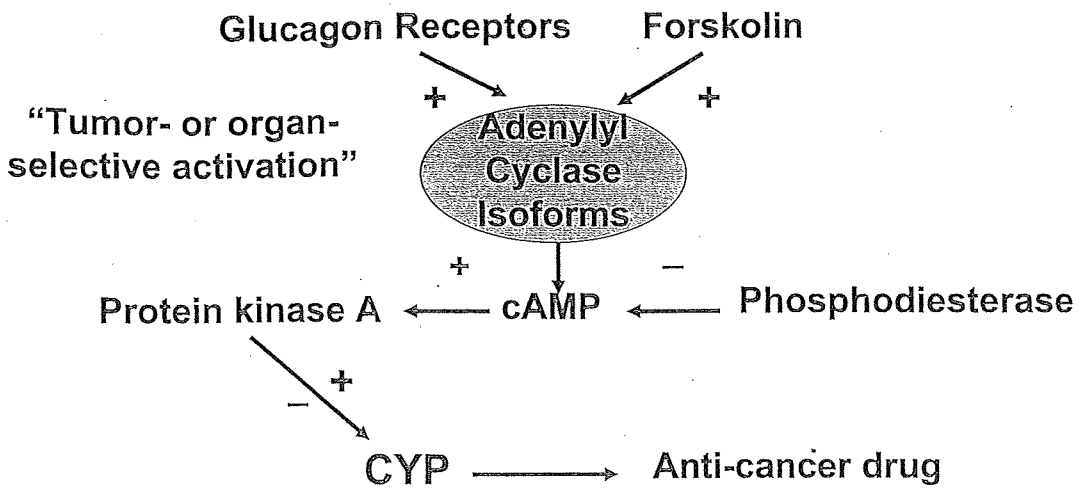

Fig. (1). A schematic concept for the proposed noble anti-cancer therapy.

metabolite may occur in hypoxic tumor cells [15]. CYP2B1 is induced by phenobarbital, a major mechanisms of CYP induction *in vivo*, and is responsible, together with CYP2C6 and CYP2C11, for conversion of the oxazaphosphorines CPA and IFA to their active metabolites [16, 17], while CYP2B1 is the most active catalyst. In human, in particular, analysis of 15 human CYP cDNAs expressed in human lymphoblasts and/or baculovirus-infected insect cells demonstrated that CYPs 2A6, 2B6, 3A4, 3A5, and three CYP2C enzymes (2C9, 2C18, 2C19) exhibited significant oxazaphosphorine 4-hydroxylase activity, with 2B6 and 3A4 displaying the highest activity toward CPA and IFA, respectively [18, 19]. These findings indicate that the expression of such CYPs are required for activating certain anti-cancer drugs.

If such CYP gene expression is not expressed in a tumor tissue, it can be introduced by gene transfer, i.e., anti-cancer gene therapy. CYP2B1 activates prodrug CPA into 4-hydroxycyclophosphamide, which ultimately degrades into acrolein and phosphoramide mustard, and serves as active (DNA-alkylating) metabolite. A recent study demonstrated that gene transfer encoding CYP2B1 indeed made tumor cells sensitive to this agent [20]. Further, such effects are not limited to tumor cells that were subject to gene-transfer. Diffusible cytotoxic metabolites can also inhibit the proliferation of surrounding tumor cells, in which the transgene was not introduced, a phenomenon called "the bystander effect". Indeed, it was demonstrated in cultured cells that CPA-sensitized, CYP-expressing C6 glioma cells transferred cytotoxicity to nonexpressing cells by releasing diffusible metabolites through the culture medium. This bystander effect occurred in the presence of CPA even when only a minor portion (~10%) of cells were gene-transferred [20]. Because the therapeutic efficacy and successful therapy with CPA is often limited by the fact that several tumor cells are not able to activate CPA or IFA, the above novel tumor-killing gene therapy with P450-based prodrug activation may be an effective method to improve the current anti-cancer therapy [21, 22] A very recent gene therapy trials using P450 provide strong support for the therapeutic potential of such gene therapy; treatment of inoperable pancreatic carcinoma patients with IFA in combination with encapsulated cells expressing CYP2B1 led to a 3-fold increase in 1-year survival [23].

REGULATION OF cAMP SIGNAL TO REGULATE CYP

It was recently demonstrated that the activation of PKA potentiated the pregnane X receptor (PXR)-mediated induction of CYP3A gene expression in cultured hepatocytes and increased the strength of PXR-coactivator protein-protein interaction [24]. This was demonstrated by the use of forskolin, a direct activator of adenylyl cyclase (AC), a membrane-bound enzyme that produces cAMP to activate PKA [25], suggesting that the induction of CYP can be achieved by regulating this cAMP producing enzyme, AC. In human, it is known that cAMP-dependent phosphorylation of CYPE1 leads to its changes in activity [14, 26].

If activation of AC is a strategy to activate of PKA, leading to the regulation of CYPs, is it possible to activate AC in a tumor tissue specific manner? Activation of AC may be made through the administration of glucagon, for example, when hepatic CYPs need to be regulated. Because glucagon receptors are expressed mostly, if not exclusively, in the liver, glucagon can activate PKA in a liver-specific manner. Other cAMP-regulating hormonal receptors, unfortunately, may not be expressed in a tissue-specific manner. Beta-adrenergic receptors, for example, that can potently stimulate the production of cAMP *via* activation of AC, are expressed in most organs, and thus the use of beta-adrenergic receptor agonist may activate PKA elsewhere. If a tumor tissue expresses a specific receptor subtype(s), agonist-medi-ated stimulation of such receptor can activate PKA in a tumor tissue specific manner. Unfortunately, the tumor tissue-specific expression of such a Gs-coupled receptor is not well known.

FORSKOLIN ANALOGUES AS TISSUE SPECIFIC ACTIVATOR OF PKA

If receptor activation may not be used, the elevation of intracellular cAMP and thus activation of PKA can be made by either the activation of AC, the cAMP producer, or the inhibition of phosphodiesterase (PDE), a key enzyme in the regulation of cAMP turnover. Because both AC and PDE

have multiple subtypes that differ in tissue distribution, a tumor cell may express a dominant subtype of either enzyme. Indeed, it is well known that AC and PDE subtype expression occurs in a much more tissue specific manner than receptor subtypes [27], and pharmacotherapy has already taken advantage of this property, and pharmacological compounds have been developed that can regulate AC and PDE in a subtype –specific manner. Subtype-specific PDE inhibitors, such as sildenafil citrate, a type 5 PDE inhibitor [28], and milrinone, a type 3 inhibitor [29], are now widely used in the treatment of erectile dysfunction and heart failure, respectively.

AC, which synthesizes cAMP, has at least 9 subtypes that differ in tissue distribution. Forskolin, a natural plant extract, was first identified as a general stimulator of AC, but a recent study has shown that 6-[3-(dimethylamino)propionyl] forskolin, a water-soluble forskolin derivative with high selectivity for type 5 AC, which is dominantly expressed in the heart, was developed and has been widely used in the treatment of acute heart failure [27, 30]. Furthermore, AC subtype specific inhibitors have been developed [31]. Adenine analogs or P-site inhibitors, which are classic, but not isoform-specific AC inhibitors, are now utilized to develop isoform-specific inhibitors [31, 32]. A novel non-nucleoside inhibitor, 2-amino-7-(2-furanyl)-7, 8-dihydro-5(6H)-quinazolinone (NKY80), was identified after virtual screening of more than 850,000 compounds by the use of crystallographic data of AC. NKY80 demonstrated a 210-fold selectivity for inhibiting type 5 AC relative to type 2 AC. Similarly, we found that some compounds, including 1R,4R-3-(6-aminopurin-9-yl)-cyclopentanecarboxylic acid hydroxyamide, potently inhibited type 5 AC, but not other subtypes [32], suggesting that various AC subtype specific inhibitors can be developed. Such efforts have been fortified by the development of computer-based screening for such subtype-selective compounds, which include the pharmacophore analysis using the crystal structure of enzyme protein. More recently, the first principle analysis, a method in theoretical physics, which analyses the molecular dynamics at the level of atoms and electrons, and has been widely used in simulation of developing new materials in semiconductor industry [33].

FUTURE DIRECTIONS

CYP, a key metabolic enzyme family for detoxification, is utilized in anti-cancer chemotherapy because it can generate, instead of detoxifying, more harmful compounds that can be used for anti-cancer therapy. Because the activity of some CYP can be regulated by AC/PKA, regulation of AC/PKA by the use of selective AC subtype activator may be utilized in future chemotherapy, which regulates CYP as a switch in a tumor tissue specific manner. Alternatively, a CYP member may be overexpressed by gene-transfer, and the activity of CYP may be regulated thereafter *via* the regulation of a AC subtype that is dominantly expressed in the tumor tissue. Nevertheless, these strategies would enable dynamic regulations in the control of the toxic metabolites and effectiveness of anti-cancer drugs in chemotherapy.

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Drug Delivery System Using Magnetic Materials

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Abstract

A drug delivery system (DDS) to deliver a drug when and where required is a powerful tool for reducing the doses of drugs administered and the side effects. Isolation of candidate materials and development of a new DDS using the materials should provide a more powerful tool in the medical field. Therefore, we are developing a new DDS using a combination of candidate magnetic materials and a magnetic field.

Introduction

Surgical therapeutic, chemotherapeutic and radiotherapeutic approaches alone or in combination have been used for treatment of cancer[1]. However, each approach has side effects such as nausea, vomiting, anorexia, diarrhea, alopecia and hepatic dysfunction[2)-5)]. Many studies aimed at the development of a DDS to solve the problems of side effects of cancer therapies have been carried out over past three decades[6),7)]. We have isolated novel magnetic materials to solve the problems of side effects of cancer chemotherapy and have studied a DDS using a novel magnetic material. Here, we introduce our study and other target-selective DDSs.

Drug Delivery Systems

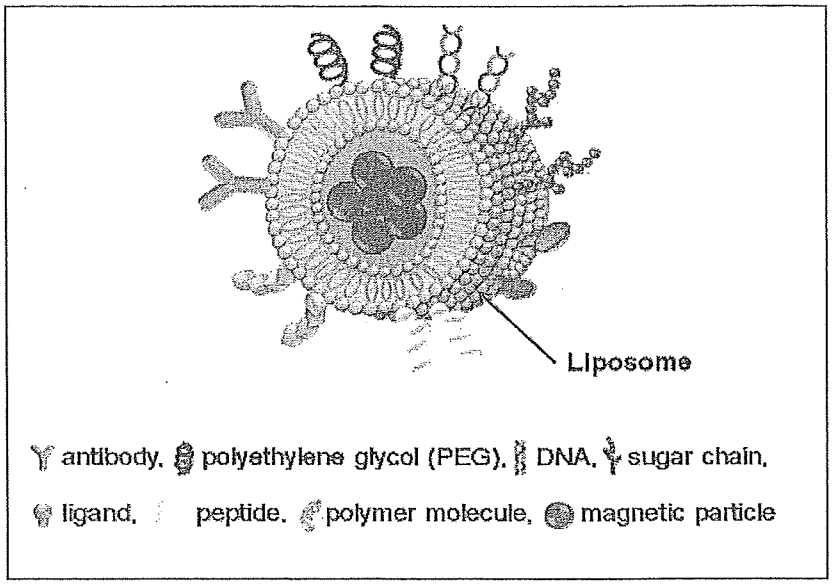
DDSs have been developed to enable drugs to safely elicit effects in target organs, tissues or cells. DDSs can be classified into 1) target-selective drug delivery systems[6)-11)], 2) controlled-release drug delivery systems[12)-14)], 3) systems for drug delivery by absorption[15),16)]. Target-selective drug delivery systems for delivering drugs to target organs, tissues and cells are expected to greatly reduce side effects in normal cells. Much interest has been shown in the use of liposomes for target-selective DDSs.

Since lisopomes are composed of lipid bilayer membranes, they can contain both aqueous and lipophilic drugs, and the surfaces of liposomes can also be modified by the addition of a specific antibody, a certain ligand, polymers, and so on[10),11)] (Scheme). A controlled-release DDS and a system for drug delivery by absorption have been developed and produced as an implantable infusion pump and a skin patch, respectively by Johnson and Johnson Company (Alza Corporation) [11), 17), 18)]

Furthermore, the use of magnetic materials in the development of DDSs has been reported in the 1970s and accumulation of albumin microspheres containing doxorubicin and magnetite (Fe3O4,) in a sarcoma by a permanent magnet led to

regression and disappearance of the sarcoma[6].

In the 1980s, liposomes containing magnetite (magnetic liposome) were developed, and accumulation of these liposomes by the use of an electronic magnet was tested in an animal study [7]. Magnetic liposomes have been improved and their anticancer effects under an alternative current magnetic field have been demonstrated in animal studies [19]. We have been attempting to isolate novel magnetic materials that also have biological properties such as cytotoxicity. Magnetic materials that also have biological properties will be useful for improving DDSs, which should lead to reduction in side effects in the near future.



Schematic illustration of liposome for a DDS. A liposome can contain probes (DNA plasmid, siRNA, virus vector) and magnetic particles.

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