Growth and survival of *A. velutinana* on diets with L-methionine supplemented with DL-lanthionine and L-cysteine

Sulphur amino acids (mg/100 g diet)	Total larvae	Percent survival to		Male (avg.)		Females (avg.)	
		Larval-pupal ecdysis	Pupal-adult ecdysis	Days to larval-pupal ecdysis	Pharate adult period (days)	Days to larval-pupal ecdysis	Pharate adult period (days)
100 Methionine	56	96.4[a]	94.6[a]	26.2[a]	7.2[a]	27.1[a]	7.5[a]
25 Methionine + 631 lanthionine	58	81.0[b]	78.9[b]	33.8[b]	7.8[a]	35.6[b]	8.1[a]
25 Methionine + 241 cysteine	55	92.7[a]	92.7[a]	25.8[a]	7.5[a]	26.5[a]	7.9[a]
25 Methionine	53	–	–	–	–	–	–

[a, b] Values not followed by same letter are significantly different ($p < 0.05$).

lanthionine and because of the possible non-utilization of the D-isomer, one and a half times of the calculated amount of lanthionine was used.

Lanthionine partially spared the dietary methionine requirement of *A. velutinana* larvae (Table), but larval survival and growth on the lanthionine supplemented diet were significantly poor compared to non-supplemented methionine (100 mg/100 g diet) and cysteine supplemented methionine diets. The replacement of cysteine and other members of the cystathionine pathway by lanthionine for partial sparing of dietary methionine was in complete agreement with the study in which lanthionine supported the growth of rats on cyst(e)ine deficient diets[7]. The conversion of cyst(e)ine to lanthionine in *B. mori* has also been reported[10]. The partial sparing of dietary methionine by a non-member of the cystathionine pathway, suggests the possibility of metabolism of sulphur amino acids by routes other than the cystathionine pathway. Radiometric studies with ^{35}S-methionine and ^{35}S-cyst(e)ine in *B. mori*[9,10], *A. pernyi*[9] and *A. velutinana*[11] also support the above suggestion.

[11] G. K. SHARMA, G. C. ROCK and E. HODGSON, J. Insect Physiol. *18*, 1333 (1972).

Some Properties of Cholinesterase of the Plant Nematode *Aphelenchoides ritzema-boosi*

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Summary. Activity and properties of cholinesterase from *Aphelenchoides ritzema-boosi*, a plant feeding nematode, were investigated by testing the reaction of the enzyme with different substrates and inhibitors. Butyrylthiocholine was a better substrate than propionyl- and acetylthiocholine. When compared with mammalian erythrocyte and plasma cholinesterase, the nematode enzyme was found to be extremely insensitive towards a number of well-known organophosphorus and carbamate inhibitors.

In contrast to a number of parasitic nematode species in animals, such as *Ascaris, Haemonchus, Nippostrongylus* and others[1], plant nematodes have not yet been tested for the presence of cholinesterase(s). The most likely reason for this lack of information is the minute size of these organisms connected with difficulties in propagating them in quantities sufficient for enzyme analysis and characterisation. By establishing a mass culture of *Aphelenchoides ritzema-boosi* on tobacco foliage, we have now been successful in obtaining enough nematodes for some in vitro studies using different substrates and inhibitors.

Materials and methods. The nematode suspension which resulted from washing the foliage with distilled water was subjected to homogenisation in an all-glass Potter-Elvehjem homogeniser in the presence of some quartz sand. The organic tissue was then further disintegrated in an ultrasonic water bath. The white milky suspension was finally centrifuged at 3000 *g* for 15 min and the supernatant stored deep-frozen in 2-ml-portions. Before the actual tests enzyme working solutions were freshly prepared by 'dissolving' a frozen portion in an appropriate amount of phosphate buffer pH 8.0 ($^1/_{15}$ *M*). A protein concentration of 100–300 µg/ml working solution was found to be convenient for the experiments. Cholinesterase activities were determined with the procedure of ELLMANN and coworkers[2], using thiocholinesters as substrates and dithio-*bis*-nitrobenzoic acid (DTNB) as sulfhydryl reagent.

Results. The results presented in Table 1 demonstrate that the cholinesterase of *Aphelenchoides ritzema-boosi* hydrolyses butyrylthiocholine (BSCh) more rapid than propionylthiocholine (PSCh) and acetylthiocholine (ASCh). For BSCh and PSCh K_m-values were graphically determined to be 4.8×10^{-4} *M* and 1.3×10^{-3} *M*, respectively. For ASCh the Lineweaver-Burk plot did not give a straight line which may indicate that two enzymes are involved in the hydrolysis of this particular substrate. There was no inhibition by excess of substrate up to a

[1] A. SILVER, in *The Biology of Cholinesterases* (North-Holland Publ. Company, Amsterdam–Oxford, American Elsevier Publ. Company, Inc., New York 1974).

[2] G. L. ELLMAN, D. K. COURTNEY, V. ANDRES and R. M. FEATHERSTONE, Biochem. Pharmac. 7, 88 (1961).

Table 1. Cholinesterase activity determined in homogenates of the plant nematode *Aphelenchoides ritzema-boosi* with 3 different substrates

Substrate concentration (M)	nmoles SH/min/mg protein produced with ASCh	PSCh	BSCh
1.25×10^{-4}	0.74	0.71	1.41
2.50×10^{-4}	1.16	1.13	2.11
5.00×10^{-4}	1.54	1.94	3.17
1.00×10^{-3}	2.22	2.96	4.05
2.00×10^{-3}	3.34	4.50	4.89

The total volume of the reaction mixture was 3 ml, the incubation temperature 20 °C. The amounts of sulfhydryl groups produced during an incubation period of 10 min were obtained from a calibration curve in which absorbance (412 nm) was plotted against amount of reduced gluthathione.

Table 2. I_{50}-values obtained with purified bovine erythrocyte acetylcholinesterase (AChE), human plasma cholinesterase (ChE), and nematode cholinesterase (N-ChE) using different carbamates and organophosphates as inhibitors

Inhibitors	I_{50}-values (M) with AChE	ChE	N-ChE
Eserine	7×10^{-8}	1×10^{-7}	8×10^{-5}
Aldicarb	2×10^{-6}	2×10^{-6}	$>10^{-4}$
Carbaryl	3×10^{-6}	1×10^{-5}	$>10^{-4}$
Dioxacarb	5×10^{-6}	1×10^{-5}	$>10^{-4}$
Carbofuran	5×10^{-8}	4×10^{-6}	6×10^{-5}
Monocrotophos	2×10^{-5}	8×10^{-7}	$>10^{-4}$
Dichlorvos	8×10^{-7}	7×10^{-8}	3×10^{-7}
Paraoxon	5×10^{-8}	1×10^{-8}	4×10^{-8}

The I_{50}-values were determined by an automated cholinesterase inhibition procedure (for details see G. Voss[3]). Enzymes and inhibitors were pre-incubated at 37 °C for 20 min and then reacted with the preferred substrates.

concentration of 2×10^{-3} M ASCh. Again, this finding indicates that the cholinester hydrolysing enzyme of *Aphelenchoides* is not a typical acetylcholinesterase.

In combination with the optimum substrate BSCh, a further characterization of the nematode enzyme was carried out by comparing its inhibition with that of 2 other types of cholinesterases, e.g. erythrocyte acetylcholinesterase and human plasma pseudocholinesterase, using a number of well-known carbamate and organophosphorus insecticides as inhibitors. The results (Table 2) demonstrate that the nematode enzyme is extremely insensitive to many inhibitors when compared with the better known types of cholinesterases from mammals. Even eserine which is a potent inhibitor of most cholinesterases in vitro requires a concentration of approximately 10^{-4} M to inhibit the enzyme by 50%. Only paraoxon resulted in inhibition values comparable to those obtained with the two other enzymes.

It is too early to conclude from the first studies on the cholinesterase of a plant nematode species that the entire group of these organisms contains a type of enzyme unique with regard to substrate specificity and inhibition properties. The present findings, however, may well initiate further investigations on additional species, since a modified enzyme often constitutes a target for more selective control agents.

[3] G. Voss, in *Advances in Automated Analysis* (Technicon Int. Congr., Chicago 1969; Mediad Inc., White Plains, N. Y. 1970).

Activity of Two Components of Serum Ribonuclease under Conditions of Physical Exercise

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Summary. Serum ribonuclease activity before and after physical exercise in healthy persons was estimated. It is found that a work load of 6000 kgm/5 min increased ribonuclease activity measured at pH 8.5 and decreased the activity of the same enzyme measured at pH 7.0 in the presence of $ZnSO_4$. The observed changes were more pronounced in untrained than in trained persons.

It is commonly known that physical activity causes a variety of biochemical changes in the blood. For example, it has been demonstrated that the activity of several enzymes in the blood serum was affected by exercise[1]. Changes in activity of AlAT, AspAT, CPK, MDH, LDH following physical exercise have been reported[2–5]. As these changes were usually less pronounced in trained than untrained subjects, the estimations of enzyme activities before and after exercise may provide information about the physical fitness of individuals.

Recently Albanese et al.[6,7] have suggested that the measurement of serum ribonuclease activity before and after exercise may indicate to what extent an organism

[1] The abbreviations used are: AlAT, alanine aminotransferase; AspAT, aspartate aminotransferase; CPK, creatine phosphokinase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; RNA, ribonucleic acid; RNAase, ribonuclease.

[2] W. M. Fowler, S. R. Chowdhury, C. M. Pearson, G. Gardner and R. Bratton, J. appl. Physiol. *17*, 943 (1962).

[3] G. W. Gardner, R. Bratton, S. R. Chowdhury, W. M. Fowler and C. M. Pearson, J. Sports med. phys. Fitn. *4*, 103 (1964).

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[7] A. A. Albanese, L. A. Orto and D. N. Zavattaro, Nutr. Rep. Int. *3*, 165 (1971).