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## c-AMP dependent protein kinase A inhibitory activity of six algal extracts from South Eastern Australia and their fatty acid composition

### Abstract

c-AMP dependent protein kinase (protein kinase A, PKA) is an important enzyme involved in the regulation of an increasing number of physiological processes including immune function, cardiovascular disease, memory disorders and cancer. The objective of this study was to evaluate the PKA inhibitory activity of a range of algal extracts, along with their fatty acid composition. Six algal species were investigated including two Chlorophyta (*Codium dimorphum* and *Ulva lactuca*), two Phaeophyta (*Phyllospora comosa* and *Sargassum* sp.) and two Rhodophyta (*Prionitis linearis* and *Corallina vancouveriensis*), with the order of PKA inhibitory activity of their extracts identified as follows: brown seaweeds > red seaweeds > green seaweeds with the brown alga *Sargassum* sp. exhibiting the highest PKA inhibitory activity (84% at 100 microg/mL). GC/MS analysis identified a total of 18 fatty acids in the six algal extracts accounting for 72-87% of each extract, with hexadecanoic acid and 9,12-octadecadienoic acid as the dominant components. The most active extract (*Sargassum* sp.) also contained the highest percentage of the saturated C14:0 fatty acid (12.8% of the total extract), which is known to inhibit PKA. These results provide the first description of the PKA inhibitory activity of marine algae along with the first description of the fatty acid composition of these six algal species from South Eastern Australian waters. Importantly, this study reveals that abundant and readily available marine algae are a new and relatively unexplored source of PKA inhibitory compounds.

### Keywords

kinase, protein, dependent, fatty, amp, composition, c, their, acid, australia, eastern, south, extracts, algal, six, activity, inhibitory, CMMB

### Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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# c-AMP Dependent protein kinase A inhibitory activity of six algal extracts from South Eastern Australia and their fatty acid composition

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c-AMP Dependent protein kinase (protein kinase A, PKA) is an important enzyme involved in the regulation of an increasing number of physiological processes including immune function, cardiovascular disease, memory disorders and cancer. The objective of this study was to evaluate the PKA inhibitory activity of a range of algal extracts, along with their fatty acid composition. Six algal species were investigated including two Chlorophyta (*Codium dimorphum* and *Ulva lactuca*), two Phaeophyta (*Phyllospora comosa* and *Sargassum* sp.) and two Rhodophyta (*Prionitis linearis* and *Corallina vancouveriensis*), with the order of PKA inhibitory activity of their extracts identified as follows: brown seaweeds > red seaweeds > green seaweeds with the brown alga *Sargassum* sp. exhibiting the highest PKA inhibitory activity (84% at 100 µg/mL). GC/MS analysis identified a total of 18 fatty acids in the six algal extracts accounting for 72-87% of each extract, with hexadecanoic acid and 9,12-octadecadienoic acid as the dominant components. The most active extract (*Sargassum* sp.) also contained the highest percentage of the saturated C14:0 fatty acid (12.8% of the total extract), which is a known to inhibit PKA. These results provide the first description of the PKA inhibitory activity of marine algae along with the first description of the fatty acid composition of these six algal species from South Eastern Australian waters. Importantly, this study reveals that abundant and readily available marine algae are a new and relatively unexplored source of PKA inhibitory compounds.

**Keywords:** protein kinase A, enzyme inhibitors, algae, fatty acids.

Kinases play a key role in cell regulation and their deregulation is a hallmark of a number of diseases including cancer [1]. PKA has received increasing attention in recent years as it is found to play a vital role in a myriad of physiological processes [2], in particular immune function [3] and memory disorders such as Alzheimer's and Parkinson's disease [4], where it has become an attractive new drug target. Interestingly, there are as yet no PKA inhibitors reported from any marine sources.

Marine algae are well known as rich sources of bioactive natural products of diverse chemical structures and with a great range of biological activities [5]. The nutritional value of marine algae is also of importance as they have a high content of vitamins and minerals and are an important source of essential fatty acids (FAs) [6,7]. Marine algae contain a complex mixture of fatty acids of greater variety than those found in vascular plants [8] and are particularly rich in polyunsaturated fatty acids (PUFAs) [9]. Fatty acids are important for a wide array of cell structure components and for many biological, physiological and chemical processes in the body [7]. Biochemical analysis, in particular fatty acid composition, has been used for the taxonomic classification of

various algal species [6,9]. Marine algae are also rich in halogenated compounds, polyphenols and polysaccharides, which display a wide range of activities including anticoagulant, antiviral, antioxidative, anticancer and immunomodulating activities [10]. A number of promising anticancer compounds are derived from algae including stypoldione from the brown alga *Stypopodium zonale*, which inhibits polymerization of microtubules, and the cytotoxic dehydrothysiferol isolated from the red alga *Laurencia viridis*, active against a number of human breast cancer cell lines [11].

Given the impressive bioactivity profiles of algal metabolites, the aim of the present work was to screen a range of algal extracts for their ability to inhibit PKA, along with obtaining their fatty acid profiles. The green seaweeds *Codium dimorphum* and *Ulva lactuca*, the brown seaweeds *Phyllospora comosa* and *Sargassum* sp. and the red seaweeds *Prionitis linearis* and *Corallina vancouveriensis*, were collected from two locations along the New South Wales coast of Australia known to be 'biodiversity hotspots' [12]. The six algal species investigated were chosen due to their high abundance in the collection area, their accessibility and as these species had not been studied previously from South Eastern Australian waters.

Table 1. Algal species collected, class, collection place and % PKA inhibitory activity.

No	Algal species	Class	Collection Place	Latitude	Longitude	% PKA inhibition
1	<i>Codium dimorphum</i>	Chlorophyta	Belambi Point, NSW	34° 21' S	150° 56' E	28 ± 0.4
2	<i>Ulva lactuca</i>	Chlorophyta	Austinmer, NSW	34° 18' S	150° 56' E	18 ± 3.0
3	<i>Phyllospora comosa</i>	Phaeophyta	Belambi Point, NSW	34° 21' S	150° 56' E	55 ± 1.6
4	<i>Sargassum</i> sp.	Phaeophyta	Austinmer, NSW	34° 18' S	150° 56' E	84 ± 5.3
5	<i>Prionitis linearis</i>	Rhodophyta	Austinmer, NSW	34° 18' S	150° 56' E	37 ± 3.4
6	<i>Corallina vancouveriensis</i>	Rhodophyta	Austinmer, NSW	34° 18' S	150° 56' E	30 ± 1.1

† All samples were collected from the South Eastern region of New South Wales (NSW), Australia. All measurements were performed in triplicate at a final concentration of 100 µg/mL. Biological data are presented as the mean values ± S.E.M from triplicate measurements.

The highest PKA inhibitory activities were displayed by the dichloromethane extracts of the two brown algae *Sargassum* sp. and

*P. comosa* (with 84% and 55% inhibition, respectively). The two red algal species, *P. linearis* and *C. vancouveriensis*, displayed

similar activities of 38% and 30% respectively, while the two green algal dichloromethane extracts showed <30% activity. A small number of algal-derived kinase inhibitors have been described previously including the tyrosine kinase inhibitor stypoquinonic acid from the brown algae *Stypopodium zonale*; sulfated triterpenoids from the green algae *Tuomoya* sp. as tyrosine kinase Tie2 inhibitors; and cycloartanol derivatives from the green algae *Tydemania expeditionis* as inhibitors of pp60<sup>v-src</sup> [13]. However, this is the first report of marine algal extracts exhibiting PKA inhibitory activity and suggests that they may be an important new source of PKA inhibitors in the future.

A total of 18 different fatty acids were identified in the samples comprising six saturated and eleven unsaturated straight chain fatty acids and one branched fatty acid. The GC-MS analysis of the six algal extracts identified hexadecanoic acid and 9,12-octadecadienoic acid as the dominant fatty acids. Hexadecanoic acid was the main acid in five of the six samples (ranging from 23.3-32.1% of the total extract), whilst only in *C. dimorphum* the major acid was 9,12-octadecadienoic acid (22.4%), followed by hexadecanoic acid (20.6%). The percentages of these two fatty acids were significantly higher than the amount of the other compounds in all extracts. These results are consistent with previous reports where hexadecanoic acid was identified in high percentages in various algal species including *U. lactuca* from China [6], *U. fenestrata* from Japan [14]; *Sargassum* sp. from Japan [7,15]; *P. linearis* from California [16]; *Corallina granifera* from the Black Sea [17], *C. dimorphum* from Chile [8], and *Phyllospora comosa* from Tasmania, Australia [18].

Some similarity was observed in the fatty acid composition of the six algal species. For example, *U. lactuca*, *P. comosa* and *C. vancouveriensis* had similar fatty acid compositions with only small differences in the type and relative percentages of some acids. The highest number of identified fatty acids (18 fatty acids), was found

in the red algae *C. vancouveriensis*, followed by 17 fatty acids in the brown algae *P. comosa*, with the lowest number (10 fatty acids), found in the green algae *C. dimorphum*. PUFAs of C<sub>18</sub>, C<sub>20</sub> and C<sub>22</sub> chain length were detected among the six species but only in low overall percentages. *C. vancouveriensis* and *P. comosa* contained the highest proportion of PUFAs (10.7% and 10.1% respectively), while a lower number were found in the *Sargassum* sp., *U. lactuca* and *P. linearis* and no PUFAs were detected at all in *C. dimorphum*. The ratio of saturated: unsaturated acids varied from 1:2 in *Codium dimorphum* and *Phyllospora comosa* to 1:1 in all others. The fatty acid compositions of some of the species investigated herein have been described previously [7,8,15,18,19], however this is the first report of the fatty acid composition of these species collected from South Eastern Australian waters, and the first description of the PKA inhibitory activity of any marine algal extracts.

As fatty acids accounted for the major part of the extracts examined (i.e. 72-87% of the total extract), it is likely that they are responsible for, or at least contribute in part to, the observed PKA inhibitory activity of the extracts.  $\omega$ -Fatty acids have been reported to inhibit related protein kinases such as PKC [21]. Furthermore, previous studies in our group [20] have shown that the saturated fatty acid C14:0 shows strong PKA inhibitory activity, and C16:0, C18:0 and C18:1 fatty acids moderate activity at the same concentration as that used herein (100  $\mu$ g/mL). Interestingly, the most active algal extract, that of the *Sargassum* sp., also contained the highest percentage (12.8%) of the more potent PKA inhibitory C14:0 fatty acid. The *Prionitis linearis* extract, which showed only moderate PKA inhibitory activity, had the highest proportion (32.1%) of the C16:0 fatty acid, which is known to inhibit PKA at a similar moderate level of activity [20]. It should, however, also be noted that the remainder of the non-polar extract examined comprised aldehydes, hydrocarbons and alcohols, which could also contribute to the observed biological activity.

**Table 2:** Fatty acid composition of the dichloromethane extracts from the SE Australian marine algae

No.	Fatty acids	Type	Chlorophyta		Phaeophyta		Rhodophyta	
			1	2	3	4	5	6
1	Tetradecanoic acid	14:0	2.1	6.0	5.4	12.8	4.0	5.8
2	12-Methyltetradecanoic acid	15:0	0.7	0.3	-	-	-	0.1
3	Hexadecanoic acid	16:0	20.6	25.5	23.3	23.3	32.1	24.9
4	7-Hexadecenoic acid	16:1 n-9	-	-	0.1	-	-	0.2
5	9-Hexadecenoic acid	16:1 n-7	15.4	3.5	4.4	3.6	6.3	2.3
6	Heptadecanoic acid	17:0	-	1.2	1.7	0.3	-	0.5
7	Octadecanoic acid	18:0	2.3	0.4	0.5	1.9	2.3	1.2
8	9-Octadecenoic acid	18:1 n-9	7.2	3.1	4.5	4.4	2.2	8.8
9	9,12-Octadecadienoic acid	18:2 n-6	22.4	14.9	20.0	16.5	26.7	19.3
10	6,9,12-Octadecatrienoic acid	18:3 n-6	1.9	0.7	0.4	1.2	1.8	0.9
11	6,9,12,15-Octadecatetraenoic acid	18:4 n-3	-	0.3	0.4	-	-	0.1
12	Eicosanoic acid	20:0	-	tr	0.1	-	-	0.4
13	11,14-Eicosadienoic acid	20:2 n-6	5.8	8.8	14.3	8.3	7.0	6.2
14	8,11,14-Eicosatrienoic acid	20:3 n-6	4.8	4.7	5.4	11.3	4.2	5.2
15	5,8,11,14-Eicosatetraenoic acid	20:4 n-6	-	0.9	1.4	0.5	0.9	0.5
16	5,8,11,14,17-Eicosapentaenoic acid	20:5 n-3	-	1.3	0.7	1.7	1.1	1.6
17	7,10,13,16,19-Docosapentaenoic acid	22:5 n-3	-	-	0.1	-	-	0.1
18	Tricosanoic acid	23:0	-	-	0.2	0.2	-	0.3
<b>% of Total extract</b>			<b>83.2</b>	<b>71.6</b>	<b>82.9</b>	<b>86.0</b>	<b>88.6</b>	<b>78.4</b>

Six algae species analysed: 1: *Codium dimorphum*; 2: *Ulva lactuca*; 3: *Phyllospora comosa*; 4: *Sargassum* sp.; 5: *Prionitis linearis*; 6: *Corallina vancouveriensis*; tr – trace amounts (< 0.1).

In summary, as part of a wider screening program aimed at identifying novel therapeutic agents from Australian marine fauna,

a range of algae from SE Australia were screened for PKA inhibitory activity, with the most potent activity (84% at 100 µg/mL) exhibited by the brown alga *Sargassum* sp. The biological activity of the algal extracts was reflected in the different algal divisions, with the order of PKA inhibitory activity identified as: brown seaweeds > red seaweeds > green seaweeds. The fatty acid composition of all six algal species investigated was dominated by hexadecanoic and 9,12-octadecadienoic acid and comprised a 2:1 to 1:1 ratio of unsaturated:saturated fatty acids. Some of these fatty acids are likely to contribute to the observed PKA inhibitory activities of the algal extracts and their further investigation is currently underway.

## Experimental

**Collection:** A summary of the species collected, taxonomic class, and collection place is provided in Table 1. The algae were collected from the intertidal zone by hand during the period March/April 2008 under Scientific Research Permit Number F95/269-4.0 issued by the New South Wales (NSW) Department of Fisheries, Australia and stored frozen (-20 °C) until extraction. Voucher specimens are held at the University of Wollongong, NSW, Australia (AZ061, AZ073, AZ056, AZ072, AZ076, and AZ077).

**Extract Preparation:** The frozen algal tissue was macerated and exhaustively extracted with CH<sub>2</sub>Cl<sub>2</sub> (20-50 ml / g tissue) and then MeOH. The solvents were combined and removed in vacuo at 30 °C. The crude extract was then extracted with CH<sub>2</sub>Cl<sub>2</sub> to yield 0.3-4.6 g of CH<sub>2</sub>Cl<sub>2</sub>-soluble material upon evaporation that was subjected to GC and GC/MS analyses. Stock solutions of 1mg/mL of the CH<sub>2</sub>Cl<sub>2</sub>-extract were prepared in 20% DMSO and further diluted by 1:10 in the assay to give a final concentration of 100 µg/mL (in 2% DMSO).

**GC:** GC Analyses were performed on a Shimadzu GC-2100 Plus system equipped with a BP-5 fused silica Rxi-5ms capillary column (5% phenyl/95% dimethyl polysiloxane, 30 m x 0.25 mm, 0.25 µm

film thickness, Restek), using hydrogen carrier gas (1.0 mL/min) and samples of 1 µL. The oven was programmed at 40 °C at 4 °C per minute to 290 °C and held at 290 °C for 5 minutes.

**GC/MS:** GC/MS Analyses were performed on a Shimadzu QP-5050A GC-MS system with a mass selective detector operated in the EI mode (70 eV) and equipped with a BP-5 fused silica Rxi-5ms capillary column (5% phenyl/95% dimethyl polysiloxane, 30 m x 0.25 mm, 0.25 µm film thickness, Restek), using helium carrier gas (1.0 mL/min) and samples of 1 µL over the temperature range 80-300 °C. The relative intensity of each peak was calculated as a percentage of the summed total. Fatty acids were detected as their methyl esters after treatment with methanolic boron trifluoride and their identification was based on comparisons of the mass spectral fragmentation patterns to the NIST 08 mass spectral database and comparison with authentic standards where possible. Replicate GC/MS analyses performed on samples gave identical results.

**Biological Activity:** PKA inhibitory activity was determined using the Kinase-Glo<sup>®</sup> Luminescent Kinase Assay (Promega Corporation, Madison, USA). Each well of an opaque white 96 well plate (Corning) contained 5 µl of sample, 25 µl of ATP (20 µM), and 20 µl of a mixture of kinase (0.1 unit/µL) and Kemptide substrate (140 µM) in reaction buffer (40 mM Tris, 20mM MgCl<sub>2</sub>, BSA 0.1mg/mL, pH 7.4). After 1 hr incubation at RT, 50 µL of the Kinase-Glo<sup>®</sup> Reagent was added, the mixture incubated for 15 min and luminescence measured on a BMG Labtech FLUOstar Optima<sup>®</sup> luminometer. Data were determined relative to positive controls that contained solvent in place of sample. Negative controls contained no substrate. Staurosporine (IC<sub>50</sub> = 7 nM) was used as an internal standard. All measurements were performed in triplicate and in at least two independent experiments for reproducibility. Data are presented as the mean values ± S.E.M.

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