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INVOLVEMENT OF SULFATIDE IN ACTIVATION OF PROTEIN KINASE C BY TUMOR PROMOTERS

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Summary: Sulfatide (cerebroside sulfate) activated protein kinase C to the same extent as phosphatidylserine did with the tumor promoters, 12-O-tetradecanoylphorbol-13-acetate (TPA), teleocidin and debromoaplysiatoxin. Sulfatide and phosphatidylserine both induced specific binding of [^{3}H]TPA to protein kinase C, although the ratios of specific to non-specific [^{3}H]TPA binding to protein kinase C with the two were not the same. It is concluded that sulfatide is involved in activation of protein kinase C by tumor promoters in a slightly different way from phosphatidylserine.

(-)-Indolactam-V, which is a biosynthetic intermediate of teleocidins A and B, is a weak tumor promoter, whereas (+)-indolactam-V and (±)-epi-indolactam-Vs are biologically and biochemically inactive (1-3). These differences are supposed to be because the stereospecificity of indolactam-Vs is recognized by a molecule in the cell membrane. Since sulfatide (cerebroside sulfate) has recently been reported to be involved in opiate binding (4) and ion transport (5), we thought that like phosphatidylserine, sulfatide might be involved in the binding of (-)-indolactam-V and other TPA-type tumor promoters to the phorbol ester receptor in the cell membrane. The phorbol ester receptor is known to be protein kinase C, which requires phosphatidylserine, tumor promoter and Ca^{++} for activation (6). Therefore, we tested whether sulfatide has the same effect as phosphatidylserine in activation of protein kinase C

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Abbreviations used: TPA, 12-O-tetradecanoylphorbol-13-acetate; sulfatide or cerebroside sulfate, ceramide ß-galactopyranose-3-sulfate.

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by the tumor promoters 12-O-tetradecanoylphorbol-13-acetate (TPA), teleocidin and debromoaplysiatoxin, and whether like phosphatidylserine, it induces specific binding of [^{3}H]TPA to protein kinase C. In this work we found that with tumor promoters sulfatide activated protein kinase C to the same extent as phosphatidylserine. However, with 1,2-diolein the activation of protein kinase C was not as great by sulfatide as by phosphatidylserine. The additional studies of the binding of [^{3}H]TPA to protein kinase C indicated that ratios of specific to non-specific [^{3}H]TPA binding to protein kinase C with sulfatide and phosphatidylserine were not the same. Therefore, the interaction of sulfatide with protein kinase C is slightly different from that of phosphatidylserine.

Materials and Methods

Chemicals: Teleocidin was isolated from mycelia of Streptomyces mediocidicus (7). The teleocidin used for experiments was a mixture of teleocidin A and teleocidin B (7). Debromoaplysiatoxin and aplysiatoxin were isolated from the blue-green alga Lyngbya majuscula, which was collected at Gushigawa, Okinawa, Japan (8). TPA was purchased from Consolidated Midland Corporation, Brewster, NY. Sulfatide, phosphatidylserine, sphingomyelin, cerebroside, ceramide and 1,2-diolein were obtained from Sigma Chemical Co., St. Louis, Mo. [20-^{3}H(N)]TPA (20 Ci/mmol) was purchased from New England Nuclear, Boston, MA and [γ-^{32}P] ATP (3000 Ci/mmol) from Amersham, U.K.

Purification of protein kinase C: Protein kinase C was purified from bovine brain by modifications of two procedures (9, 10) involving chromatographies on DEAE-cellulose, Octyl-Sepharose CL-4B and Ultrogel AcA 44. The specific activity of the partially purified enzyme was 22.5 units/mg protein/min.

Assay of protein kinase C: The assay mixture (250 µl) contained 20 µM $CaCl_2$, 7.5 µg each of sulfatide, phosphatidylserine, ceramide, sphingomyelin or cerebroside, and various concentrations of tumor promoters or 1,2-diolein with 0.05 unit of purified enzyme. Enzyme activity was assayed by measuring the incorporation of ^{32}P into histone H1 from [γ-^{32}P]ATP during incubation for 3 min at 30 °C.

[^{3}H]TPA binding to partially purified protein kinase C: [^{3}H]TPA binding was assayed by the cold acetone-filter method (11). The enzyme (82 µg) and 30 µg of sulfatide or phosphatidylserine were incubated with the various concentrations of [^{3}H]TPA in 1 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 2 mM 2-mercaptoethanol and 25 uM $CaCl_2$ at 0° C for 2 h. Non-specific binding was measured in the presence of 500-fold excess of unlabeled TPA.

Protein-determination: Protein was determined by the method of Bradford (12) with bovine serum albumin as a standard.

Results and Discussion

Previously we reported that three TPA-type tumor promoters, TPA, teleocidin and debromoaplysiatoxin, activated protein kinase C isolated from rat brain (13). These tumor promoters are all supposed to form a quaternary complex with protein kinase C, Ca^{++} and phosphatidylserine (6). We investigated whether sulfatide could replace phosphatidylserine in activation of protein kinase C in the presence of tumor promoters. As Table 1 shows, additions of 7.5 ug of sulfatide and phosphatidylserine resulted in similar strong activations of protein kinase C with TPA, teleocidin and debromoaplysiatoxin. Moreover, with either sulfatide or phosphatidylserine, TPA activated protein kinase C at a concentration of one tenth of those of teleocidin and debromoaplysiatoxin.

Since with tumor promoters sulfatide, like phosphatidylserine, activated protein kinase C, we examined whether several other sphingolipids could also replace sulfatide in activation of protein kinase C with various concentrations of teleocidin. Fig. 1a shows that additions of 7.5 ug of sulfatide and phosphatidylserine both resulted in activation of protein kinase C with various concentrations of teleocidin, but that the same amount of cerebroside, sphingomyelin or ceramide did not cause any activation of protein kinase C with teleocidin at concentrations of up to 100 ng/ml. These results indicate that the $-OSO_3$ group of sulfatide is necessary for activation of protein kinase C.

Table 1. Activation of protein kinase C with tumor promoters

Tumor promoter	Concentration ug/ml	Protein kinase activity (cpm x 10^{-3}) in the presence of sulfatide	phosphatidylserine
TPA	0.1	9.9	10.2
Teleocidin	0.1	5.6	6.5
	1	11.8	11.6
Debromoaply-siatoxin	0.1	1.9	3.1
	1	9.6	8.0

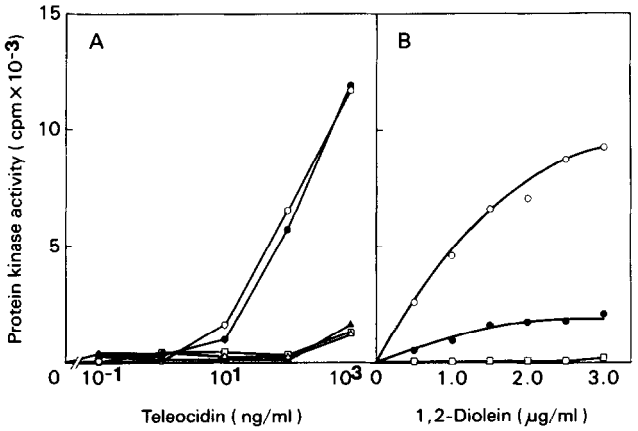

Fig. 1. Dose-dependent activations of protein kinase C by teleocidin (A) and 1,2-diolein (B). Assays were carried out in the presence of sulfatide (●—●), phosphatidylserine (○—○), cerebroside (△—△), sphingomyelin (□—□) and ceramide (▲—▲).

Protein kinase C was reported to be activated by a synthetic diacylglycerol, 1,2-diolein (14). Fig. 1b shows the activation of protein kinase C by various concentrations of 1,2-diolein in the presence of 7.5 ug of phosphatidylserine. Lower activation of protein kinase C was observed with sulfatide than with phosphatidylserine at the same concentration (Fig. 1b). Sphingomyelin had no activating effect. Thus, although both sulfatide and phosphatidylserine activated protein kinase C to the same extent in the presence of tumor promoters, their activations were different in the presence of 1,2-diolein.

Since protein kinase C serves as a phorbol ester receptor in the cell membrane (6), we measured specific [^{3}H]TPA binding to partially purified protein kinase C in the presence of sulfatide or phosphatidylserine by the cold acetone-filter method. The results in Fig. 2 show that total [^{3}H]TPA binding was the same with sulfatide as with phosphatidylserine. However, these compounds induced different ratios of specific to non-specific [^{3}H]TPA binding to protein kinase C: specific [^{3}H]TPA binding as a percentage of total binding was 53.9% with sulfatide and 93.6% with phosphatidylserine with 10 nM [^{3}H]TPA. Therefore, sulfatide caused very high non-specific [^{3}H]TPA binding. It

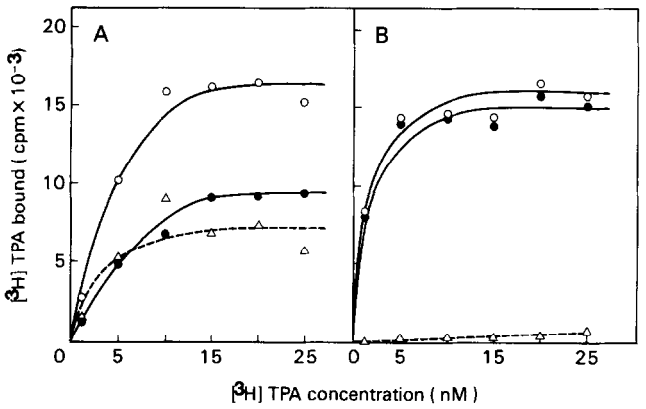

Fig. 2. Binding of [^{3}H]TPA to 82 ug of partially purified protein kinase C at various concentrations of [^{3}H]TPA. Binding was carried out with sulfatide (A) and phosphatidylserine (B). Total binding (o—— o), specific binding (●—— ●) and non-specific binding (Δ---Δ).

is interesting that the dose-response curve of specific [^{3}H]TPA binding to protein kinase C with phosphatidylserine seems to be similar to that of specific [^{3}H]TPA binding to a mouse particulate fraction. These results indicated that the binding of sulfatide to protein kinase C is different from that of phosphatidyserine in the presence of a tumor promoter, although sulfatide and phosphatidylserine are involved in a similar way in activation of protein kinase C with tumor promoters. Further studies are required on how the stereospecificity of tumor promoters can be identified.

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