dimethylpyrrole and later revised the structure to the α-hydroxy isomer, 5-hydroxy-3, 5-dimethyl-3-pyrrolin-2-one. The only reported product of autooxidation of kryptopyrrole is a dimer[7]. We were thus quite surprised to discover **5** as a photoproduct of **1**, especially because none of the equivalent was product detected following photooxidation of the related 3, 4-diethyl-2-methylpyrrole[15]. At present the only other instance of a similar product originating from photooxygenation of a pyrrole may be found in the photooxidation of 3-methylpyrrole in methanol[3] from which low yields of both 3-hydroxy-3-methyl-4-pyrroline-2-one and 3-methoxy-3-methyl-4-pyrrolin-2-one were isolated among other products. For kryptopyrrole as well as 3-methylpyrrole, we propose a dioxetane intermediate (e.g. **8**) arizing from 1, 2-cycloaddition of $^1O_2$ to one of the enamine-like double bonds[18] of the pyrrole. We could find no analogous products arizing from attack of $^1O_2$ at the other double bond of **1**, nor could we detect a methoxylactam corresponding to **5**. We presume that an intramolecular rearrangement in **8** leads to **5**. Such a reaction course is abnormal in that similar dioxetane intermediates lead mainly to carbonyl products after C–C bond clevage[19], and apparent solvolysis products are usually minor components[19,20]. Further work on the mechanistic details of these reactions and studies on the photooxidation of other alkylated pyrroles are currently under investigation in our laboratories.

Zusammenfassung. Die durch Rose Bengal sensibilisierte Photooxygenierung des Kryptopyrrols in Methanol ergab Äthylmethylmaleimid, 4-Äthyl-5-methoxy-3, 5-dimethyl-3-pyrrolin-2-on, 4-Äthyl-5-hydroxy-3, 5-dimethyl-3-pyrrolin-2-on und 4-Äthyl-3-hydroxy-3, 5-dimethyl-4-pyrrolin-2-on.

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A Cholinesterase from Bean Roots and its Inhibition by Plant Growth Retardants

Acetylcholine (ACh) levels have been shown to be related to phytochrome-mediated processes in several plant systems[1,2], which suggested that a cholinesterase (ChE) might play a regulatory role in plant development. The occurrence of ACh-hydrolyzing activity of plant extracts has been reported in several works[3–5], but a detailed characterization of the enzyme(s) involved is still missing, and the question of whether or not ChE and especially acetylcholinesterase (AChE) exist in plants has not yet been answered. The present paper describes the purification and characterization of a ChE with high affinity for ACh from mung bean (*Phaseolus aureus*) roots and the effects of various plant growth retardants on the activity of the bean ChE.

Materials and methods. The ChE was purified from roots of 12-day-old light-grown seedlings. The roots were first extracted with 10 m*M* potassium phosphate buffer, pH 7.0, to remove soluble proteins and the ChE was then extracted from the plant residue with 4% $(NH_4)_2SO_4$ (w/v) in phosphate buffer. After concentration with $(NH_4)_2SO_4$ at 80% saturation followed by dialysis, the enzyme was further purified on a Sephadex G-200 column. Overall purification was 36-fold.

ChE activity was determined by the method of ELLMAN et al.[6] using thiocholine esters as a substrate, and

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Table I. Properties of bean root ChE

Characteristics	Bean ChE
Localization	Membrane-bound
Rate of hydrolysis of choline esters	Acetyl > propionyl = butyryl
Hydrolysis of non-choline esters	+
pH optimum with acetylthiocholine and ACh	8.5, 8.7
Shape of activity curve (ACh or acetylthiocholine as a substrate)	Bell-shaped (inhibition by excess substrate)
Km with ACh and acetylthiocholine (μM)	72, 84
Mol. Wt.	> 200,000 (evidence for smaller mol. wt. forms)
Concentration of eserine causing 50% inhibition (m*M*)	0.42
Concentration of neostigmine causing 50% inhibition (μ*M*)	0.60
Effect of choline	Stimulation

the method of REED et al.[7] using 1-^{14}C-acetylcholine (New England Nuclear) as a substrate.

For measurement of growth retardant activity, mung bean seeds were soaked overnight in running tap water and planted in petri dishes on Whatman No. 1 paper wetted with 5 ml of test solution and adding 2 ml of the same solution on the 3rd day. The seedlings were grown under continuous light at 25°C. The number of secondary roots was determined on the 6th day.

The growth retardants used were: tributyl-2,4-dichlorobenzylphosphonium chloride (Phosfon-D); 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride (AMO-1618); (2-chloroethyl) trimethylammonium chloride (CCC); 1-*p*-mentanol, 2-dimethylamino-4-bromobenzyl bromide (Q76) and 1-*p*-mentanol, 2-dimethylamino-2,5-dimethylbenzyl chloride (Q80).

Results and discussion. The properties of the bean ChE are summarized in Table I. The data in this table represent only one ChE, as confirmed by the similarity in the effects of a number of inhibitors on the rates of hydrolysis of several choline esters by the enzymatic preparations used. Table I shows that the bean enzyme is not identical with any animal ChE. However, it is similar in some important properties to AChE. Although the physiological role of this enzyme is not yet clear and there is no evidence that ACh is the natural substrate of the enzyme, preliminary observations (M. J. JAFFE, unpublished) indicate that it probably functions as AChE to regulate the content of ACh in the root system.

NEWHALL[8,9] reported an excellent positive correlation between the ability of quaternary ammonium derivatives of (+)-limonene to inhibit human serum-ChE and their ability to retard the growth of various plant seedlings. Following his observations, we studied the effects of recognized plant growth retardants on the activity of bean ChE in comparison to their retardation of the production of secondary bean roots. Table II shows that there was a significant positive correlation between the two effects studied; the more effective a compound was at retarding root production, the more it inhibited the enzyme. At present, the meaning of these observations is still obscure, but two possible explanations may be offered: 1. Endogenous ACh may be a native growth retardant and inhibition of the ChE allows its accumulation in the root system. 2. The enzyme or enzymes which are responsible for root growth are biochemically similar to the ChE and the ChE is acting as a representative model when in vitro. More research is needed to clarify the observations reported[10,11].

Table II. Effect of plant growth retardants on the in vitro activity of bean root ChE and on the production of secondary roots of mung bean seedlings

Growth retardant	I_{50}[a] (*M*)	
	ChE activity[b]	Secondary root production[c]
Phosfon-D	3.3×10^{-5}	2.0×10^{-5}
Q80	2.3×10^{-4}	6.6×10^{-4}
AMO-1618	2.1×10^{-4}	7.6×10^{-4}
Q76	3.5×10^{-3}	1.2×10^{-3}
CCC	9.0×10^{-2}	4.3×10^{-2}
Coefficient of correlation (r)		$+0.99$[d]

[a] I_{50} = 50% inhibition. [b] ChE activity was assayed by the method of ELLMAN et al.[6], using acetylthiocholine as a substrate. [c] Each datum of root production represents the average of 5 replicates, 10 plants per replicate. [d] $P < 0.01$.

Résumé. Une nouvelle cholinestérase des racines des haricots a été isolée et partiellement purifiée. L'affinité de la cholinestérase des haricots pour l'acétylcholine est comparable à celle de l'acétylcholinestérase animale, mais elle diffère de cette dernière par sa réaction aux inhibiteurs et par d'autres propriétés. Une corrélation positive entre l'inhibition de la production des racines des haricots par plusieurs retardants de la croissance végétale et leurs effets inhibitoires sur la cholinestérase a été établie dans cette étude.

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Adrenergic Mechanism in *Tetrahymena*. I. Changes in Monoamine Oxidase Activity During Growth

JANAKIDEVI et al.[1,2] first found biogenic amine, such as noradrenaline, adrenaline and serotonin, in the ciliated protozoan, *Tetrahymena pyriformis* W. Recently, we reported that this protozoan possesses monoamine oxidase (MAO) and diamine oxidase[3]. It was suggested previously[1-6] that catecholamine (CA) may participate in metabolism in *Tetrahymena* as it does in mammals, though its precise role in the growth of the protozoan is still unknown. This paper reports the relationship between MAO activity and cell proliferation and the influence of adrenaline on enzyme activity.

Materials and methods. Tetrahymena pyriformis W, was cultured in a medium containing 2% polypepton and 0.1% yeast extract at pH 7.2, 26°C. After growth for 96 h in this medium, cells were inoculated into fresh medium and the growth rate was determined turbidimetrically, as described previously[6]. Synchronization was carried out by the heat shock method of SCHERBAUM and ZEUTHEN[7], with a slight modification: cells were subjected to 8 heat shocks, each of 30 min heat shocks at 32°C, separated by intervals of 30 min at 26°C. The transfer to inorganic medium (50 m*M* NaCl, 1 m*M* $MgSO_4 \cdot 7H_2O$ and 10 m*M* KH_2PO_4) was done by centrifugation prior to the 7th shock by washing the cells 3 times in inorganic medium. The synchronized cultures were then incubated with and without L-adrenaline (5×10^{-5} *M*) at 26°C, and samples were removed from the medium by pipette every 15 min for 2 h. The procedures used for enzyme preparation and