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活動報告

平成１５年度後期－１６年度前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及びＢＬ６Ｃ

１．利用状況

平成１５年度後期のビームタイムは平成１６年１月１６日（金）午前９時に開始され３月２３日（火）午前９時に終了した。又、平成１６年度前期のビームタイムは平成１６年４月１３日（火）午前９時に開始され７月１日（木）午前９時に終了した。平成１５年度後期のBL6B及びBL6Cの予約状況をそれぞれ表１及び表２に示す。又、平成１６年度前期のBL6Bの予約状況を表３に示す。尚、平成１６年度にはBL6Bは主としてSuper Galaxy設計に必要なテストを行うため、予約表は用いず利用希望者が坂部知平までメールで連絡する形式をとった。その結果６月２９日エイザイの後藤田さんが利用された。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。

２．装置の状況

１）　ＢＬ６Ｂ

ビームラインの故障はなかった。測定装置はIP vaccume errorが２回出た。低温吹付け装置は全く正常に稼動した。

Station check sheetから状況を拾うと

５月１４日	RS232C error
６月２４日	The IP reader gives null pixel value throughout the IP area
６月３０日	Frost heavily deposits on the cryocooler’s nozzle

また使用波長を調べると

６月１３日　0.9587Åを使用、

上記以外は全て1.0 Åの波長を使用している。

６月２４日のトラブルは修理が必要であったため㈱リガクのサービスに連絡した、原因不明で修理できず結局２９日になって読み取り装置内のHV1ケーブルの断線及びCPU-ASユニットコネクターの接続不良の２つの原因が分かり修理していただいた。原因が複数になると原因の発見が難しくなる。６月２４日～６月２９日間に予約されていた方々には大変ご迷惑をお掛けしてしまいました。お詫び申し上げます。

２）　ＢＬ６Ｃ

低温吹付け装置の冷却用He圧が下がったので供給した。

IPカセット２が読みより後、クラッチが外れずに停止することがたびたび起こったが、調整は難しく未だ治っていない。

表１．平成１５年度後期BL6Bビームタイム使用状況

1/16	FRI	R	bonus_time (day) / bonus_time (night)
1/17	SAT	R	- (day) / - (night)
1/18	SUN	R	- (day) / - (night)
1/19	MON	R	machine_study (day) / machine_study (night)
1/20	TUE	R	bonus_time (day) / bonus_time (night)
1/21	WED	R	Sankyo_Co._Ltd_c (day) / - (night)
1/22	THU	R	Yamanouchi_Pharm._c (day) / - (night)
1/23	FRI	R	- (day) / - (night)
1/24	SAT	R	- (day) / - (night)
1/25	SUN	R	- (day) / - (night)
1/26	MON	R	machine_study (day) / machine_study (night)
1/27	TUE	R	bonus_time (day) / bonus_time (night)
1/28	WED	R	Kyowa_Hakko_Kogyo_c (day) / - (night)
1/29	THU	R	Ajinomoto_Co._Inc_c (day) / - (night)
1/30	FRI	R	- (day) / - (night)
1/31	SAT	R	- (day) / - (night)
2/ 1	SUN	R	- (day) / - (night)
2/ 2	MON	R	machine_study (day) / machine_study (night)
2/ 3	TUE	R	bonus_time (day) / bonus_time (night)
2/ 4	WED	R	- (day) / - (night)
2/ 5	THU	R	- (day) / - (night)
2/ 6	FRI	R	Chugai_Pharm._c (day) / - (night)
2/ 7	SAT	R	Chugai_Pharm._c (day) / - (night)
2/ 8	SUN	R	- (day) / - (night)
2/ 9	MON	R	machine_study (day) / machine_study (night)
2/10	TUE	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/11	WED	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/12	THU	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/13	FRI	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/14	SAT	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/15	SUN	R	3GeV_single-bunch (day) / 3GeV_single-bunch (night)
2/16	MON	R	machine_study (day) / machine_study (night)
2/17	TUE	R	Sankyo_Co._Ltd_c (day) / - (night)
2/18	WED	R	Kyowa_Hakko_Kogyo_c (day) / - (night)
2/19	THU	R	Ajinomoto_Co._Inc_c (day) / - (night)
2/20	FRI	R	Eisai_Co._Ltd_c (day) / Eisai_Co._Ltd_c (night)
2/21	SAT	R	- (day) / - (night)
2/22	SUN	R	Daiichi_Pharm._c (day) / - (night)
2/23	MON	R	machine_study (day) / machine_study (night)
2/24	TUE	R	- (day) / - (night)
2/25	WED	R	Yamanouchi_Pharm._c (day) / - (night)
2/26	THU	R	- (day) / - (night)
2/27	FRI	R	Chugai_Pharm._c (day) / - (night)
2/28	SAT	R	- (day) / - (night)
2/29	SUN	R	- (day) / - (night)

3/ 1 MON R	machine_study (day)	machine_study (night)
3/ 2 TUE R	- (day)	- (night)
3/ 3 WED R	Fujisawa_Pharm._c (day)	- (night)
3/ 4 THU R	Kyowa_Hakko_Kogyo_c (day)	- (night)
3/ 5 FRI R	Eisai_Co._Ltd_c (day)	Eisai_Co._Ltd_c (night)
3/ 6 SAT R	- (day)	- (night)
3/ 7 SUN R	- (day)	- (night)
3/ 8 MON R	machine_study (day)	machine_study (night)
3/ 9 TUE R	- (day)	- (night)
3/10 WED R	- (day)	- (night)
3/11 THU R	Ajinomoto_Co._Inc_c (day)	- (night)
3/12 FRI R	- (day)	- (night)
3/13 SAT R	- (day)	- (night)
3/14 SUN R	- (day)	- (night)
3/15 MON R	machine_study (day)	machine_study (night)
3/16 TUE R	Daiichi_Pharm._c (day)	- (night)
3/17 WED R	- (day)	- (night)
3/18 THU R	- (day)	- (night)
3/19 FRI R	- (day)	- (night)
3/20 SAT R	- (day)	- (night)
3/21 SUN R	- (day)	- (night)
3/22 MON R	- (day)	- (night)

表２．平成１５年度後期 BL6C ビームタイム使用状況

1/16 FRI A	bonus_time (day)	bonus_time (night)
1/17 SAT A	- (day)	- (night)
1/18 SUN A	- (day)	- (night)
1/19 MON A	machine_study (day)	machine_study (night)
1/20 TUE A	bonus_time (day)	bonus_time (night)
1/21 WED A	- (day)	- (night)
1/22 THU A	- (day)	- (night)
1/23 FRI A	- (day)	- (night)
1/24 SAT A	- (day)	- (night)
1/25 SUN A	- (day)	- (night)
1/26 MON A	machine_study (day)	machine_study (night)
1/27 TUE A	bonus_time (day)	bonus_time (night)
1/28 WED A	- (day)	- (night)
1/29 THU A	- (day)	- (night)
1/30 FRI A	- (day)	- (night)
1/31 SAT A	- (day)	- (night)
2/ 1 SUN A	- (day)	- (night)
2/ 2 MON A	machine_study (day)	machine_study (night)
2/ 3 TUE A	bonus_time (day)	bonus_time (night)
2/ 4 WED A	- (day)	- (night)
2/ 5 THU A	- (day)	- (night)
2/ 6 FRI A	- (day)	- (night)

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2/ 7 SAT A      Chugai_Pharm._c (day)      Chugai_Pharm._c (night)
2/ 8 SUN A                  - (day)                  - (night)
2/ 9 MON A        machine_study (day)        machine_study (night)
2/10 TUE A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/11 WED A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/12 THU A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/13 FRI A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/14 SAT A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/15 SUN A    3GeV_single-bunch (day)    3GeV_single-bunch (night)
2/16 MON A        machine_study (day)        machine_study (night)
2/17 TUE A           bonus_time (day)           bonus_time (night)
2/18 WED A                  - (day)                  - (night)
2/19 THU A                  - (day)                  - (night)
2/20 FRI A                  - (day)                  - (night)
2/21 SAT A                  - (day)                  - (night)
2/22 SUN A   Sakabe_Noriyoshi_d (day)                - (night)
2/23 MON A        machine_study (day)        machine_study (night)
2/24 TUE A           bonus_time (day)           bonus_time (night)
2/25 WED A   Sakabe_Noriyoshi_d (day)                - (night)
2/26 THU A                  - (day)                  - (night)
2/27 FRI A                  - (day)                  - (night)
2/28 SAT A      Sakabe_Kiwako_d (day)      Sakabe_Kiwako_d (night)
2/29 SUN A       Sasaki_Kyoyu_d (day)       Sasaki_Kyoyu_d (night)
3/ 1 MON A        machine_study (day)        machine_study (night)
3/ 2 TUE A    Asahi_Kasei_Co._c (day)    Asahi_Kasei_Co._c (night)
3/ 3 WED A    Asahi_Kasei_Co._c (day)    Asahi_Kasei_Co._c (night)
3/ 4 THU A    Asahi_Kasei_Co._c (day)    Asahi_Kasei_Co._c (night)
3/ 5 FRI A             co_users (day)             co_users (night)
3/ 6 SAT A                  - (day)                  - (night)
3/ 7 SUN A                  - (day)                  - (night)
3/ 8 MON A        machine_study (day)        machine_study (night)
3/ 9 TUE A           bonus_time (day)           bonus_time (night)
3/10 WED A                  - (day)                  - (night)
3/11 THU A  Ajinomoto_Co._Inc_c (day)                - (night)
3/12 FRI A             co_users (day)                - (night)
3/13 SAT A                  - (day)                  - (night)
3/14 SUN A                  - (day)                  - (night)
3/15 MON A        machine_study (day)        machine_study (night)
3/16 TUE A           bonus_time (day)           bonus_time (night)
3/17 WED A                  - (day)                  - (night)
3/18 THU A             co_users (day)                - (night)
3/19 FRI A             co_users (day)                - (night)
3/20 SAT A             co_users (day)                - (night)
3/21 SUN A             co_users (day)                - (night)
3/22 MON A             co_users (day)                - (night)
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表３．平成１６年度前期 BL6B ビームタイム使用状況

4/13	TUE	R	- (day) - (night)
4/14	WED	R	Yamanouchi_Pharm._c (day) - (night)
4/15	THU	R	Daiichi_Pharm._c (day) - (night)
4/16	FRI	R	Sankyo_Co._Ltd_c (day) - (night)
4/17	SAT	R	- (day) - (night)
4/18	SUN	R	- (day) - (night)
4/19	MON	R	machine_study (day) machine_study (night)
4/20	TUE	R	- (day) - (night)
4/21	WED	R	- (day) - (night)
4/22	THU	R	Ajinomoto_Co._Inc_c (day) - (night)
4/23	FRI	R	- (day) - (night)
4/24	SAT	R	- (day) - (night)
4/25	SUN	R	- (day) - (night)
4/26	MON	R	- (day) - (night)
4/27	TUE	R	bonus_time (day) bonus_time (night)
4/28	WED	R	Sankyo_Co._Ltd_c (day) - (night)
4/29	THU	R	- (day) - (night)
4/30	FRI	R	NO_beam (day) NO_beam (night)
5/ 1	SAT	R	NO_beam (day) NO_beam (night)
5/ 2	SUN	R	NO_beam (day) NO_beam (night)
5/ 3	MON	R	NO_beam (day) NO_beam (night)
5/ 4	TUE	R	NO_beam (day) NO_beam (night)
5/ 5	WED	R	NO_beam (day) NO_beam (night)
5/ 6	THU	R	NO_beam (day) NO_beam (night)
5/ 7	FRI	R	machine_study (day) machine_study (night)
5/ 8	SAT	R	machine_study (day) machine_study (night)
5/ 9	SUN	R	machine_study (day) machine_study (night)
5/10	MON	R	Banyu_Pharm._c (day) - (night)
5/11	TUE	R	- (day) - (night)
5/12	WED	R	- (day) - (night)
5/13	THU	R	Sankyo_Co._Ltd_c (day) - (night)
5/14	FRI	R	Sankyo_Co._Ltd_c (day) - (night)
5/15	SAT	R	- (day) - (night)
5/16	SUN	R	- (day) - (night)
5/17	MON	R	machine_study (day) machine_study (night)
5/18	TUE	R	- (day) - (night)
5/19	WED	R	- (day) - (night)
5/20	THU	R	Ajinomoto_Co._Inc_c (day) - (night)
5/21	FRI	R	- (day) - (night)
5/22	SAT	R	- (day) - (night)
5/23	SUN	R	- (day) - (night)
5/24	MON	R	machine_study (day) machine_study (night)
5/25	TUE	R	- (day) - (night)
5/26	WED	R	Yamanouchi_Pharm._c (day) Tada_Toshiharu_d (night)
5/27	THU	R	- (day) - (night)
5/28	FRI	R	- (day) - (night)
5/29	SAT	R	- (day) - (night)

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

３．その他

これまで長期に亘りBL6Bの世話をお願いしていた㈱リガクの三浦俊典さんが４月末日をもって配置換えになり、その後任にＰＦが人材派遣会社㈱日本アクシスの出村一貴氏が来てくださった。ＰＦとの話し合いで、１１時以降及びシャットダウン前後の全ての操作を受け持って下さることになった。ビーム合わせを行う９時～１１時の要員をFAISで雇い上げ坂部知平が付き添ってトレーニングしたが、適性が合わず短期間で止められた。その後は坂部知平が光軸合わせを行った。（本来なら次号に載せるべきであるが本誌の発行が年２回では遅くなるので、その後のこともここに書かせて頂く。）平成１６年１０月１日より１７年３月３１日迄の期間の契約が三菱システムサーサービス㈱とＦＡＩＳ間で取り交わされ、土、日、月、祭日及びshingle bunchのビームタイム時を除く９時から１１時までサポートをして頂くことになった。教育に関しては上記の時間外でも行って下さいと言うことで、実際には契約時間外でも作業してくれている。担当者は以前からＰＦの蛋白質ビームラインを受け持っておられた渡邉一樹氏で大変助かっている。

Ⅱ．コンピュータ関係

１．平成１５年後期のネットワークとデータサーバの状況

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。今期が始まる前にネットワークとデータサーバが更新され､データの短期保存領域(/home)が 606GB に増強されたこととユーザの自主的な不要ファイル消去によりビームタイム中のファイル消去が必要なくなった。今後とも不要なファイルの消去にご協力をお願いしたい。

２．平成１６年前期の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。今後とも不要なファイルの消去にご協力をお願いしたい。

Ⅲ．委員会報告

１．編集委員会

第２５回編集委員会が平成１６年５月２５日（火）BSBPハウスにて開催された。
出席者：幾田まり氏、坂部貴和子氏、坂部知平氏、櫻井正博氏、松本拓男氏の５名。
１）構造生物Vol.10,No.2の内容及び執筆者について検討および執筆者に依頼する役の割り当てがなされた。
２）SBSPの財政が緊迫しているため、当分の間「構造生物」の発行回数を年２回にすることが了承された。
３）第２５編集委員会のあと、幾田まり委員が配置換えのため委員を辞任することになり、後任に角南智子氏（万有製薬㈱）が就任した。

Ⅳ．業績紹介

１．祥雲　弘文（東京大学）

Kinetic analysis of hydroxylation of saturated fatty acids by recombinant P450foxy produced by an *Escherichia coli* expression system

Eur. J. Biochem., **269**, 2075-2082, (2002)

Tatsuya Kitazume[1], Akinori Tanaka[1], Naoki Takaya[1], Akira Nakamura[1], Shigeru Matsuyama[1], Takahisa Suzuki[1] and Hirofumi Shoun[2]

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Summary

Cytochrome P450foxy (P450foxy, CYP505) is a fused protein of cytochrome P450 (P450) and its reductase isolated from the fungus *Fusarium oxysporum,* which catalyzes the subterminal (ω-1~ω-3) hydroxylation of fatty acids. Here, we produced, purified and characterized a fused recombinant protein (rP450foxy) using the *Escherichia coli*

expression system. Purified rP450foxy was catalytically and spectrally indistinguishable from the native protein, but most of the rP450foxy was recovered in the soluble fraction of *E. coli* cells unlike the membrane-bound native protein. The results are consistent with our notion that the native protein is targeted to the membrane by a post-translational modification mechanism. We also discovered that P450foxy could use shorter saturated fatty acid chains (C9 and C10) as a substrate. The regiospecificity (ω-l~ω-3) of hydroxylation due to the enzymatic reaction for the short substrates (decanoate, C10; undecanoate, C11) was the same as that for longer substrates. Steady state kinetic studies showed that the k_{cat}values for all substrates tested (C9-C16) were of the same magnitude (1200-1800 min^{-1}), whereas the catalytic efficiency (k_{cat}/K_m) was higher for longer fatty acids. Substrate inhibition was observed with fatty acid substrates longer than C13, and the degree of inhibition increased with increasing chain length. This substrate inhibition was not apparent with P450BM3, a bacterial counterpart of P450foxy, which was the first obvious difference in their catalytic properties to be identified. Kinetic data were consistent with the inhibition due to binding of the second substrate. We discuss the inhibition mechanism based on differences between P450foxy and P450BM3 in key amino acid residues for substrate binding.

2．祥雲　弘文（東京大学）

The B' Helix Determines Cytochrome P450nor Specificity for the Electron Donors NADH and NADPH

The Journal of Biological Chemistry, **277** (37), 33842-33847, (2002)

Li Zhang[1], Takashi Kudo[1], Naoki Takaya[1] and Hirofumi Shoun[2]

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Summary
Nitric oxide reductase (Nor) cytochrome P450nor (P450nor) is unique because it is catalytically self-sufficient, receiving electrons directly from NADH or NADPH. However, little is known about the direct binding of NADH to cytochrome. Here, we report that oxidized pyridine nucleotides (NAD^+ and $NADP^+$) and an analogue induce a spectral perturbation in bound heme when mixed with P450nor. The P450nor isoforms are classified according to electron donor specificity for NADH or NADPH. One type (Fnor, a P450nor of Fusar*ium oxysporum)* utilizes only NADH. We found that NAD^+ induced a type I spectral change in Fnor, whereas $NADP^+$ induced a reverse type I spectral change, although the K_d values for both were comparable. In contrast, $NADP^+$ as well as NAD^+ caused a type I spectral change in Tnor, a P450nor isozyme from *Trichosporon cutaneum* that utilizes both NADH and NADPH as electron donors. The B' helix region of Tnor (^{73}SAGGKAAA^{80}) contains some Ala and Gly residues, whereas the sequence is replaced at a few sites with more bulky amino acid residues in Fnor (^{73}SASGKQAA^{80}). A single mutation (S75G) significantly improved the NADPH- dependent Nor activity of Fnor, and the overall activity was accelerated via the NADPH-enhanced reduction step. These results showed that pyridine nucleotide cofactors can bind P450nor and that only a few residues in the B' helix

region determine cofactor specificity. We further showed that a poor electron donor (NADPH) could also bind Fnor, but an appropriate configuration for electron transfer is blocked by steric hindrance mainly by Ser^{75} against the 2'-phosphate moiety. The present results along with previous observations together revealed a novel motif for cofactor binding.

3．祥雲　弘文（東京大学）

Transcriptional Control of Nitric Oxide Reductase Gene (CYP55) in the Fungal Denitrifier *Fusarium oxysporum*

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Summary
Fungal denitrification is a dissimilating metabolic mechanism for nitrate and was first described in *Fusari*um *oxysporum*. Here we investigated regulatory systems of expression of *CYP55,* which encodes cytochrome P450 (P450nor) and is essential for the fungal denitrification. Promoter-reporter analysis of *F. oxysporum CYP55* using *Escherichia coli* *β*-galactosidase showed that the region between nucleotides ―526 and ―515 was critical for induction by nitrate. It contained a nucleotide sequence similar to the binding consensus sequence of the pathway-specific transcriptional factor NirA, which induces expression of the nitrate-assimilatory genes of *Aspergillus nidulans* in the presence of nitrate. This indicates that expression of the nitrate dissimilatory gene *(CYP55)* is concomitantly regulated with the nitrate-assimilatory genes. The deletion studies also indicated that the nucleotide sequence between ―118 and ―107, which was similar to the binding consensus of the yeast Rox1p, which represses the anoxic genes under aerobic conditions, was responsible for repression of *CYP55* under aerobic conditions. These results indicate that the fungus adapts to the denitrifying conditions by a combination of NirA- and Rox1-like transcription factors.

4．伏信　進矢・祥雲　弘文（東京大学）

Crystal structures of a meta-cleavage product hydrolase from *Pseudomonas fluorescens* IP01 (CumD) complexed with cleavage products

Protein Science , **11**, 2184-2195, (2002)

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Summary
2-Hydroxy-6-oxo-7-methylocta-2,4-dienoate hydrolase (CumD) from *Pseudomonas fluorescens* IP0l hydrolyzes a meta-cleavage product generated in the cumene (isopropylbenzene) degradation pathway. The crystal structures of the inactive S103A mutant of the CumD enzyme complexed with isobutyrate and acetate ions were determined at 1.6 and 2.0Å resolution, respectively. The isobutyrate and acetate ions were located at the same position in the active site, and occupied the site for a part of the hydrolysis product with CumD, which has the key determinant group for the substrate specificity of related hydrolases. One of the oxygen atoms of the carboxyl group of the isobutyrate ion was hydrogen bonded with a water molecule and His252. Another oxygen atom of the carboxyl group was situated in an oxyanion hole formed by the two main-chain N atoms. The isopropyl group of the isobutyric acid was recognized by the side-chains of the hydrophobic residues. The substrate-binding pocket of CumD was long, and the inhibition constants of various organic acids corresponded well to it. In comparison with the structure of BphD from *Rhodococcus* sp. RHA1, the structural basis for the substrate specificity of related hydrolases, is revealed.

5．祥雲　弘文（東京大学）

Hybrid Respiration in the Denitrifying Mitochondria of *Fusarium　oxysporum*

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Summary
Induction of the mitochondrial nitrate-respiration (denitrification) system of the fungus *Fusarium oxysporum* requires the supply of low levels of oxygen (O_2). Here we show that O_2 and nitrate (NO_3^-) respiration function simultaneously in the mitochondria of fungal cells incubated under hypoxic, denitrifying conditions in which both O_2 and NO_3^- act as the terminal electron acceptors. The NO_3^- and nitrite (NO_2^-) reductases involved in fungal denitrification share the mitochondrial respiratory chain with cytochrome oxidase. *F. oxysporum* cytochrome c_{549} can serve as an electron donor for both NO_2^- reductase and cytochrome oxidase. We are the first to demonstrate hybrid respiration in respiring eukaryotic mitochondria.