Pergamon

PII: S0031-9422(98)00371-9

Phytochemistry Vol. 49, No. 7, pp. 1875–1877, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

THE PRESENCE OF CHOLINESTERASE IN MARINE ALGAE

A. GUPTA, M. R. VIJAYARAGHAVAN† and R. GUPTA*

Department of Botany, University of Delhi, Delhi 110007, India

(*Received in revised form* 11 *May* 1998)

Key Word Index—*Gracilaria corticata*; Chlorophyta; Phaeophyta; Rhodophyta; marine algae; presence; acetylcholine; cholinesterase.

Abstract—Cholinesterase (ChE) activity was tested spectrophotometrically in cell free extracts of ten marine algae belonging to Chlorophyta, Phaeophyta and Rhodophyta. All the algae showed ChE activity. Of the algae screened, *Gracilaria corticata* (Rhodophyta) showed the highest ChE activity.

INTRODUCTION

Acetylcholine (ACh) the well-known neurotransmitter in higher animals is also known to be ubiquitously present in higher plants [1–3]. There is no direct evidence of the presence of ACh in any algae and the enzyme for its biosynthesis, choline acetyltransferase (ChAT), has been reported only from *Oscillatoria agardhii*, a blue–green alga [4]. Similarly, cholinesterase (ChE), the enzyme for hydrolysis of ACh has widespread occurrence in higher plants [5–8] but is reported in just one alga *Nitella flexilis* [9]. Since there is little information on ChE in algae and because of the importance of such information for discerning the natural role of ACh in plants as well as for possible use of algae as a source of enzyme and medicines, the present work was undertaken to study the presence of ChE in 10 marine algae.

RESULTS AND DISCUSSION

Neostigmine (Nst) is a potent anti-ChE agent in animals [10] and plants [1, 2]. Complete inhibition of acetylthiocholine (ATCh) hydrolysis by 25 μM Nst has been used as a marker for the presence of ChE [5, 6, 8]. In the present study ChE activity was found in 10 marine algae for the first time (Table 1). ChE was associated mostly with the pellet of the centrifuged homogenates. The association of ChE with pelletable material is consistent with similar reports from higher plants [5, 11, 12]. However, in a red alga *Gracilaria corticata* significantly high ChE activity was found in the supernatant. The results indicate widespread distribution of ChE in marine algae, as in other groups of plants. Lack of demonstrable ChE in the supernatant of some algae may not imply its absence for several reasons, including the possible presence of anti-ChE compounds [8]. Algae should be further explored as a source of ChE as well as of naturally occurring anti-ChE compounds for use in medicine and agriculture.

EXPERIMENTAL

Experiments were carried out using fresh algae collected from Okha coast (Gujrat, India) in April.

Preparation of extract

Algae were crushed in liquid N_2, homogenized in 0.1 M K–Pi buffer, pH 8 (1:2–10 w/v) and centrifuged at 5000*g* for 20 min at 4°C. The supernatant was removed and tested for ChE. The pellet was repeatedly washed with buffer and centrifuged to remove pigments. The pellet was finally resuspended in a small volume of pre-chilled 0.1 M K–Pi buffer, pH 8, for testing ChE.

Ellman's test for ChE activity

ChE activity was measured spectrophotometrically by employing a minor modification of the method of Ref. [13] as described earlier [8]. In the original method, the time course of the enzymatic activity was recorded, whereas in the present study

*Author to whom correspondence should be addressed.
†Present address: Bhavan's Vidyashram, Post Box 9, Golf Club Road, Kodaikanal, Tamil Nadu 624101, India.

Table 1. Neostigmine inhibited hydrolysis of ATChI in marine algae

Family	Algae	Cholinesterase activity* ATChI hydrolysed (pmol s^{-1} g^{-1} fr.wt)	
		pellet	supernatant
Chlorophyta			
Cladophoraceae	*Spongomorpha indica* Thivy and Visalakshmi	15	ND
Codiaceae	*Udotea indica* A. and E.S. Gepp.	164	ND
Caulerpaceae	*Caulerpa racemosa* (Forsk.) van Bosse	ND	21
	Caulerpa scalpelliformis (R.Br.) van Bosse	21	25
Phaeophyta			
Punctariaceae	*Iyengaria stellata* (Boergs.) Boergs.	30	ND
Rhodophyta			
Chaetangiaceae	*Galaxaura oblongata* Lamour.	22	ND
Gracilariaceae	*Gracilaria corticata* J. Ag.	5	235
Grateloupiaceae	*Halymenia venusta* Boergs.	23	ND
Helminthocladiaceae	*Helminthocladia calvadosii* (Lamour.) Setchell f.	19	14
Rhodomelaceae	*Laurencia pedicularioides* Boergs.		
	sample I	134	107
	sample II	17	15
Positive control			
Leguminosae	*Cicer arietinum* L.		
	roots of 7-days old plants	660	48
	roots of 15-days old plants	128	ND

*Values of controls containing anti-ChE Nst (25 μM) were subtracted from the values in tests.
ND: not detected.

the enzyme activity was tested after a fixed time of 30 min for each sample. It was ascertained that the enzyme activity remains linear for at least 30 min even in samples containing high ChE activity. The test is based on the hydrolysis of ATCh, a thiol analogue of ACh, to acetate and thiocholine and the reaction of the thiocholine with sulphydryl detection reagent 5:5′-dithio-bis(2-nitrobenzoate) (DTNB) to yield a yellow coloured anion of 5-thio-2-nitrobenzoate having a molar absorbance coefficient equal to 1.36×10^4 [13]. Besides thiocholine produced as a result of enzymatic hydrolysis of acetylthiocholine iodide (ATChI), some other factors may also contribute to the yellow colour produced in the test, e.g. autohydrolysis of ATChI to thiocholine, other thiol compounds including sulphydryl groups of proteins or some yellow coloured pigments present in the samples. To check for the above factors, A was recorded in controls containing the specific anti-ChE compound, Nst. The data of control sets was deducted from the corresponding test data. Experiments were carried out at 30°C. The reaction medium in a final volume of 5 ml contained K–Pi buffer, pH 8, 0.1 M, DTNB 0.1 mM, ATChI 1 mM and pellet suspension or supernatant of algal homogenates. Controls were preincubated with anti-ChE Nst 25 μM for 30 min before addition of ATChI. 30 min after addition of ATChI, A was recorded at 412 nm directly (for tubes containing supernatant of algal homogenates) or after centrifuging the reaction medium (for tubes containing pellet of algal homogenates). A positive control was also included in the tests, i.e. extracts of chick pea roots with well-proven ChE activity [14] were tested for comparison with extracts of algae.

Acknowledgements—We are grateful to the University Grants Commission (UGC), New Delhi, for a research grant (R. G.) and a Junior Research Fellowship (A. G.). We thank Professor S. C. Gupta for his interest and use of Shimadzu spectrophotometer.

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