Syntheses and Antimicrobial Activities of Five-membered Ring Heterocycles Coupled to Indole Moieties

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Indole-substituted oxazolidinones, oxazolones, pyrrolidinone, imidazolidinone and imidazolones were synthesized. Their inhibitory potencies towards protein kinase C and protein kinase A were determined and their *in vitro* activities against *Streptomyces chartreusis*, *Streptomyces griseus*, *Bacillus cereus*, *Candida albicans* and *Escherichia coli* were examined. The inhibition of *Streptomyces* sporulation observed for some of them could not be linked to *in vitro* protein kinase C inhibition. All proved inactive against *C. albicans* but three of them exhibited a marked activity towards *E. coli*. This effect extends to other Gram-negative bacteria.

In the course of a search for new protein kinase C (PKC) inhibitors, structurally related to staurosporine[1], rebeccamycine[2], bis-indolyl maleimide[3] and MDL 27,032[4] (Fig. 1), we investigated the syntheses and biological activities of new heterocyclic compounds **A**~**H** (Fig. 2) possessing a five-membered ring system that includes a carbamate function like MDL 27, 032 (**A**, **B**, **C**, **D**), an amide function like staurosporine aglycone (**E**) or an ureylene function (**F**, **G**, **H**), attached to monoindolyl, bis-indolyl or indolocarbazolyl moieties.

Staurosporine and structurally related K-252a, both

Fig. 1. PKC inhibitors.

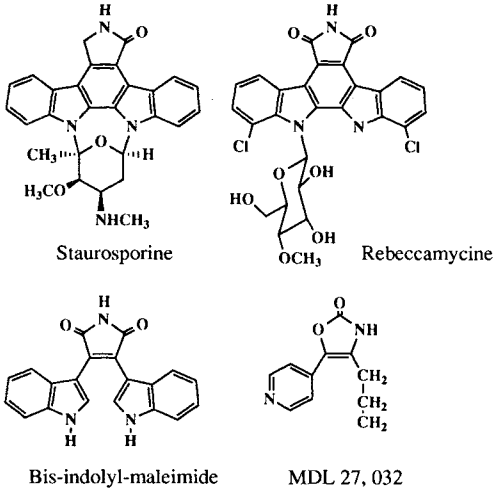
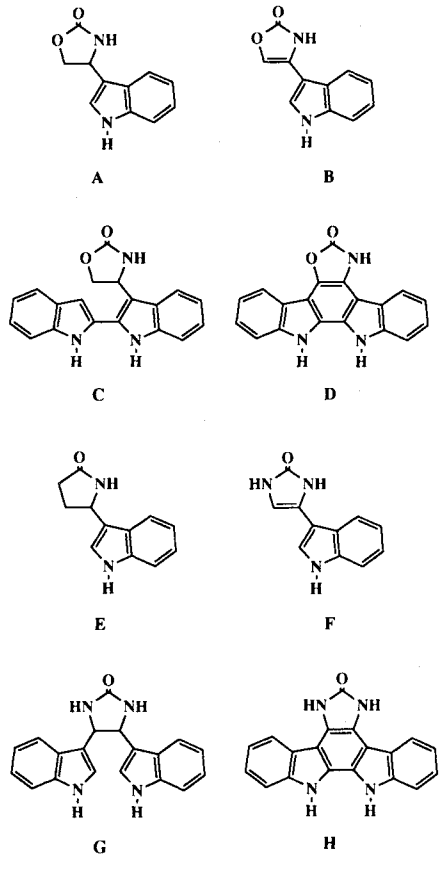


Fig. 2. New compounds **A**~**H** tested.



strong PKC inhibitors, were found to inhibit growth and aerial mycelium formation of different *Streptomyces* species[5]. In previous papers[6,7] we reported the antimicrobial activities of indolocarbazole and bis-indole compounds against *Streptomyces chartreusis* and *Streptomyces griseus*. Some of them were found to strongly inhibit growth and aerial mycelium formation of these *Streptomyces* without exerting any inhibitory activity against protein kinase C. Our results suggested that the inhibition of sporulation may be induced *via* pathways other than protein phosphorylation by PKC.

In this paper, we report the activities of compounds **A**～**H** towards *S. chartreusis* and *S. griseus* together with their activities against *Bacillus cereus*, *Escherichia coli* and *Candida albicans*. Commercial oxazolidin-2-one and oxazol-2-one (Fig. 3) were tested as references.

Results and Discussion

A and **B** were readily prepared from oxazolidin-2-one *via* electrochemical pathways[8,9]. The synthesis of **C** and **D** was performed from 2,2′-bis-indole and N-Boc-oxazol-2-one[10]. **E** was prepared by reaction of 5-methoxypyrrolidin-2-one with indolyl-magnesium bromide in the presence of BF_3-etherate according to the method described by ISHIZUKA *et al.*[11]. **F**, **G** and **H** were obtained from imidazolidin-2-one according to the synthetic scheme shown in Fig. 4. Electrochemical oxidation of imidazolidin-2-one in methanol by application of the method described by STAHL *et al.*[12] led to 4,5-dimethoxy-imidazolidin-2-one, which by reaction with indolyl-magnesium bromide in the presence of a Lewis acid, provided **G** and **F** with concomitant elimination of methanol. Oxidation of **G** using DDQ in refluxing toluene yielded carbazole **H**.

Fig. 3. Oxazolidin-2-one **I** and oxazol-2-one **J**.

The *in vitro* growth inhibitory effects of compounds **A**～**J** against *S. chartreusis*, *S. griseus*, *E. coli*, *B. cereus* and *C. albicans* were tested and the inhibition of sporulation of *S. chartreusis* and *S. griseus* was examined (Table 1).

The inhibitory potencies of compounds **A**～**H** towards protein kinase C and protein kinase A were determined using histones IIIs and IIa respectively as substrates[13]. They were inactive except for **D** which was more active against PKA than PKC (IC_{50} PKC: 89 μM; IC_{50} PKA: 24 μM) and **C** (IC_{50} PKC: >150 μM; IC_{50} PKA: 81 μM).

B and **G** exhibited the strongest activity against *S. chartreusis* and *S. griseus* without showing any inhibitory effect towards PKC and PKA. These results confirm those reported in our previous papers. No correlation could be demonstrated between inhibition of PKC and inhibition of growth and sporulation of the *Streptomyces* strains tested.

Since **I** and **J** had no effect on growth and sporulation of the two *Streptomyces* tested, the activity observed for **B** and **C** could not be due solely to the presence of oxazolidin-2-one or oxazol-2-one heterocycle.

D and **H** with rigid indolocarbazole frameworks were inactive while more flexible structures, **C** and **G**, bearing the same non-indolic heterocycles, strongly inhibited growth and aerial mycelium formation, which was already observed in our previous series[6].

In contrast to findings for all the compounds in our

Fig. 4. Synthetic scheme of **F**, **G** and **H**.

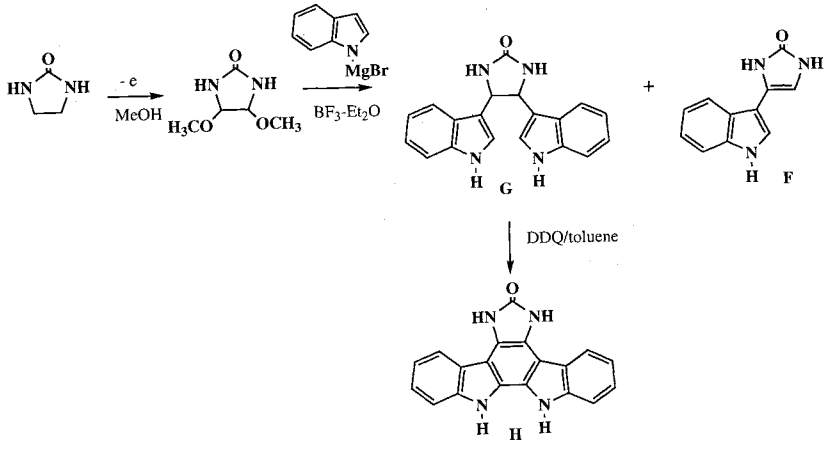


Fig. 5. Effects of compounds **F** and **G** on sporulation of *S. chartreusis.* and *S. griseus.*

Scanning electron microscopy photographs. a) *S. chartreusis* as control. b) Inhibition of sporulation by **F** (zone of partial inhibition). c) Inhibition of sporulation by **G** (zone of total inhibition). d) *S. griseus* as control. e) Inhibition of sporulation by **F**. f) inhibition of sporulation by **G**. Bar represents 5.0 μm.

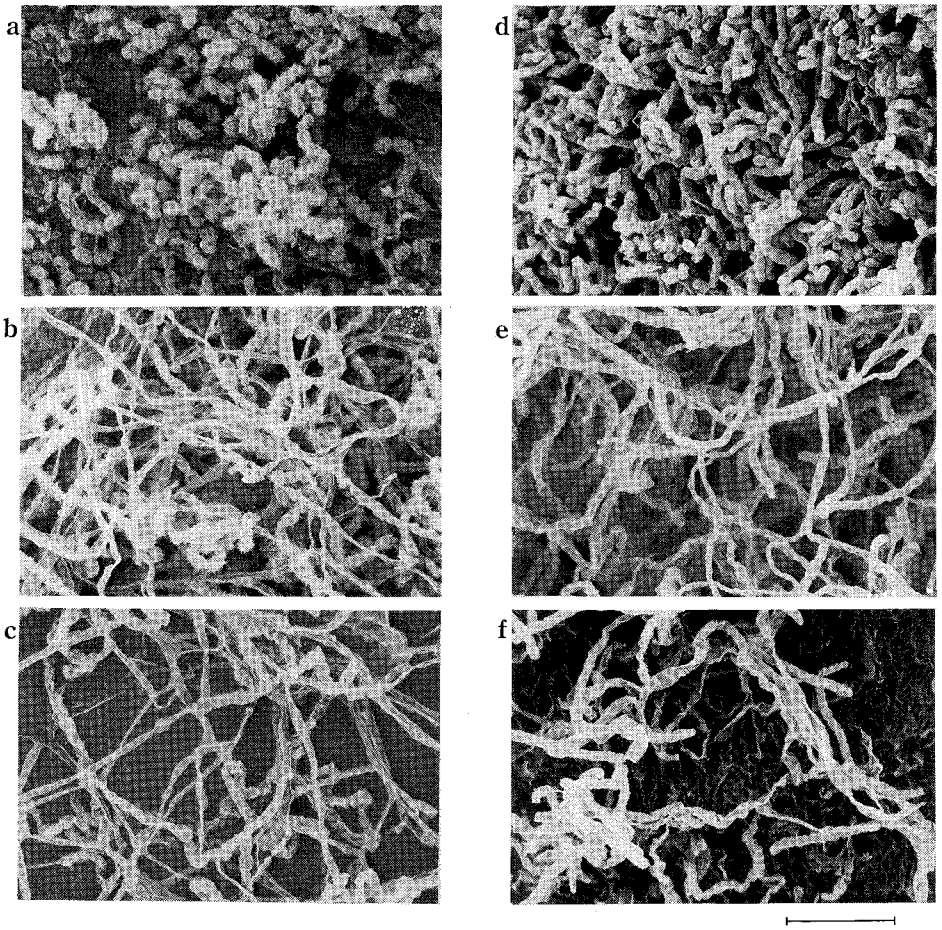


Table 1. *In vitro* growth inhibitory effect against different microorganisms.

Compound	*S. chartreusis* NRRL 11407		*S. griseus* ATTC 23345		*E. coli* ATCC 11303	*B. cereus* ATCC 14879	*C. albicans* IP 444
	g.	sp.	g.	sp.			
A	±	−	±	−	+++	−	−
B	+++	+++	++	+++	p. +	+	−
C	++	++	++	+++	−	++	−
D	+	−	+	−	p. +	+	−
E	±	−	±	−	−	−	−
F	+	+ p. +++	+	+	−	+	−
G	+++	+++	+++	+++	−	+	−
H	±	−	−	−	−	+	−
I	±	−	±	−	−	−	−
J	±	−	±	−	−	−	−

For the two *Streptomyces species*: g. means inhibition of growth, sp. means inhibition of sporulation. (+++), (++), (+), (±) and (−) mean strong, medium, weak, very weak and no activity. The size of zones of growth inhibition was 12~15 mm (+++), 10~12 mm (++), 7~9 mm (+), 6~7 mm (±). Data for staurosporine: *S. chartreusis* g. +, sp. +++, *S. griseus* g. ±, sp. ++. When there is only a partial inhibition, the size of the inhibition zone is preceded by p.

Table 2. *In vitro* growth inhibitory effect against different Gram-negative bacteria.

Compound	*E. coli* ATCC 11303	*E. coli* ATCC 10798	*Pseudomonas aeruginosa* ATCC 17504	*Proteus mirabilis* CIP 7515
A 300 μg	++++	+++ p. ++++	++	p. +
A 150 μg	+++	++ p. +++	+	−
D 300 μg	p. +			
D 150 μg	p. +			
D 50 μg	p. +	++ p. +++	++	p. +

E. coli (ATCC 11303), *E. coli* (ATCC 10798), *Pseudomonas aeruginosa* (ATCC 17504) and *Proteus mirabilis* (CIP 7515) The size of zones of growth inhibition was >15 mm (++++), 12~15 mm (+++), 10~12 mm (++), 7~9 mm (+), 6~7 mm (±). When there is only a partial inhibition, the size of the inhibition zone is preceded by p.

previous series[6,7], three substances, **A**, **B** and **D**, were active against *E. coli*. This activity cannot be due to PKC nor PKA inhibition since **A** and **B** did not inhibit these enzymes. Oxazolidinone **A** strongly inhibited growth of *E. coli* while inhibition of growth by oxazolones **B** and **D** was only partial. Since **I** and **J** were inactive, the effects of **A**, **B** and **D** are not linked solely to the presence of the heterocycle oxazolidin-2-one or oxazol-2-one. To test for activities against other Gram-negative strains, the antibacterial activities of **A** and **D** were also examined against *E. coli* (ATCC 10798), *Pseudomonas aeruginosa* (ATCC 17504) and *Proteus mirabilis* (CIP 7515) at different concentrations (Table 2).

The results in Table 2 show that the activity observed against *E. coli* (ATCC 11303) can be extended to several other Gram-negative strains. What remains unexpected is (i) the strong activity of **A** against *E. coli* though it is almost inactive against Gram positive bacteria, and (ii) the activity of **A**, **B** and **D** against *E. coli* though oxazolidinone **C** is inactive.

Their mode of action and their targets remain to be determined. Since **A** has a very weak or no activity against the Gram-positive bacteria tested, its activity against the Gram-negative bacteria may be due to interactions with the outer membrane.

Experimental

IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}), NMR spectra on a Bruker AC 400 (^1H: 400 MHz, ^{13}C: 100 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), tertiary carbons (C tert.), quaternary carbons (C quat.)). Mass spectra (EI) were determined at CESAMO (Talence) on a high resolution FISONS Autospec-Q spectrometer. Chromatographic purifications were performed with flash Geduran SI 60 (Merck) 0.040~0.063 mm.

5-Methoxypyrrolidin-2-one

Electrochemical oxidation of pyrrolidin-2-one (2 g, 23.5 mmol) in methanol yielded, after purification by flash chromatography (eluent: AcOEt-MeOH 9:1), 5-methoxypyrrolidin-2-one (2.07 g, 18 mmol, 76% yield) as a white solid.

mp 50~52°C. IR $\nu_{C=O}$ 1700 cm^{-1} ν_{NH} 3210 cm^{-1}. HR-MS (EI) calcd. for $C_5H_9NO_2$ 115.0633, found 115.0634. ^1H NMR (400 MHz, CD_3OD): 2.20 (1H, m), 2.38 (1H, m), 2.49 (1H, m), 2.64 (1H, m), 3.49 (3H, s), 5.03 (1H, s, NH), 5.08 (1H, d, J=5.9 Hz). ^{13}C NMR (100 MHz, CD_3OD): 29.1 (CH_2); 29.4 (CH_2); 55.1 (CH_3O); 89.0 (CH); 181.8 (C=O).

5-(3-Indolyl)pyrrolidin-2-one **E**

To magnesium (1.25 g) in THF (30 ml) was added dropwise ethyl bromide (4.76 g), the mixture was warmed to allow the beginning of the reaction. Indole (5.08 g, 43.4 mmol) in THF (40 ml) was then slowly added and the mixture was warmed at 45°C for 45 minutes, then cooled at −30°C. 5-methoxypyrrolidin-2-one (1.27 g, 11.0 mmol) was added then BF_3-Et_2O (2.36 ml) and the mixture was stirred overnight at −30°C. After quenching with saturated aqueous NH_4Cl, extraction with AcOEt, the organic phase was dried over $MgSO_4$. After removal of the solvent, purification by flash chromatography (eluent: cyclohexane-AcOEt 5:5) yielded 5-(3-indolyl)pyrrolidin-2-one **E** as a white powder (1.475 g, 7.37 mmol, 66.7% yield).

mp 160~162°C. IR (KBr) $\nu_{C=O}$ 1680 cm^{-1} ν_{NH} 3250, 3400 cm^{-1}. HR-MS (EI) calcd. for $C_{12}H_{12}N_2O$ 200.0949, found 200.0953. ^1H NMR (400 MHz, methanol-d_4): 2.15 (1H, m), 2.33 (2H, m), 2.54 (1H, m), 4.95 (1H, t, J=6.2 Hz), 6.98 (1H, t, J=8.1 Hz), 7.09 (1H, t, J=8.1 Hz), 7.25 (1H, d, J=2.4 Hz), 7.38 (1H, d, J=8.1 Hz), 7.60 (1H, d, J=8.1 Hz), 8.03 (1H, s, NH), 10.20 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, DMSO-d_6): 30.0 (CH_2); 30.6 (CH_2); 50.7 (CH); 111.7, 118.5, 118.7,121.2, 122.1 (C tert. arom.); 116.6, 125.2, 136.8 (C quat. arom.); 176.6 (C=O).

4,5-Dimethoxyimidazolidin-2-one

Anodic oxidation of imidazolidin-2-one (2 g, 23.2 mmol), in methanol (60 ml) containing Et_4NOTs (500 mg), using glassy carbon electrodes was performed in a cell externally cooled by an ice bath at a constant intensity of 0.3 A and yielded, after purification by flash chromatography (eluent AcOEt) 4,5-dimethoxyimidazolidin-2-one (620 mg, 4.25 mmol, 18% yield) as a white solid.

mp 102~105°C. IR (KBr) $\nu_{C=O}$ 1720 cm^{-1} ν_{NH} 3250 cm^{-1}. HR-MS (EI) calcd. for $C_4H_7N_2O_3$ ($M-CH_3$) 131.0456, found 131.0453. ^1H NMR (400 MHz, DMSO-d_6): 3.25 (6H, s), 4.56 (2H, s), 7.75 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): 52.8 (OCH_3); 88.4 (CH); 160.7 (C=O).

4,5-Bis(3-indolyl)imidazolidin-2-one (**G**) and 4-(3-Indolyl)imidazol-2-one (**F**)

To magnesium (410 mg) in THF (10 ml) was added dropwise ethyl bromide (1.3 ml). After warming to start the reaction, indole (1.97 g, 16.9 mmol) in THF (18 ml) was slowly added. The mixture was warmed at 45°C for 45 minutes. After cooling to −30°C, 4,5-dimethyloxyimidazolidin-2-one (330 mg, 2.03 mmol) was added then BF_3-Et_2O (1.02 ml) and the mixture was stirred at −30°C for 12 hours. After quenching with saturated aqueous NH_4Cl, extraction with AcOEt, the organic phase was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent: AcOEt) to give **G** (240 mg, 0.76 mmol, 37% yield) as a pale yellow solid and **F** as a yellow solid (eluent: AcOEt - MeOH 9:1) (54 mg, 0.27 mmol, 12% yield).

G: mp 143~145°C. IR (KBr) $\nu_{C=O}$ 1690 cm^{-1} ν_{NH} 3250 and 3400 cm^{-1}. HR-MS (EI) calcd. for $C_{19}H_{16}N_4O$ 316.1324, found 316.1324. ^1H NMR (400 MHz, DMSO-d_6): 5.01 (2H, s), 6.89 (2H, s), 6.99 (2H, t, J=7.8 Hz), 7.12 (2H, t, J=7.8 Hz), 7.29 (2H, d, J=2.4 Hz), 7.41 (2H, d, J=7.8 Hz), 7.53 (2H, d, J=7.8 Hz), 10.95 (2H, s, N_{indole}-H). ^{13}C NMR (100 MHz, DMSO-d_6): 57.1 (CH); 111.7, 118.5, 119.2, 121.2, 123.3 (C tert. arom.); 115.5, 125.5, 136.9 (C quat. arom.); 162.2 (C=O).

F: mp >230°C. IR (KBr) $\nu_{C=O}$ 1700 cm^{-1} ν_{NH} 3420 cm^{-1}. HR-MS (EI) calcd. for $C_{11}H_9N_3O$ 199.0745, found 199.0742.

^1H NMR (400 MHz, DMSO-d_6): 6.68 (1H, s), 7.05 (1H, t, J=7.2 Hz), 7.12 (1H, t, J=7.2 Hz), 7.40 (1H, d, J=7.5 Hz), 7.58 (1H, d, J=2.8 Hz), 7.72 (1H, d, J=7.2 Hz), 9.89 (1H, s), 10.40 (1H, s), 11.21 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6): 102.5, 111.7, 119.3, 119.6, 121.5, 121.7 (C tert.); 105.9, 118.1, 123.6, 136.5 (C quat.); 154.6 (C=O).

5*H*-Indolo[2,3-a]imidazolo[4,5-c]-carbazol-6(7*H*)-one (**H**)

To a solution of **G** (120 mg, 0.38 mmol) in toluene (13 ml) was added DDQ (102 mg). The mixture was refluxed for 9 hours. After removal of the solvent, the residue was purified by flash chromatography (eluent cyclohexane - AcOEt 1:9) to give **H** as a brown powder (40 mg, 0.13 mmol. 34% yield).

mp >295°C. IR (KBr) $\nu_{C=O}$ 1680 cm^{-1} ν_{NH} 3420 cm^{-1} HR-MS (EI) calcd. for $C_{19}H_{12}N_4O$ 312.1011, found 312.1010. ^1H NMR (400 MHz, DMSO-d_6): 7.16 (2H, t, J=7.5 Hz); 7.37 (2H, t, J=7.5 Hz); 7.66 (2H, d, J=8 Hz); 8.58 (2H, d, J=8 Hz); 10.97 (2H, s, N_{amide}-H); 11.32 (2H, s, N_{indole}-H). ^{13}C NMR (100 MHz, DMSO-d_6): 105.8, 114.5, 121.3, 121.4, 138.8 (C quat.); 111.1, 118.6, 121.4, 124.2 (C tert. arom.); 156.5 (C=O).

Antimicrobial Tests

Eight strains were tested: three Gram-positive bacteria (*B. cereus* ATCC 14579, *S. chartreusis* NRRL 11407, *S. griseus* ATCC 23345), four Gram-negative bacteria (*E. coli* ATCC 11303, *E. coli* ATCC 10798, *Pseudomonas aeruginosa* ATCC 17504, *Proteus mirabilis* CIP 7515) and a yeast (*C. albicans* 444 from Pasteur Institute). Antibacterial activity was determined by the conventional paper disk (Durieux, N° 268, 6 mm in diameter) diffusion method using the following nutrient media: Mueller Hinton (Difco) for *B. cereus*, *E. coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*, Sabouraud agar (Difco) for *C. albicans* and Emerson agar (0.4% beef extract, 0.1% yeast extract, 0.4% peptone, 1% dextrose, 0.25% NaCl, 2% agar, pH 7.0) for the *Streptomyces* species. Growth inhibition was examined after 24 hours incubation at 27°C (37°C for *E. coli* and *P. mirabilis*). Inhibition of sporulation was examined 3 to 5 days later. Products **A**~**J** were dissolved in DMSO and a paper disk containing each of the products (300 μg) was placed on agar plates. For the inhibition of Gram negative bacteria different concentrations were tested 300, 150 or 50 μg.

Measurements of PKC and PKA Inhibition

Histones IIIs and IIa, phosphatidylserine and diacylglycerol were purchased from Sigma, [γ^{32}P] ATP was from Amersham. Protein kinase A was purchased from Sigma and protein kinase C from Calbiochem.

Protein kinase C phosphorylation assays were performed in a reaction mixture (80 ml) containing histone IIIs (2.4 mg/ml), $MgCl_2$ (10 mM), $CaCl_2$ (0.1 mM), phosphatidylserine (10 mg/ml), diacylglycerol (10 mg/ml), ATP (10 mM), [γ^{32}P] ATP (10^6 cpm/80 ml), Tris buffer (50 mM, pH 7.5), protein kinase C (0.5 mg/ml) and inhibitors at different concentrations.

Protein kinase A phosphorylation assays were performed in a reaction (80 ml) mixture containing histone IIa (1 mg/ml), $MgCl_2$ (5 mM), ATP (10 mM), [γ^{32}P] ATP (10^6 cpm/80 ml), Tris buffer (50 mM, pH 7.0), protein kinase A (1 mg/ml) and inhibitors at different concentrations.

For each kinase, reactions were run at 30°C for 12 minutes and stopped with trichloroacetic acid (12% w/v) in the presence of bovine serum albumin (0.9 mg) as a carrier protein. After centrifugation (10 minutes at

3000 rpm), the pellet was dissolved in 1 M NaOH and precipitated a second time with trichloracetic acid. Radioactivity incorporated into histones was counted by scintillation spectrometry (Tri-Carb 4530, Packard). All experiments were carried out in triplicate.

Scanning Electron Microscopy

Pieces of mycelium were cut out and fixed under osmium tetroxide vapour for 15 hours then allowed to dry for 12 hours. After sputter coating with gold for 2 minutes under vacuum, the mycelium was examined under a Cambridge stereoscan 360 scanning electron microscope.

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