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Biochemical characterization and inhibitory effects of dinophysistoxin-okadaic acid and microcystine l-r on protein phosphatase 2a purified fı the mussel *Mytilus chilensis*.

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ABSTRACT

Protein phosphatases are involved in many cellular processes. One of the most abundant and best studied members of this cl phosphatase type-2A (PP2A). In this study, PP2A was purified from the mussel *Mytilus chilensis*. Using both SDS-PAGE and size filtration under denaturant conditions, it was confirmed that the PP2A fraction was essentially pure. The isolated enzyme is a hetero molecular estimated masses of the subunits are 62 and 28 kDa. The isolated PP2A fraction has a notably high *p*-NPP phosphatase acti inhibited by NaCl. The hydrolytic *p*-NPP phosphatase activity is independent of the $MgCl_2$ concentration. The time courses of the inl PP2A activity of *p*-NPP hydrolysis by increasing concentrations of three phycotoxins that are specific inhibitors of PP2A are show caused by Okadaic acid, dinophysistoxin-1 (DTX1, 35-methylokadiac acid) and Microcystine L-R are dose-dependent with inhibi (K_i) of 1.68, 0.40 and 0.27 nM respectively. Microcystine L-R, the most potent phycotoxin inhibitor of PP2A isolated from *Mytilus* an IC_{50} = 0.25 ng/ml, showed the highest specific inhibition effect an the *p*-NPP hydrolisis. The calculated IC_{50} for DTX1 and OA w and 1.8 ng/ml respectively.

Key words: Chile, Diarrhetic Shellfish Poisoning (DSP), Mussel, *Mytilus chilensis*, Protein Phosphatase 2A, Microcystine L-R

INTRODUCTION

Inhibitors of protein serine/threonine phosphatases have proven extremely useful in understanding the role phosphorylation. Protein phosphatase inhibitors such as pyrophosphate (Cohen, et al. 1988a), b-glycerophosphate (Ci 1988), sodium fluoride (Anderson, et al. 1990), *p*-nitrophenyl phosphate (*p*-NPP) and sodium vanadate (Pelech, et al. 19 the phosphorylated state of proteins in cell extracts and prevent interference from protein phosphatases in protein kinase

Two specific heat-stable protein inhibitors I1 and I2 (Cohen, et al. 1988b) have been used to classify protein phosphata main groups (Ingebritsen and Cohen, 1983; Cohen and Cohen, 1989; Cohen, et al. 1988b): protein phosphatase 1 (P phosphatase 2A (PP2A), protein phosphatase 2B (PP2B) and protein phosphatase 2C (PP2C). Of these, PP1 and PP2A predominant forms of protein ser/thr phosphatases found in most mammalian cells (Cohen and Cohen, et al. 1989; C 1988b). Moreover, in rabbit skeletal muscle, three sub-classes of protein phosphatase 2A have been separated and i chromatography on DEAE-cellulose, termed $2A_0$, $2A_1$ and $2A_2$ (Ingebritsen and Cohen, 1983).

Since diarrhetic shellfish poisoning (DSP) was first described (Murata, et al. 1982; Yasumoto, et al. 1978; Yasumoto, et new generation of protein phosphatase inhibitors has been isolated from marine phytoplankton (Bialojan and Takai, 198 al. 1990; Haystead, et al. 1989; Holmes, 1990; Sasaki, et al. 1994; Takai, et al. 1987; Yasumoto, et al. 1980). Okadaic ac dinophysistoxin-1 (DTX1, 35-methylokadiac acid) are two of the principal toxins associated with this syndrom polyethers are produced by dinoflagellates, which are concentrated by shellfish during filter feeding (Yasumoto, Yasumoto and Murata, 1993).

In addition to these marine toxins, hepatotoxic cyclic peptides known as microcystins, such as Microcystin L-R (Carmi 1994; Falconer, 1993, 1996; Honkanen, et al. 1990; Sivonen, 1996), have been purified from freshwater cyanobacteria algae) and also exhibit strong inhibitory activity against both PP1 and PP2A enzymes (MacKintosh, et al. 1990; Yosh 1992).

Diarrhetic shellfish poisoning has been an endemic problem in southern Chile since 1970 (Lagos, 1998). Every spring ar harmful algal bloom of *Dinophysis sp*. produces the contamination of native shellfish with these toxins. Knowing t DTX1 are strong inhibitors of PP2A, it was interesting to look for and study the properties of PP2A isolated from the nat filter bivalve *Mytilus chilensis*, harvested from areas in which this phenomenon occurs frequently (Bialojan and Takai, 1

In the present study, we used the specific inhibitory effects of OA, DTX1 and Microcystin L-R to purify and characteriz phosphatase type 2A isolated from the mussel *Mytilus chilensis*, an endemic Chilean filter bivalve. The PP2A activity by taking advantage of the enzyme's ability to dephosphorylate a colorless substrate (*p*-nitrophenyl phosphate, *p*-NPI yellow product (*p*-nitrophenol, *p*-NP). This paper also shows the characteristic inhibitory effects of OA, DTX1 and Mic against PP2A under identical experimental conditions. This is the first description, purification and biochemical charac PP2A isolated from this filter bivalve. The time course of inhibition of PP2A *p*-NPP hydrolysis caused by DTX1 concentrations is shown; these data allowed the calculation of the IC_{50} and the inhibition constant (K_i) for DTX1 under conditions for the first time. The inhibition sensitivities of PP2A activity produced by the three phycotoxins are compar IC_{50} and the inhibition constant (K_i) determined in this study.

MATERIALS AND METHODS

All reagents were analytical grade or better, solvents were purchased from MERCK (Santiago, Chile) and salts and Mic from SIGMA Chemical Co. (St. Louis, MO, USA). Low molecular weight protein standards were purchased fron (Richmond, CA, USA). Okadaic acid and DTX1 were obtained from Research Biochemical International (Natick, MA conductivity of the solutions used to study the effect of ionic salts was measured by a Conductivity Meter (Radiometer, Denmark).

Purification of Protein Phosphatase 2A

The enzymes were isolated from non-toxic mussel (*Mytilus chilensis*) tissue, following the procedure described by (1984). Protein content was measured by Bradford (1976) (BIO-RAD, Richmond, CA, USA) with Bovine Serum Alb used as a calibration standard. The protein composition of the enzyme fraction was analyzed by SDS-PAGE (SDS-con polyacrylamide gels) according to Laemmli (1970) and using the BIO-RAD Mini-Protean II electrophoresis chaml standard markers used were low range SDS-PAGE molecular weight standards (BIO-RAD, Richmond, CA, USA). enzyme was immediately aliquoted and cooled in liquid nitrogen and stored at -20 ^{O}C until use. All steps were carried ou

Assay of phosphatase activities

The enzymatic assay was conducted according to Takai and Mieskes (1991), Simon and Vernoux (1994) and Tubaro, All assays were carried out at 22-24 ^{O}C in a final volume of 550 µl. The reaction mixtures contained 22 mM *p*-NI TRIS/HCl, 20 mM EDTA, 20 mM DTT, and 2 mM $MgCl_2$, pH 8.31. To assay p-NPP phosphatase activities, the started by adding an enzyme, and the initial rate of liberation of *p*-nitrophenol was measured by recording the change ir at 420 nm in a Beckman spectrophotometer model 25 with a pen recorder. The assay took advantage of PP2A dephosphorylate a colorless substrate (*p*-nitrophenyl phosphate, *p*-NPP) to a yellow product (*p*-nitrophenol, *p*-NP) in

medium (Takai and Mieskes, 1991). Each determination was performed twice. The reaction was stopped with 25 µl of n calculate the amount of *p*-NP produced in nmol an $E_{420} = 1.78 \times 10^4\ M^{-1} \times cm^{-1}$ was used.

To assure that all the *p*-NP produced came from PP2A activity, the enzymatic activities were tested for total inhibitic DTX1 and Microcystin L-R. The control percentage (0%) of inhibition, which corresponds to the absence of the inhibi the calculation of the total PP2A activity. The control percentage of 100% inhibition corresponds to the total inhibition of OA and/or Microcystin L-R. The inhibition of protein phosphatase activity by inhibitors was determined by adding t (phycotoxins) to the enzyme mixture 5 min prior to initiating the reaction with the addition of the substrate.

Practical Analysis of Dose-Inhibition Relationships

OA is a tightly-bound non-competitive inhibitor (or mixed inhibitor) whose K_i is extremely low (=30 pM) according t Mieskes (1991). In our experimental conditions $E_t / K_i > 0.01$, the Michaelis and Menten kinetic analysis is not valid, anc a general kinetic model is recommended (Henderson, 1972). According to this model, if V_0 and V_i are, respectivel steady state velocities of the reaction in the absence and presence of the inhibitor, and E_t and I_i are, respectively, the con the enzyme and inhibitors, then we have the following equations (Henderson, 1972):

$$I_t = K_i [(V_0 / V_i) - 1] \qquad (1)$$

and

$$I_b = E_t [1 - (V_i / V_0)] \qquad (2)$$

where K_i [= K_i (s)] is the apparent dissociation constant for the inhibitor, which in general is a function of t concentration S. That given, the conservation equation for the inhibitor is:

$$I_t = I_f + I_b \qquad (3)$$

where I_t is the total concentration of the inhibitor. The expression I_f is the free inhibitor concentration, and I_b is the enzy inhibitor concentration.

When $E_t / K_i = 0$ and is less than 10, I_f tends to I_t, and hence equation (1) becomes $V_i / V_0 = K_i / (K_i + I_t)$ (4)

This is a Hill function with a Hill coefficient of 1.0.

The concentration of the inhibitor required to obtain 50% inhibition corresponds to IC_{50}, and is given by equations (1), as the value of I_t at which $V_i/V_0 = 0.5$; i.e.

$$Ic_{50} = K_{i(s)} + (Et/2) \quad (5)$$

The value of K_i can be estimated using equation (4) and plotting the experimental data as shown in Figure 4. Note that E be as small as possible for the accurate estimation of K_i by this method.

Preparation of substrates and inhibitors

The *p*-Nitrophenyl Phosphate, *p*-NPP (Di(Tris) salt, from SIGMA Chemical Co. (St. Louis, MO) was dissolved in reac

just before use.

Two mg of OA or DTX1 were dissolved in 200 µl of ethanol and then diluted with an aqueous buffer to the final conc 1% ethanol. The maximal concentration of ethanol in reaction mixtures was 0.1% (V/V). Control activities were not affected by the addition of this amount of ethanol.

HPLC analysis of Phycotoxins

In order to quantify and calibrate the amount of OA and DTX1 measured by the inhibition activity of PP2A, these toxi measured by HPLC with fluorescence detection on-line. The pre-column derivatization method was used as described b (1987), with a slight modification of Pereira, et al. (1995) in the extraction procedure was used. Analogously, the Microcystin L-R that inhibits PP2A activity was measured and confirmed using the HPLC methods described by Wat (1988).

Determination of M_r subunits of PP2A by gel filtration chromatography

The M_r of the subunits of the native enzyme were determined by HPLC gel filtration on a RoGel SEC column (250 x size 17 nm, particle size 5 mm) (BIO-RAD, Richmond, CA, USA), equilibrated with 25 mM NaCl, 4 mM 2-mercaptoetl SDS, 10% glycerol, 20 mM Bis-Tris buffer (pH 6.2). The enzymatic fraction was diluted in 200 µl of equilibrium bu 10% SDS and 20 µl of 2-mercaptoethanol. The mixture was sonicated for 30 sec and then injected into the column. T was 1.0 ml/min. The protein standards used were carbonic anhydrase and BSA with M_r values of 31,000 and respectively.

RESULTS AND DISCUSSION

Purification and biochemical characterization of PP2A

According to the SDS-PAGE analysis of the PP2A fraction purified from the filter bivalve *Mytilus chilensis* (Fig. 1A protein bands with intense Coomasie blue staining were observed. The apparent molecular masses of the subunits estima SDS-polyacrylamide gels (Fig. 1A) were approximately 62 kDa and a 28 kDa, both calculated from the apparent mole protein standards shown in Figure 1A, line 1. All the results in the present study were obtained using this isolated fracti referred to as the PP2A fraction.

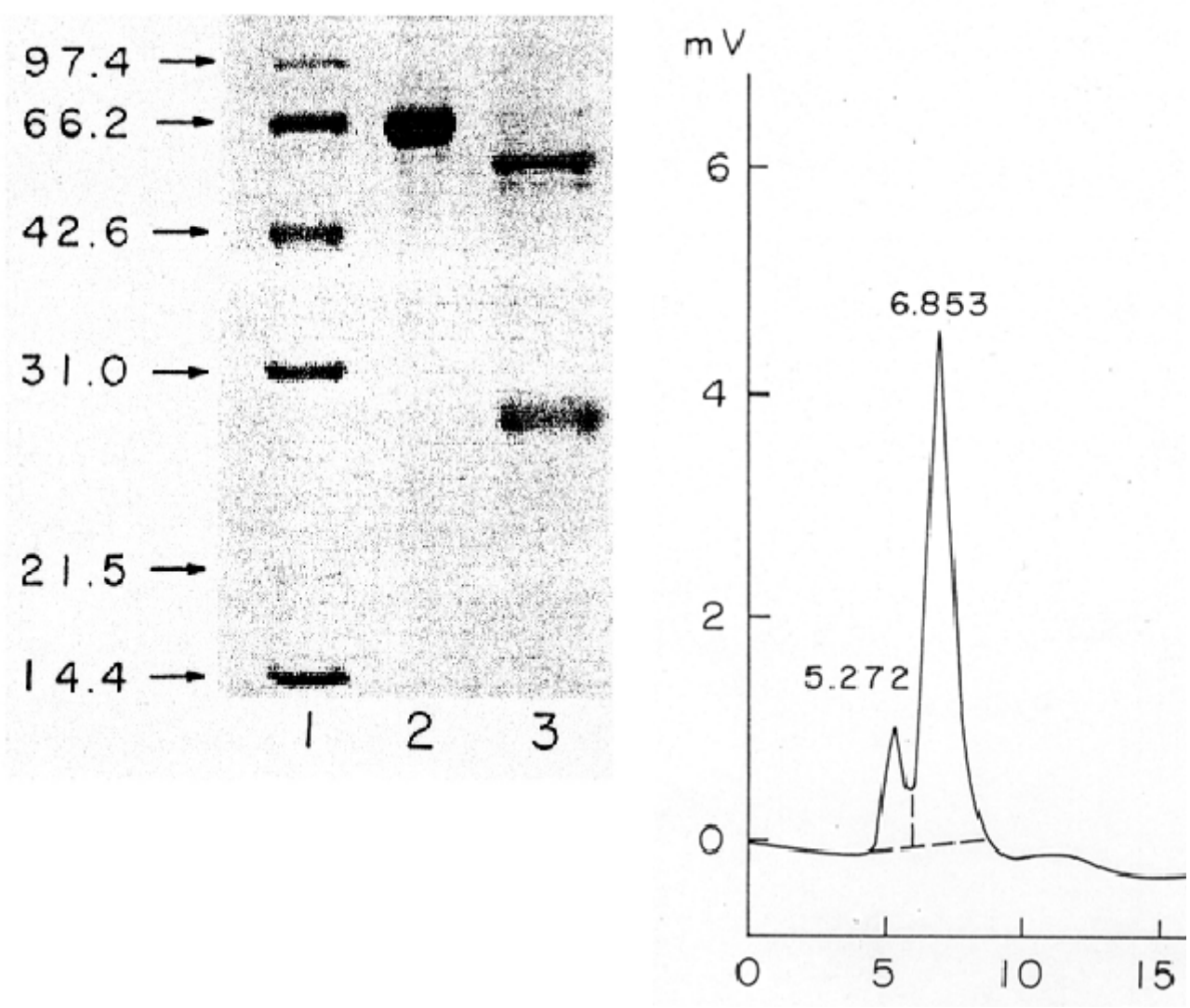


Figure 1. A. *12% SDS-polyacrylamide gel electrophoresis of PP2A fraction*. The gel was stained with Coomassie blue. Migration bottom. *Line 1*. Standard protein markers, phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.6 k anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). *Line 2*. Bovine serum albumin (66.2 kDa). fraction isolated according to the method described in Materials and Methods.
B. *Chromatogram of PP2A fraction analyzed by size exclusion gel filtration in high performance liquid chromatography und conditions.* PP2A fraction was injected on a gel filtration RoGel SEC column (250 x 7 mm, pore size 17 nm, particle size 5 mm), a signal was follow by absorbance to 280 nm. The pick of R_t = 6.853 min co-migrated with the molecular weight marker carbonic ar kDa). The pick corresponding to R_t = 5.272 min showed a retention time similar to the standard BSA (66.2 kDa).

When this PP2A fraction was analyzed by size exclusion gel filtration in high performance liquid chromatography (I denaturant conditions, two peaks were also seen in the chromatogram. The largest had the highest absorbance 280 nm a retention time R_t at 6.853 min (Fig. 1B). This peak co-migrated with the molecular weight marker carbonic anhydrase (agreed well with the low molecular weight subunit of PP2A, termed subunit C and corresponding to the catalytic subunit and Cohen, 1983). The peak corresponding to the lower R_t at 5.272 min showed a retention time similar to that of the s (66.2 kDa, data not shown) and correlated well with the subunit PP2A of approximately 60 kDa, termed subunit A and the regulatory one (Ingebritsen and Cohen, 1983; Kamibayashi, et al. 1992). The M_r subunits obtained using the size e filtration compare well with the values determined on 12% SDS-containing gels. Both values are in close agreemer

reported for PP2A from other species (Ingebritsen and Cohen, 1983; Tung, et al. 1984).

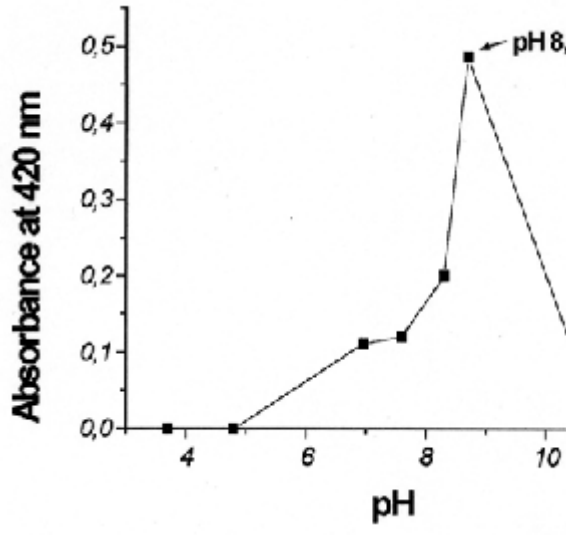
In our experiment using size exclusion gel filtration under denatured conditions, the PP2A fraction showed that the e dimer, as was initially resolved by SDS-PAGE (Fig. 1A, line 3). We thus concluded that the PP2A enzyme isolated *chilensis* tissue was a heterodimer, and both methodologies confirmed that the PP2A fraction was essentially pure.

Comparing the SDS-PAGE profile of the protein phosphatase 2A isolated in this study with species isolated from r muscle by Ingebritsen, et al. (1983), a profile similar to the $PP2A_2$ species was found; both exhibit only two Coomassi and with similar migration patterns (Fig. 1A; Ingebritsen, et al. 1983).

The average specific activity of the purified PP2A fraction from five different preparations was 6.42 ± 0.8 nmol of *p*-N min^{-1} per mg of protein. This PP2A fraction has a notably high *p*-NPP phosphatase activity, and its value is very clo described in rabbit skeletal muscle, 11.7 ± 1.3 nmol of Pi produced x min^{-1} per mg of protein (Takai and Mieskes, 1991)

Figure 2A shows the effects of increasing the concentration of NaCl. PP2A exhibited great sensitivity to NaCl. As is sho 2A, a dramatic decrease in the PP2A activity was observed. At 100 mM NaCl (solution conductivity, 4.5 mMHO) dropped below 40% of the initial total activity. We considered this inhibition effect on the *p*-NPP phosphatase activity the NaCl concentration itself more than to the ionic strength, because a similar experiment done in the presence of incre concentrations (ranging from 5 to 25 mM) showed no variation in the *p*-NPP phosphatase activity, given a constant acti 5.72 ± 0.4, n=5, nmol of *p*-NP produced x min^{-1} per mg of protein for all the $MgCl_2$ concentrations assayed. The demonstrate that the *p*-NPP phosphatase activity is independent of the $MgCl_2$ concentration and moreover, that the inhil by high NaCl was not related to the ionic strength, as the highest $MgCl_2$ concentrations tested (25 mM) disp conductivity 5.2 mMHO than the NaCl incubation mixtures. The three species of protein phosphatases isolated from r muscle, termed A_0, A_1 and A_2 do not require divalent cations (Cohen, et al. 1989; Honkanen, et al. 1990).

Figure 2B shows the pH-dependence of the *p*-NPP phosphatase activity of the PP2A fraction. This pH-dependence profile reaching its optimal pH value at 8.7, then dropping abruptly to nearly zero activity at pH 10.7. The optimal pH activity of the PP2A fraction agreed very well with the optimal pH range (8.0 - 8.5) described for the catalytic subun protein phosphatase prepared from rabbit skeletal muscle using *p*-NPP as the substrate (Takai and Mieskes, 19 cyanobacterial phosphoprotein phosphatase family protein phosphatases using [^{32}P] phosphoseryl casein as the substra 1999).



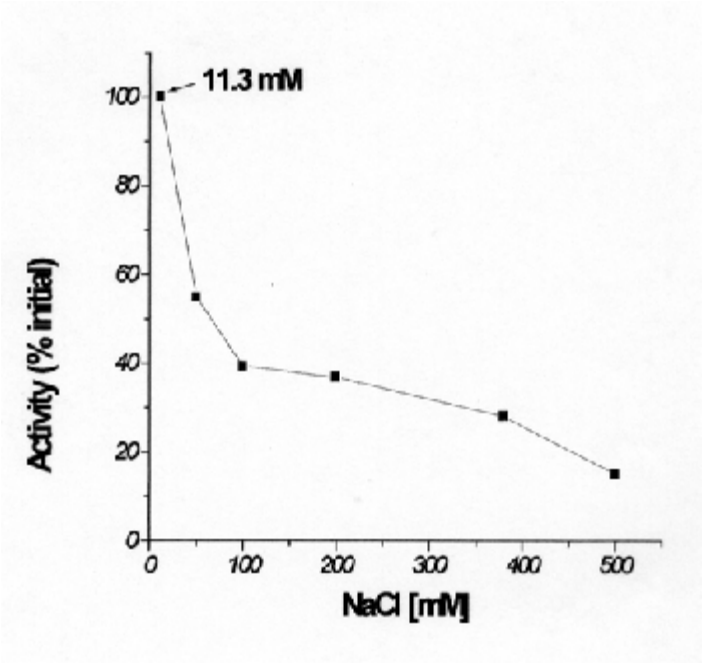


Figure 2. A. *Effect of NaCl concentrations in the PP2A activity of p-NPP hydrolysis*. PP2A fractions were assayed as described in Methods using increasing concentrations of NaCl ranging from 11.3 to 500 mM.
B. *pH-dependence of the p-NPP phosphatase activity of the PP2A*. PP2A fractions were assayed as described in Material and Metho pH and then incubated in the same buffer. For pH 3.7 and 4.8 the buffer acetate-acetic acid was used; for the pH ranging from 6.9 buffer TRIS-HCl was used.

Inhibition studies on the p-NPP phosphatase activity of PP2A phosphatase by OA, DTX1 and Microcystine L-R

The time course of the purified PP2A activity on *p*-NPP hydrolysis was studied by recording the kinetic of absorbanc 420 nm. As shown in Figure 3A, the hydrolysis of *p*-NPP increases linearly during the first 5 min of incubation, and afte reaction slows progressively, reaching a plateau at approximately 35 min (control, without presence of inhibitors, Fig. presence of increasing concentrations of OA, the absorbance was dose-dependent, showing inhibition with respect to co OA inhibits the PP2A activity, purified from *Mytilus chilensis*, in a dose-dependent manner at concentrations ranging 2.00 ng/ml. The concentration of OA required to inhibit the PP2A activity by 50% (IC_{50}) was 1.8 ng/ml or 2.20 nM. Sir commercially-available PP2A isolated from human red blood cells, OA showed a IC_{50} = 0.32 nM (Tubaro, et al. 1996).

Although in European DSP episodes, OA is the most significant and frequent toxin involved, the major DSP toxin found samples from Chile, Japan and Canada is DTX1 (Hallegraeff, 1993; Quilliam and Wright, 1995). Moreover, from diarrhetic marine toxin contents in natural samples collected in southern Chile, DTX1 proved to be ten times higher thai et al. 1993).

In order to study the effects of DTX1 over the *p*-NPP phosphatase activity of the PP2A fraction, the time course of inh NPP hydrolysis caused by DTX1 is shown in Figure 3B. Similarly to OA, its analogue (35-methylokadaic acid) also sh dependent inhibition at concentrations ranging from 0.04 to 1.20 ng/ml. The PP2A activity showed a high sensi inhibition effect caused by DTX1. The DTX1 concentration required to inhibit the PP2A activity by 50% (IC_{50}) was 0 0.92 nM. To the best of our knowledge, no data on the inhibition time-course caused by DTX1 over PP2A activit published elsewhere. Therefore these are the first data published that permit the calculation of the IC_{50} and the inhibit (K_i) for DTX1 under these assay conditions. The DTX1 inhibition constant was 0.40 ± 0.079 nM (Fig. 4).

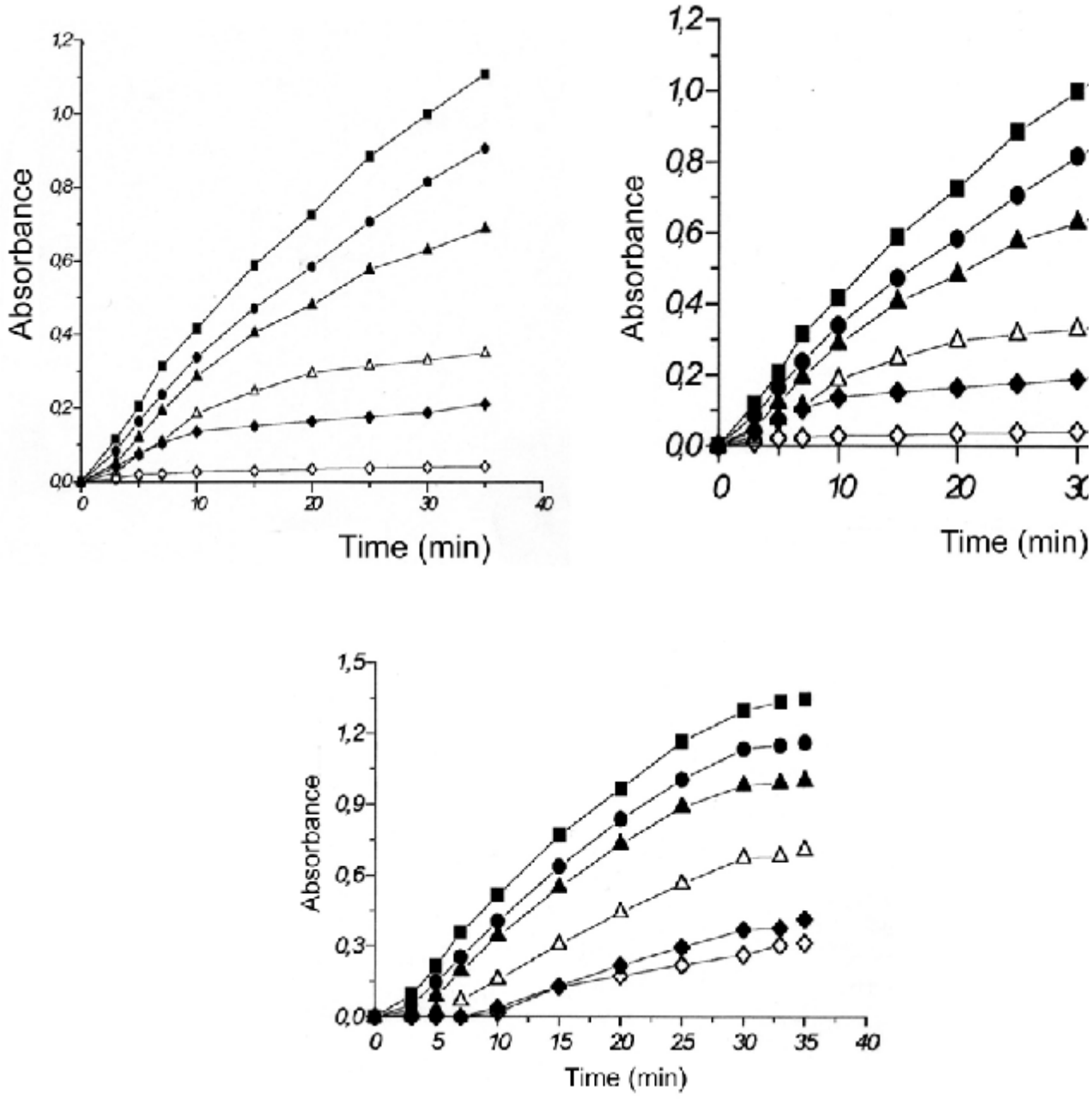


Figure 3. *Time-course of the enzymatic reaction of p-NPP hydrolysis in the presence of different concentrations of phycotoxi inhibitors of PP2A activity.* Assays were conducted as described in Materials and Methods. Enzyme fractions were mixed with the min prior to the addition of substrate.

A. *In presence of Okadaic acid (OA)*. Filled squares, control curve, without OA. Filled circles, 0.075 ng/ml of OA. Filled triangles, OA. Empty triangles, 0.5 ng/ml of OA. Filled diamonds, 1 ng/ml of OA. Empty diamonds, 2 ng/ml of OA.

B. *In presence of DTX1*. Filled squares, control curve, without DTX1. Filled circles, 0.04 ng/ml of DTX1. Filled triangles, 0.20 ng Empty triangles, 0.4 ng/ml of DTX1. Filled diamonds, 0.8 ng/ml of DTX1. Empty diamonds, 1.2 ng/ml of DTX1

C. *In presence of Microcystine L-R*. Filled squares, control curve, without Microcystine L-R. Filled circles, 0.075 ng/ml of Microcysti triangles, 0.15 ng/ml of Microcystine L-R. Empty triangles, 0.5 ng/ml of Microcystine L-R. Filled diamonds, 1.0 ng/ml of Microcystir diamonds, 2.0 ng/ml of Microcystine L-R.

Microcystins are cyclical peptides that are potent liver toxins. They are produced by several genera of cianobacteria algae) and have been detected in surface waters worldwide. Microcystine L-R, the most common member of the fami over 40 microcystin analogues already described and has also been shown to be a potent tumor promoter (Dawson, 199 1996; Sivonen, 1996). This phycotoxin is water soluble and has an entirely different chemical nature than the other tw marine toxins. It also displays strong inhibitory activity against both PP1 and PP2A (MacKintosh, et al. 1990; Yosh 1992). The time course of the purified PP2A activity on *p*-NPP hydrolysis in the presence of Microcystine L-R was stu 3C shows the effect of increasing concentrations of Microcystine L-R over the PP2A activity. The PP2A activity p *Mytilus chilensis* again showed a dose-dependent inhibition at Microcystine L-R concentrations ranging from 0.075 to The concentration of toxin required to inhibit the PP2A activity by 50% (IC_{50}) was 0.25 ng/ml or 0.25 nM. Of the three tested, Microcystine L-R was the most potent inhibitor of PP2A isolated from *Mytilus chilensis*, and showed the highe effect on the *p*-NPP hydrolysis (Fig. 4). The inhibition constants for the three phycotoxins were obtained from Figur values were 0.27, 0.40 and 1.68 nM for Microcystine L-R, DTX1 and OA respectively (Table I).

Table I

Parameters of the inhibition of PP2A by phycotoxins

	Ic_{50} nM	$Ki_{(S)}$* nM	E_T**/Ki
OA	2.20	1.68	0.62
DTX_1	0.92	0.40	2.60
Mcyst-LR	0.25	0.27	3.90

* 28.2 mM *p*-NPP.
** 1.04 nM purified enzyme.
We considered the reaction to have ocurred with a Hill coefficient of 1.0.

We observed an unusually high quantity of PP2A per gram of mussel tissue in *Mytilus chilensis*. Furthermore, knowing t harmful algal bloom of *Dinophysis sp*. produced the contamination of native shellfish with these toxins every spring and was of interest to measure the yield in mg of PP2A isolated per gram of the filter bivalve tissue harvested from areas in v phenomenon occurs frequently. Our intention was also to compare this yield with the protein phosphatases type-2A isola rabbit skeletal muscle. We normally began the enzymatic preparation with 8 grams of mussel tissue, obtaining an averag 0.3 mg (N = 12 different preparations) of PP2A fraction. This yield is remarkably high in comparison with the amounts c species of protein phosphatases $2A_0$, $2A_1$ and $2A_2$ purified to homogeneity from rabbit skeletal muscle, 1 mg of phospha and $2A_1$ and 0.5 mg of phosphatase $2A_2$ starting from 4000 grams of muscle tissue (Tung, et al. 1984). We can assume t PP2A fraction, which showed only two bands in SDS-PAGE (loaded with 15 ng of total protein), and the size exclusion method, which also showed two bands (50 mg of total protein were injected into the column), may have some contamina visible by either method. However, even if this contamination were to reach 25% of the total protein, it would still not be explain this amazing yield. We therefore hypothesize that these high amounts of protein phosphatase type 2A are more c related to the periodical harmful algal blooms of *Dinophysis sp*. that contaminate native shellfish with OA and DTX1 an specific PP2A inhibitors. We suggest that this unusual production of PP2A by the mussel *Mytilus chilensis* can be associ physiological response to the high accumulation of DSP toxins produced by the *Dinophysis sp*. filtrate during the bloom are inhibiting and/or sequestering the PP2A.

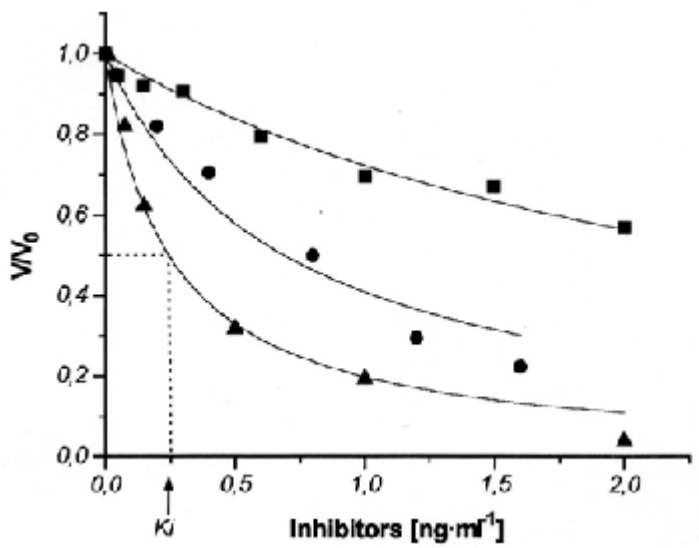


Figure 4. *Inhibition of PP2A activity isolated from* Mytilus chilensis *by phycotoxins*. Assays were carried out with 22 mM *p*-NPP a described in Materials and Methods. Filled squares, inhibition by Okadaic acid, filled circles, inhibition by DTX1 and filled triangles, Microcystine L-R. In the figure, at a value of $V_i / V_0 = 0.5$, the K_i for Microcistine L-R is obtained. The $K_{i(s)}$ for OA and DTX1 were similar way.

CONCLUSIONS

Studies done with SDS-polyacrylamide gels and the size exclusion gel filtration under denaturant conditions showed th fraction is a dimer and that the estimated molecular masses of the *Mytilus chilensis* PP2A subunits are 62 kDa (terme and 28 kDa (termed subunit C). Comparing the SDS-PAGE profiles of the three protein phosphatase 2A species isolate skeletal muscle with the one isolated in this research, the data suggest a close correspondence with the $PP2A_2$ s inhibition caused by OA, DTX1 and Microcystine L-R was dose-dependent, with inhibition constants (K_i) in the nM r for Microcystine L-R, DTX1 and OA were 0.27, 0.40 and 1.68 nM respectively. The PP2A fraction activity sh inhibition sensitivity to DTX1 than to OA. Microcystine L-R showed the highest inhibition effect on the PP2A activity *p*-NPP hydrolysis.

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