

Notes

AN INHIBITOR FOR INOSITOL-
SPECIFIC PHOSPHOLIPASE C
FROM *Actinomadura* sp.

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(Received for publication February 13, 1992)

Inositol-specific phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate inositol 1,4,5-trisphosphate and diacylglycerol. These two compounds, in turn, serve as intracellular second messengers¹⁾. Inositol-specific phospholipase C, therefore, plays an important role in the signal transduction pathway of various systems. Thus, an inhibitor of phospholipase C would be a possible candidate for cancer chemotherapy and also a hopeful reagent to explore the mechanism of the signal transduction in these systems. In this line of screening program, we isolated Q12713 substance from fermentation broth of *Actinomadura* sp. This report describes the isolation of Q12713 substance from *Actinomadura* sp., which is an inhibitor of inositol-specific phospholipase C.

The microorganism producing the inhibitor was isolated from a soil sample. Based on the cultural and physiological properties, strain Q12713 was identified as *Actinomadura* sp.

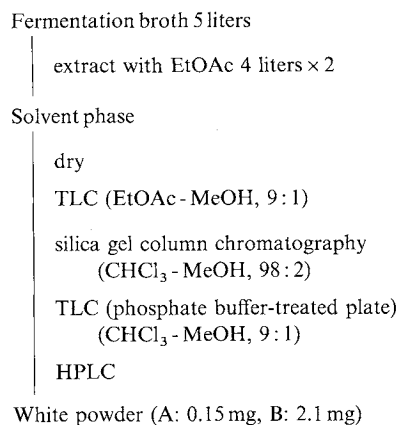
Phospholipase C activity of bovine brain was determined in a final volume of 50 μ l containing [inositol-2-³H(N)]phosphatidylinositol 4,5-bisphosphate (0.2 μ Ci/ml, 38 nM), 0.8 mg/ml phosphatidylcholine, 0.1 M HEPES-NaOH pH 7.0, 1% sodium cholate, 1 mM MgCl₂, 1 mM CaCl₂, 0.1% bovine serum albumin, 1 mM dithiothreitol, 50 mM KCl, partially purified bovine brain phospholipase C and the inhibitor. After 15 minutes at 37°C, the

reaction was terminated by the addition of 200 μ l of 10% trichloroacetic acid and kept at 4°C for 30 minutes. Then the reaction mixture was centrifuged for 5 minutes at 16,000 rpm and the radioactivity in the supernatant was measured by a liquid scintillation counter. The inhibitory activity was calculated from the remaining phospholipase C activity as follows: $(a-b)/(a-c) \times 100$, here 'a' is the radioactivity of a sample without an inhibitor; 'b' is the radioactivity of a sample with an inhibitor; and 'c' is the radioactivity of a sample without the enzyme.

A loopful of cells of the strain Q12713 from an agar slant was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a medium composed of potato starch 1%, Polypepton 1%, roast wheat germ 0.5%, copra meal 1.0%, carrot meal 0.5% and CaCO₃ 0.15%. The pH was adjusted to 7.0 before sterilization. The flasks were incubated in a rotary shaker at 28°C for 120 hours at 220 rpm.

The purification procedure outlined in Fig. 1 yielded A and B substances as inositol-specific phospholipase C inhibitors. Whole fermentation broth (5 liters) was extracted with 4 liters of ethyl acetate twice, and the solvent phases were combined and evaporated to dryness under reduced pressure. The solid thus obtained was dissolved in ethyl acetate and purified by preparative thin-layer chromatography on silica gel plates followed by silica gel column chromatography and preparative thin-layer chromatography on 0.5 M NaH₂PO₄-treated silica gel plates. Then, the active fraction was

Fig. 1. Purification procedure of Q12713 substance.



dissolved in methanol and the solution was applied to a 20 mm × 250 mm Inertsil prepODS column and developed with 80% acetonitrile. A and B substances were obtained at retention times of 9.968 and 12.405 minutes, respectively. The melting point of B substance was 152 to 156°C and $[\alpha]_D^{20}$ was -49.7° (*c* 0.1, methanol). RYDON-SMITH reaction was positive but ninhydrin, anthrone and SAKAGUCHI reactions were negative. When the isolated B substance was injected to a column of HPLC, B as well as A substances were detected in the eluants. On the other hand, when the isolated A substance was injected to a column of HPLC, A as well as B substances were eluted. It is concluded, therefore, that A substance was in an equilibrium with B substance. The ^3H and ^{13}C NMR spectra, IR spectrum and the mass spectrum of B substance indicated that they were very similar to those of MH755-CF2²⁾ and BU-3983T³⁾. The direct comparison with these substances by HPLC confirmed that these three compounds were identical. These two compounds were isolated as cytotoxic substances against tumor cells.

Q12713 substance showed IC_{50} for inositol-specific phospholipase C from bovine brain at 8.5×10^{-6} M, and K_i of 2.6×10^{-5} M in a competitive manner with the substrate. However, it showed no inhibitory activity against phospholipase C from *Bacillus cereus*. This enzyme is rather specific for lecithin. Only one compound named vinaxanthone was isolated as a phospholipase C inhibitor so far⁴⁾. However, detailed report on its activity has not been published. Therefore, Q12713 substance should be

a useful reagent for the analysis of the mechanism of signal transduction and might be a helpful drug for cancer and other diseases in which the signal transduction works.

Acknowledgment

We are grateful to T. TAKEUCHI of the Institute of Microbial Chemistry and T. OKI of the Bristol-Myers Squibb Co., Ltd. for their kind gifts of MH755-CF2 and BU-3983T, respectively.

This work is supported in part by the Grant-in-Aid for cancer research from the Ministry of Education, Science and Culture, Japan.

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