Carbohydrate Research, 149 (1986) 13–21
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE EFFECT OF PAPULACANDIN B ON (1→3)-β-D-GLUCAN SYNTHETASES. A POSSIBLE RELATIONSHIP BETWEEN INHIBITION AND ENZYME CONFORMATION

MOHINDER S. KANG, PAUL J. SZANISZLO*, VICENTE NOTARIO†, AND ENRICO CABIB‡

Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205 (U.S.A.)

(Received January 4th, 1985; accepted for publication, March 7th, 1985)

ABSTRACT

The antibiotic, papulacandin B, inhibited growth or (1→3)-β-D-glucan synthetase (or both) in the fungi *Saccharomyces cerevisiae, Hansenula anomala, Neurospora crassa, Cryptococcus laurentii, Schizophyllum commune* and *Wangiella dermatitidis*. No efect was observed on *Achlya ambisexualis*. There was no apparent correlation between the inhibition of growth and that of the synthetase. With most of the fungal extracts, the inhibition of glucan synthetase by papulacandin B became less pronounced as the substrate (UDP-glucose) concentration was decreased. At very low levels of UDP-glucose, with the enzymes from *S. cerevisiae* and *W. dermatitidis*, the antibiotic stimulated the activity of glucan synthetase. As further studied with the *W. dermatitidis* enzyme, those low concentrations of UDP-glucose corresponded to a sigmoidal portion of the rate *vs.* substrate curve. The sigmoid segment of the curve extended to higher concentrations of UDP-glucose as the temperature was increased. Concomitantly, the range of substrate concentrations at which papulacandin B stimulated the reaction or was noninhibitory was broadened. It is tentatively concluded that glucan synthetase may exist in more than one interconvertible form. The stimulatory effect of papulacandin B is possibly due to preferential binding to the active form of the enzyme. The equilibrium between these forms could be shifted by structural changes in the membrane in which the enzyme is embedded. The lack of correlation between the effects of papulacandin B in whole cells and in extracts is discussed in terms of the variations in membrane structure in the two situations.

*Permanent address: Department of Microbiology, University of Texas, Austin, TX 78759 (U.S.A.).
†Present address: Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205 (U.S.A.).
‡To whom correspondence should be addressed at National Institutes of Health, Building 10, Room 9N-115, Bethesda, MD 20205, U.S.A.

0008-6215/86/$ 03.50

INTRODUCTION

Antifungal drugs that interfere with the biosynthesis of cell walls hold great promise for therapy, because the wall often contains polysaccharides that are not found in the infected tissues. Therefore, there is a good chance that drugs that inhibit enzymes responsible for the synthesis of cell wall components may be relatively harmless to the host. Papulacandin B, an antibiotic produced by the deuteromycete *Papularia sphaerosperma*, appeared to be such a drug[1,2]. Papulacandin B contains both a D-glucosyl and a D-galactosyl residue, two fatty acids, and an aromatic ring[1]. The antibiotic seems to inhibit, with some degree of specificity, the formation of alkali-insoluble D-glucan [predominantly β-D-(1→3)] in *Saccharomyces cerevisiae*[2], *Schizosaccharomyces pombe*[3], and *Geotrichum lactis*[4]. In the latter two organisms, it also inhibits the enzyme (1→3)-β-D-glucan synthetase[3,5,*]. Nevertheless, we found very little inhibition of *S. cerevisiae* (1→3)-β-D-glucan synthetase by papulacandin B, despite the strong inhibitory effect of the antibiotic on growth. Our survey of several other fungi showed a similar lack of correlation between the effect of papulacandin B *in vivo* and *in vitro*. On the other hand, a fairly general finding was a decline in the inhibition of (1→3)-β-D-glucan synthetase as the concentration of UDP-glucose, the substrate, decreased. At very low concentrations of UDP-glucose, papulacandin B even acted as a stimulator. We decided to investigate further this puzzling phenomenon, in the hope of gaining some insight into the discrepancy between the *in vivo* and *in vitro* effects of papulacandin B.

EXPERIMENTAL

Chemicals. — Sources for chemicals were the same as previously described[6]. Papulacandin B was a generous gift of Dr. W. Wehrli (CIBA–Geigy, Basel, Switzerland).

Fungal strains and growth conditions. — The strains of *Achlya ambisexualis, Saccharomyces cerevisiae, Hansenula anomala, Neurospora crassa; Cryptococcus laurentii, Schizophyllum commune,* and *Wangiella dermatitidis* were the same as previously used[6]. The media and conditions of growth have been described[6]. When added to the growth medium, papulacandin B was in a dimethyl sulfoxide solution. The final concentration of dimethyl sulfoxide in the medium was 1%. Control media received the same concentration of the solvent. Growth of *S. cerevisiae. H. anomala, C. laurentii,* and *W. dermatitidis* was monitored by measuring A_{660} in a Coleman Junior Spectrophotometer with a light path of 1 cm. With *N. crassa, S. commune,* and *A. ambisexualis,* growth was followed by measuring dry weight.

(1→3)-β-D-Glucan synthetase preparation and assay. — Particulate fractions

*The name recommended by the Nomenclature Committee of I.U.B. is 1,3-β-D-glucan synthase (EC 2.4.1.34).

were isolated from extracts of cells broken with glass beads as described[6]. Glucan synthetase was measured by the incorporation of radioactivity from UDP-D-[^{14}C]glucose into a water- and trichloroacetic acid-insoluble product[6]. The reaction mixture was as described[6], except that the specific activity of UDP-D-[^{14}C]glucose was varied between 2.3×10^5 and 2.3×10^7 c.p.m. · μmol^{-1}, depending on the concentration of substrate and, consequently, on the amount of activity to be measured. GTP (20 μM) was included in all assays as stimulator of the enzymic activity[6]. The incubation temperature was 30°, except where indicated otherwise. Papulacandin was added to assay mixtures as a solution in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the mixture was 2.5%, and controls with the same concentration of solvent were run simultaneously. Except where otherwise noted, the enzyme was incubated for 2 min at room temperature with papulacandin B before addition of substrate.

RESULTS AND DISCUSSION

Effect of papulacandin B on growth. — The effect of papulacandin B, at 10 μg/mL concentration, on growth varied widely in various fungi. There was no change with *C. laurentii, S. commune,* or *A. ambisexualis,* strong inhibition with *S. cerevisiae, H. anomala,* and *N. crassa,* and moderate inhibition with *W. dermatitidis.* An example of each of the three cases is shown in Fig. 1. When an effect on growth was present, it was evident after 2 to 4 h.

Effect of papulacandin B on (1→3)-β-D-glucan synthetase. — When added to assay mixtures for glucan synthetase in the presence of cell-free extracts of various fungi, papulacandin B was inhibitory (Fig. 2), except in the case of *A. ambisexualis* (data not shown). There was no apparent correlation, however, between the inhibitory power of antibiotic on growth and that on glucan synthetase. Thus, in the three organisms, *S. cerevisiae, N. crassa,* and *H. anomala,* that showed the greatest depression of growth, the lowest inhibition of glucan synthesis was found in cell-free extracts (Fig. 2). The activity of the enzyme was more sensitive to the antibiotic in *C. laurentii* and *S. commune,* organisms that seemed unaffected by papulacandin B *in vivo.* Finally, the enzyme from *W. dermatitidis,* an organism that was only moderately affected by the antibiotic *in vivo,* was the most strongly inhibited (Fig. 2).

Additional studies on the kinetics of the inhibition uncovered a behavior that is shared by the enzymes from each of the several organisms, and is illustrated for *S. cerevisiae* in Fig. 3. As the concentration of substrate (UDP-glucose) was decreased, papulacandin B became a less efficient inhibitor and, at very low concentrations of substrate, it even stimulated the activity. Similar results were obtained whether ATP or GTP was used as a stimulator (Fig. 3). The enzymes from *W. dermatitidis* and *H. anomala* behaved similarly to that from *S. cerevisiae.* Those of *C. laurentii* and *N. crassa* also showed decreased inhibition at lower substrate concentration, but no stimulation of the activity by papulacandin B was

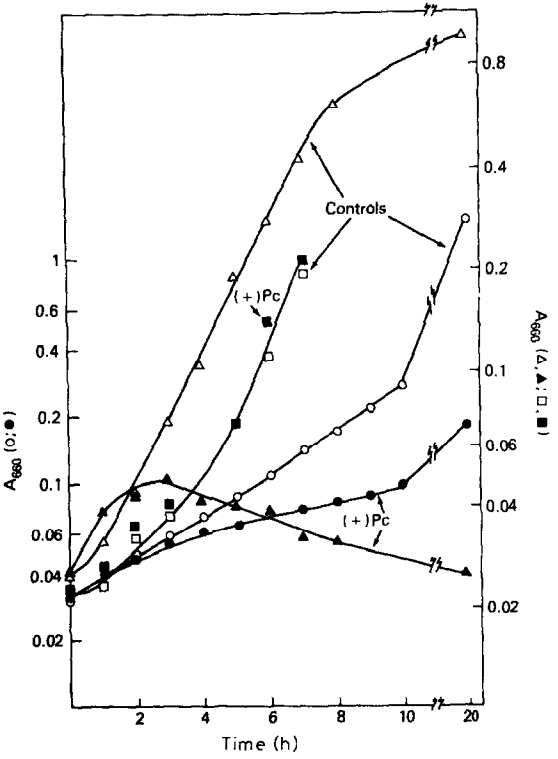

Fig. 1. Effect of papulacandin B, at 10 μg/mL concentration, on growth of *S. cerevisiae*, *W. dermatitidis*, and *C. laurentii*: Pc, papulacandin B; ○, ● *W. dermatitidis*; △, ▲ *S. cerevisiae*; and □, ■ *C. laurentii*.

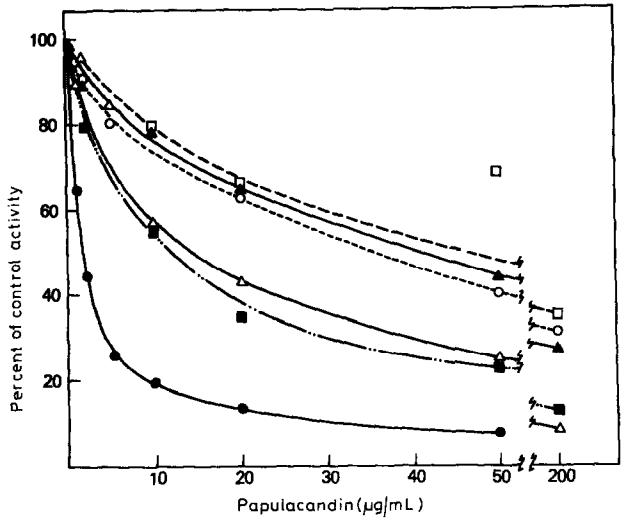

Fig. 2. Inhibition of (1→3)-β-D-glucan synthetase of various fungi by papulacandin B: -□--□- *S. cerevisae*, —▲—▲— *N. crassa*, --○--○-- *H. anomala*, —△—△— *S. commune*, ···■···■· *C. laurentii*, and —●—●— *W. dermatitidis*. The concentration of UDP-glucose was 5mM.

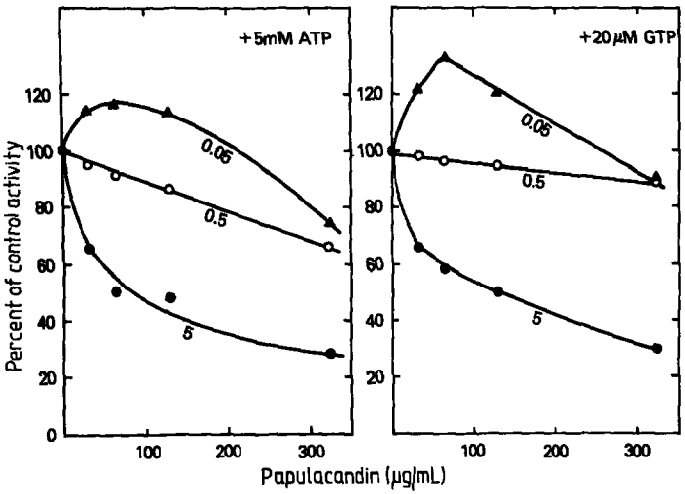

Fig. 3. Effect of papulacandin B on (1→3)-β-D-glucan synthetase of *S. cerevisiae*, at various substrate concentrations and in the presence of ATP or GTP. Concentration of UDP-glucose: ▲, 0.05; ○, 0.5; and ●, 5mM.

detected. With the preparation from *S. commune*, there was very little change in the inhibition.

Kinetics of papulacandin B inhibition with (1→3)-β-D-glucan synthetase from W. dermatitidis. — Further studies were carried out with the enzyme from *W. dermatitidis*, because it was the most sensitive to inhibition by the antibiotic (Fig. 2) while still showing a very clear stimulation at low UDP-glucose concentrations (Fig. 4). Inhibition was observed at the shortest time intervals tested and did not vary appreciably during incubation (not shown). Within the range of substrate concentrations in which papulacandin B was inhibitory, the inhibition appeared to

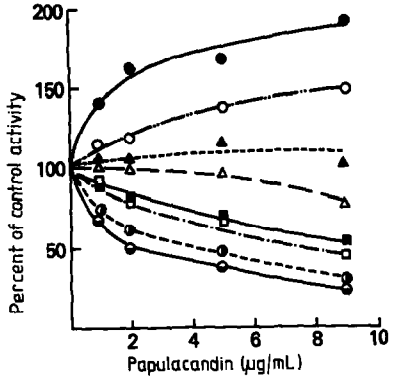

Fig. 4. Effect of papulacandin B on (1→3)-β-D-glucan synthetase of *W. dermatitidis* at various concentrations of UDP-glucose: ●, 0.0125; ○, 0.025; ▲, 0.05; △, 0.1; ■, 0.25; □, 0.5; ◑, 1.0; and ⊖, 5.0mM. All reaction mixtures contained 20μM GTP.

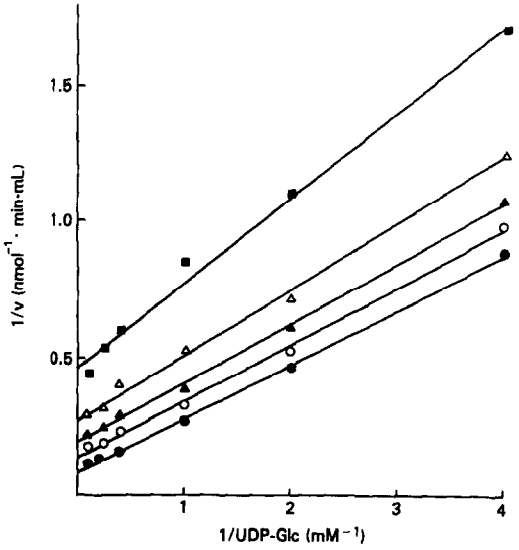

Fig. 5. Inverse plot of reaction rate *vs.* substrate concentration at various concentrations of papulacandin B for the (1→3)-β-D-glucan synthetase of *W. dermatitidis*. The concentration of antibiotic, in μg/mL, was: 0 (—●—●—), 1 (—○—○—), 2 (—▲—▲—), 5 (—△—△—), and 10 (—■—■—).

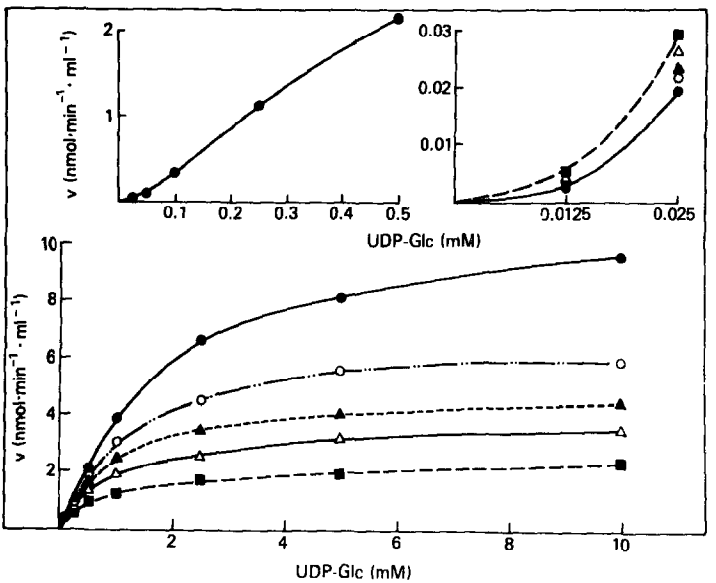

Fig. 6. Effect of UDP-glucose concentration on the reaction rate of (1→3)-β-D-glucan synthetase of *W. dermatitidis* at various levels of papulacandin B. The concentration of added antibiotic was: 0 (—●—●—), 1 (···○···○···), 2 (--▲--▲--), 5 (—△—△—), and 10 μg/mL (--■--■--). Inset at *upper left* shows the sigmoidal shape of the control curve at low concentration of substrate with an expanded scale. Inset at *upper right*, with an even larger scale, shows that higher activity is obtained in the presence of papulacandin B at very low substrate concentrations. The symbols in the insets are the same as in the main graph.

be of the mixed type, although very close to noncompetitive (Fig. 5). A similar type of inhibition was reported by Perez *et al.*[4] for the glucan synthetase of *Geotrichum lactis*.

In order to gain some insight into the effect of papulacandin B at low substrate levels, the activity of glucan synthetase was carefully determined over a large range of UDP-glucose concentrations in the absence and in the presence of the antibiotic (Fig. 6). It was observed that at a concentration of UDP-glucose <0.1mM, the reaction rate showed a sigmoid dependence on substrate concentration (Fig. 6, inset at upper left). It is in this same range of low substrate levels that papulacandin B ceased to inhibit and became stimulatory (Fig. 4 and Fig. 6, inset at upper right). Similar results were obtained with the enzyme from *S. cerevisiae* (data not shown). A simple explanation of this behavior would be that UDP-glucose acts as an allosteric ligand, shifting the enzyme from one conformation to (at least) another one as its concentration increases. In such cases, inhibitors that bind preferentially to the conformation favored by high substrate concentrations may become stimulators at low substrate levels by shifting the allosteric equilibrium towards the active form[7]. If this hypothesis were correct, a broadening of the sigmoid part of the curve should coincide with an increased range in which the antibiotic would cease to be an inhibitor or become an activator.

In a search for conditions that would modify the sigmoid character of the rate *vs.* substrate curve, we found that changes in pH or omission of GTP were without effect (data not shown). Increasing the temperature from 15 to 37° resulted, however, in a gradual widening of the sigmoid portion of the curve (Fig. 7). In the figure, the curves were normalized by plotting the rate at each concentration as the percentage of the rate at 10mM UDP-glucose, which is in the saturation range. Measurement of the inhibition by papulacandin B at various temperatures and UDP-glucose concentrations yielded the expected result. At higher temperatures,

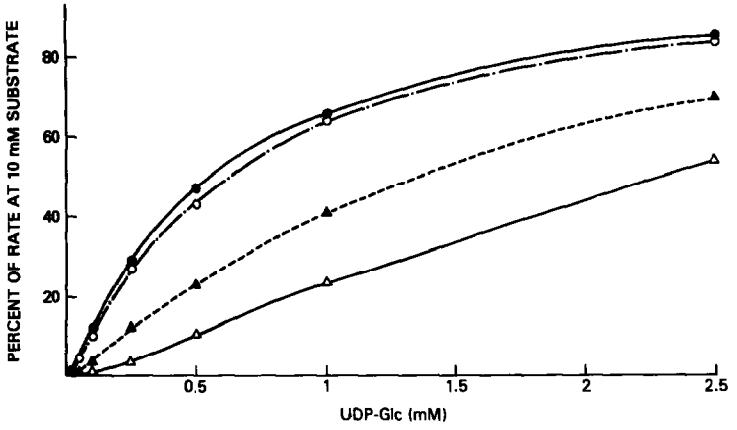

Fig. 7. Effect of temperature on the sigmoidicity of the rate *vs.* substrate curve for (1→3)-β-D-glucan synthetase of *W. dermatitidis*: 15 (—●—●—), 20 (·-O-·-O-·), 30 (--▲--▲--), and 37° (—Δ—Δ—).

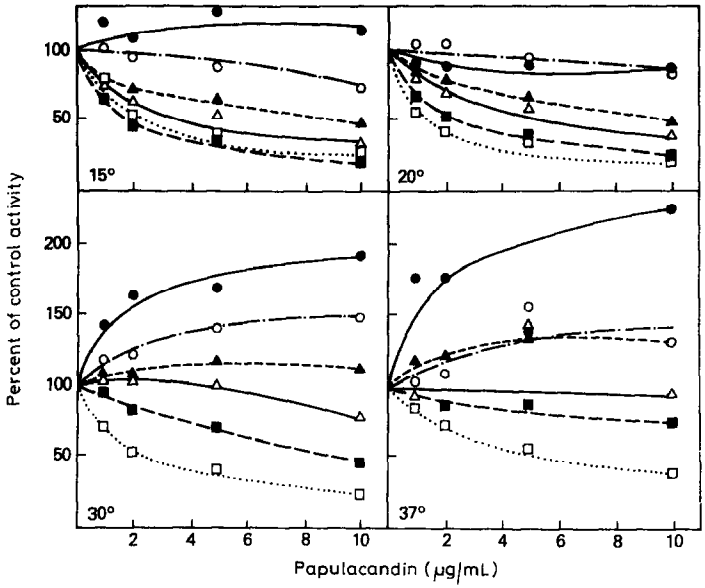

Fig. 8. Effects of papulacandin B on the activity of (1→3)-β-D-glucan synthetase of *W. dermatitidis* at various concentrations of UDP-glucose and at different temperatures: 0.0125 (—●—●—), 0.025 (··○··○··), 0.05 (--▲--▲--), 0.1 (—△—△—), 0.5 (--■--■--), and 5.0mM (···□···□···).

where the sigmoidicity of the curve was more pronounced, stimulation by the antibiotic was also greater and the range of substrate concentrations at which papulacandin B stimulated or did not inhibit reaction was more extended (Fig. 8).

These results are compatible with the existence of two or more interconvertible forms of the synthetase. Since the enzyme is membrane-bound[6,8], it is possible that the shift in equilibrium between the various forms observed with changes in temperature are caused by conformational changes in the membrane itself. Furthermore, there is no direct evidence that papulacandin B binds directly to the synthetase, inasmuch as the observed inhibition is close to the non-competitive type. It may well be that the antibiotic binds to another membrane component that interacts with the enzyme.

To what extent can our results provide an explanation for the lack of correlation between the effects of papulacandin B *in vivo* and *in vitro*? If membrane structure can influence the conformation of the enzyme, hence its susceptibility to papulacandin B, it would be necessary to work with membranes that have conserved the native state in order to mimic the conditions in the cell. In at least two aspects this is not possible; the broken membranes have lost the electrochemical potential existing in the intact cell and they are accessible to papulacandin B on both sides. A requirement for maintenance of the membrane potential in the biosynthesis of cell wall components have been reported for cellulose[9,10] and for teichoic acids[11].

We suggest that the general lack of consistency between results *in vivo* and *in*

vitro might result from variability in membrane structure in the various organisms, as well as in intact cells *vs.* broken cell preparations, that would affect the sensitivity of glucan synthetase to papulacandin B. To test this possibility, it would be desirable to conserve the original configuration of the membrane after cell disruption, *i.e.*, to prepare right-side out vesicles from the plasma membrane. It might be difficult, however, to obtain glucan synthesis under these conditions. If the process is vectorial, as for chitin synthetase[12], the substrate would have to be inside the vesicles.

There are of course other possible interpretations for the observed effects of papulacandin B *in vivo* and *in vitro*. It may be, for instance, that in addition to some inhibitory action on glucan synthetase, papulacandin B has a hitherto unknown effect on some other essential function of the fungal cell that explains its ability to inhibit growth in certain organisms. Variations in permeability to papulacandin B of the membranes of various organisms may also account in part for the differences observed *in vitro* and *in vivo*.

ACKNOWLEDGMENTS

The authors thank W. Wehrli of CIBA-Geigy for a gift of papulacandin B and W. B. Jakoby and P. McPhie for a critical reading of the manuscript. We are also indebted to the University of Texas University Research Institute and the National Institutes of Health for providing funds for Paul J. Szaniszlo to be a Visiting Research Scientist at the NIADDK.

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