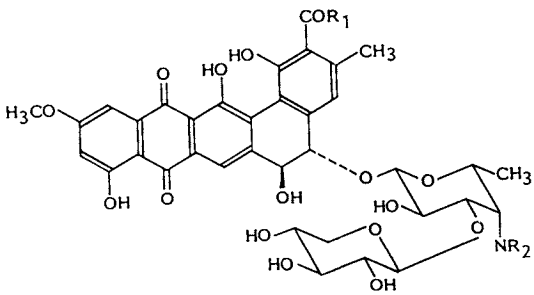
MANNAN-MEDIATED ANTICANDIDAL ACTIVITY OF BMY-28864, A NEW WATER-SOLUBLE PRADIMICIN DERIVATIVE

Sir:

Pradimicin A is a representative antifungal antibiotic among naturally-occurring pradimicin congeners[1], but its experimental uses *in vitro* and *in vivo* are relatively limited because of its low water solubility. To overcome this disadvantage, we attempted to develop a variety of pradimicin derivatives by biological and chemical modifications, leading to BMY-28864 (Fig. 1), which was prepared from pradimicins FA-1 and FA-2, fermentation products, by *N*-methylation[2,3]. The new pradimicin derivative has a water solubility of more than 20 mg/ml in PBS containing $CaCl_2$ 0.9 mM and $MgCl_2$ 0.5 mM, pH 7.2, at 25°C (pradimicin A: 0.02 mg/ml). The essential role of calcium in expression of anticandidal activity of pradimicin A has been reported in connection with its avid binding to the cell surface of *Candida albicans*[4,5]. In this communication, the mode of anticandidal action of pradimicins on *C. albicans* A9540 was studied more precisely using the water soluble derivative BMY-28864.

Fig. 1. Structures of pradimicin derivatives.



	R_1	R_2
Pradimicin A	$-NHCH(CH_3)COOH$ (D)	$-H$, $-CH_3$
17-Epipradimicin A	$-NHCH(CH_3)COOH$ (L)	$-H$, $-CH_3$
BMY-28864	$-NHCH(CH_2OH)COOH$ (D)	$-CH_3$, $-CH_3$

Comparative antimicrobial activities of pradimicin A, 17-epipradimicin A and BMY-28864 (Fig. 1) are shown in Table 1. Like pradimicin A, BMY-28864 exhibits good *in vitro* antimicrobial activity against yeast, fungi and *Micrococcus luteus*, while 17-epipradimicin A has no antimicrobial activity at all. Even in the presence of 200 μM $CaCl_2$, 17-epipradimicin A showed a negligible amount of binding to the cell surface of *C. albicans* (about 2% of pradimicin A binding), which seemingly resulted in no anticandidal activity[5]. In contrast, BMY-28864 and pradimicin A were avidly adsorbed on the candidal cell surface, expressing potent *in vitro* anticandidal activity. Accordingly, the amino acid moiety at C-17 of pradimicins is considered to be one of the key elements in binding to the cell surface components and subsequent candidacidal activity.

The essential role of mannan among cell surface components of *C. albicans* in the cell surface binding of BMY-28864 was confirmed using a commercially available preparation of yeast mannan as follows: 5 g of acrylamide in 12.5 ml of 150 mM Na^+, K^+-phosphate buffer, pH 7.2, was mixed with 1.15 g of bis-acrylamide in 50 ml of the buffer and 100 mg of mannan (M 7504, Sigma) suspended in 25 ml of the buffer. To the suspension, 600 μl of 10%

Table 1. *In vitro* antimicrobial activity of pradimicin derivatives.

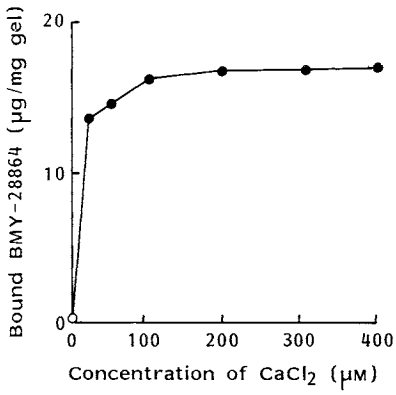
Test organism	MIC (μg/ml)[a]		
	Pradimicin A	17-Epipradimicin A	BMY-28864
Saccharomyces cerevisiae ATCC 9763	12.5	ND	3.1
Candida albicans A9540	50	>100	6.3
Trichophyton mentagrophytes No. 4329	1.6	>100	3.1
Escherichia coli NIHJ JC-2	>100	>100	>100
Bacillus subtilis ATCC 6633	>100	>100	>100
Micrococcus luteus ATCC 9341	3.1	ND	12.5

[a] Determined by the agar dilution method on Sabouraud dextrose agar, pH 7.0, for fungi and yeast, and on nutrient agar, pH 7.0, for bacteria.

ND: Not determined.

ammonium persulfate and 60 μl of TEMED were added and left to stand for 2 hours. The resulting gel was soaked in water and then lyophilized to yield 6.67 g of a solid which was ground in a motor to give a fine white powder (1 mg of the powder contained 15 μg mannan). In the presence of 200 μM $CaCl_2$, 1 mg of the mannan-immobilized gel adsorbs 17 μg of BMY-28864, while in the absence of calcium, no substantial binding is observed (Fig. 2).

Fig. 2. Effect of $CaCl_2$ on BMY-28864 binding to immobilized yeast mannan.

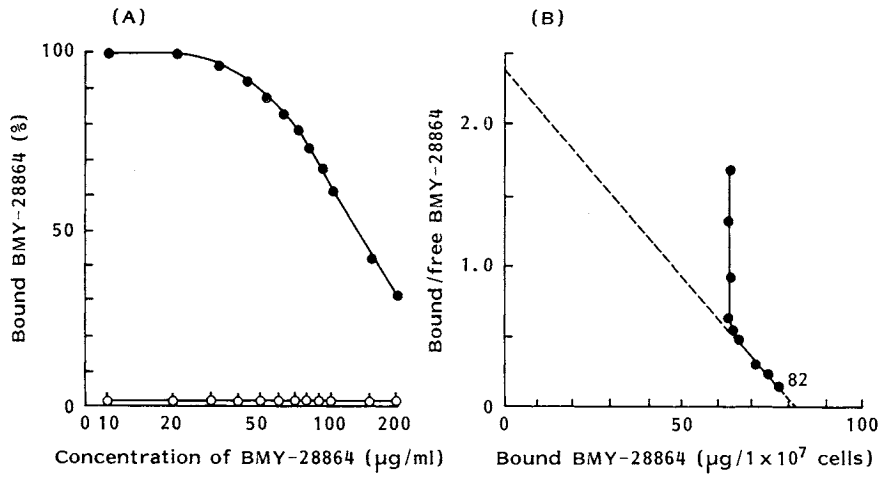


The mannan-immobilized polyacrylamide gel powder (1 mg) was suspended in 840 μl of 50 mM Na^+-phosphate buffer, pH 7.0, in the presence (●) and absence (○) of 100 μl $CaCl_2$ at various concentrations and mixed with 60 μl of BMY-28864 (1,000 μg/ml). The mixture was incubated at 25°C for 30 minutes. Amounts of bound BMY-28864 were determined spectrophotometrically at 490 nm.

The binding kinetics of BMY-28864 to *C. albicans* cells was examined with and without 200 μM $CaCl_2$. Fig. 3(A) shows the binding percentage of added BMY-28864 as a function of antibiotic concentration. In the presence of calcium, 60 μg/ml BMY-28864 results in 83% (50 μg) binding to 1×10^7 *Candida* cells. Below 30 μg/ml, surprisingly, over 95% of added BMY-28864 is adsorbed to the cells. This binding was irreversible upon washings with water containing no calcium. In the absence of calcium, on the contrary, binding of BMY-28864 to the cells is insignificant. Scatchard analysis of this calcium-dependent binding indicates an unusual two-phase pattern of regression line. If only the lower linear portion of the regression line was used, a Bmax value of 82 μg per 1×10^7 cells would be obtained (Fig. 3B).

Time course of the candidacidal action of BMY-28864 was studied: 60 μg/ml BMY-28864 killed *C. albicans* cells by about 90% in 60 minutes and by 99.9% in 4 hours at an initial cell density of 1×10^7 cells/ml in 50 mM Na^+-phosphate, pH 7.0, containing 200 μM $CaCl_2$. The killing effect and binding to cell surface mannan were completely reversed with 2 mM EGTA. This cause-effect relationship of the cell surface binding of BMY-28864 with the candidacidal activity was also supported by measuring the potassium leakage from *C. albicans* cells in the presence and absence of 200 μM calcium with or without 2 mM EGTA.

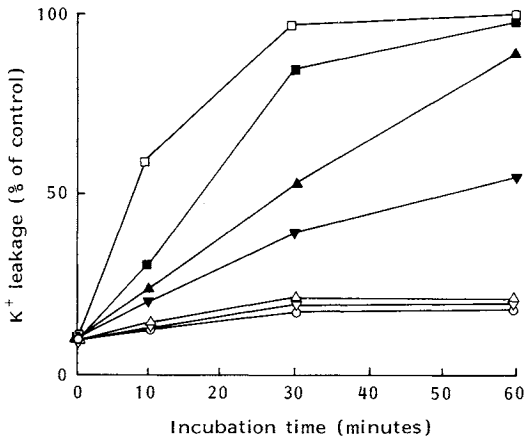
Fig. 3. Binding of BMY-28864 to *Candida albicans* (A) and the Scatchard analysis (B).



C. albicans (2×10^7 cells/2 ml) was incubated at 28°C with indicated concentrations of BMY-28864 in the presence (●) and absence (○) of 200 μM $CaCl_2$ in 50 mM Na^+-phosphate buffer, pH 7.0. Amounts of bound BMY-28864 were determined spectrophotometrically at 490 nm.

Fig. 4. Effect of calcium on the BMY-28864-induced K^+ leakage from *Candida albicans*.

□ Amphotericin B 1 μg/ml + 200 μM Ca^{++}, ■ BMY-28864 60 μg/ml + 200 μM Ca^{++}, ▲ BMY-28864 30 μg/ml + 200 μM Ca^{++}, ▼ BMY-28864 15 μg/ml + 200 μM Ca^{++}, △ BMY-28864 60 μg/ml + 200 μM Ca^{++} + 2 mM EGTA, ▽ BMY-28864 60 μg/ml without Ca^{++}, ○ BMY-28864 0 μg/ml.



C. albicans (2×10^7 cells/2 ml) was incubated at 37°C with and without BMY-28864 or amphotericin B in the presence and absence of 200 μM $CaCl_2$ in 50 mM Na^+-phosphate buffer, pH 7.0. Concentrations of K^+ were determined with a flame photometer as described previously[4]. 100% = 4.2 ppm.

Without calcium, 60 μg/ml BMY-28864 produces no potassium leakage, whereas the addition of 200 μM calcium rapidly induces pradimicin dose-dependent leakage of potassium (Fig. 4). *Trichophyton mentagrophytes* and *M. luteus*[6] are known to contain glucomannan and lipomannan, respectively. Although the bindings of 60 μg/ml of BMY-28864 to these pathogens were less abundant than to *Candida* cells, significant BMY-28864-dependent potassium leakage was observed (data not shown).

Human erythrocytes and various cultured mammalian cells were also tested for BMY-28864 binding and subsequently for potassium leakage and cell viability. Even in the presence of 200 μM calcium, BMY-28864 (60 μg/ml) treated-erythrocytes and-cultured mammalian cells showed neither BMY-28864 binding nor potassium leakage and cell death.

In conclusion, the anticandidal activity of BMY-28864 in the presence of calcium is considered to be mediated by cell surface mannan which hence brings about action selectivity of pradimicins. The detailed mechanism of action of BMY-28864 on *Candida* cells is now under study.

Acknowledgment

We thank Drs. M. KAKUSHIMA and K. NUMATA of our Institute for their helpful advice and comments.

YOSUKE SAWADA
TSUTOMU MURAKAMI
TOMOKAZU UEKI
YASUO FUKAGAWA
TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

YOSHINORI NOZAWA

Department of Biochemistry,
Gifu University School of Medicine,
40 Tsukasamachi, Gifu 500, Japan

(Received August 22, 1990)

References

1) OKI, T.; M. KONISHI, K. TOMATSU, K. TOMITA, K. SAITOH, M. TSUNAKAWA, M. NISHIO, T. MIYAKI & H. KAWAGUCHI: Pradimicin, a novel class of potent antifungal antibiotics. J. Antibiotics 41: 1701~1704, 1988
2) SAWADA, Y.; M. HATORI, H. YAMAMOTO, M. NISHIO, T. MIYAKI & T. OKI: New antifungal antibiotics pradimicins FA-1 and FA-2: D-Serine analogs of pradimicins A and C. J. Antibiotics 43: 1223~1229, 1990
3) OKI, T.; M. KAKUSHIMA, M. NISHIO, H. KAMEI, M. HIRANO, Y. SAWADA & M. KONISHI: Water-soluble pradimicin derivatives, synthesis and antifungal evaluation of *N,N*-dimethyl pradimicins. J. Antibiotics 43: 1230~1235, 1990
4) SAWADA, Y.; K. NUMATA, T. MURAKAMI, H. TANIMICHI, S. YAMAMOTO & T. OKI: Calcium-dependent anticandidal action of pradimicin A. J. Antibiotics 43: 715~721, 1990
5) KAKUSHIMA, M.; M. NISHIO, K. NUMATA, M. KONISHI & T. OKI: Effect of stereochemistry at the C-17 position on the antifungal activity of pradimicin A. J. Antibiotics 43: 1028~1030, 1990
6) TONN, S. J. & J. E. GANDER: Biosynthesis of polysaccharides by prokaryotes. Annu. Rev. Microbiol. 33: 169~199, 1979