tract), 1000 g for 5 min and 3000 g for 15 min. Both the 1000 g and 3000 g resuspended pellets were layered on 40–75% (w/v) sucrose gradients, which were centrifuged for 3 h in a Beckman SW-27 swinging bucket rotor at 25000 rpm in a Spinco L2-65 B preparative ultracentrifuge.

Table 1 shows the contamination of the different organelle fractions and their isopycnic densities. The estimation of the percent organelle cross contamination is based on chlorophyll contents and the activity of organelle marker enzymes (NADP-glyceraldehyde-3-phosphate dehydrogenase for intact chloroplasts, cytochrome c oxidase for mitochondria and catalase for peroxisomes). The isopycnic densities are comparable to those found by other workers[14].

Figure 1 shows a peak of OAS-S activity in the fractions of intact chloroplasts. It contains about 25% of the enzyme activity of the 1000 g pellet. The OAS-S activity found in the supernatant of the gradient may be explained as consequence of chloroplast breakage during preparation. Figure 2 shows a gradient of the 3000 g pellet. There is no appreciable OAS-S activity banding with the mitochondrial and peroxisomal marker enzymes.

In the gradients both of the 1000 g and 3000 g pellets, no OAS-S activity bands with the broken chloroplasts. Thus we assume that the OAS-S localized in the chloroplasts is not a thylakoid-bound, but a soluble, stromal enzyme.

The values of Table 2, taken from a typical experiment, show that on a chlorophyll basis 20.39% of the total OAS-S activity present in the 250 g crude extract are associated with the intact chloroplasts. The rest of the activity is found in a non-particulate form and may be attributed to the cytoplasm.

Our results are consistent: 1. with the reported formation of cysteine in chloroplasts[10–12, 15]; 2. the proposed localization of the OAS-S in the cytoplasm[7–9] and 3. the results from light-induced chloroplast development experiments with *Euglena* which indicate that OAS-S is not exclusively or predominantly in the chloroplasts[20]. In contrast to *Euglena* however, Spinach leaves have not appreciable amounts of OAS-S activity banding with mitochondria[20].

The reason for the exclusive cytoplasmic localization of OAS-S by other workers[7–9] may be due to difficulties in the estimation of the OAS-S contents of chloroplasts.

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Partial Sparing of Dietary Methionine by Lanthionine in *Argyrotaenia velutinana* Larvae

G. K. Sharma[1] and G. C. Rock[2]

Department of Entomology, University College of Agriculture, Udaipur 313001 (Rajasthan, India); and Department of Entomology, N. C. State University, P. O. Box 5215, Raleigh (North Carolina, USA), 31 May 1976.

Summary. Lanthionine was able to partially spare the dietary methionine requirement of *Argyrotaenia velutinana* (Walker) larvae but the sparing of methionine by lanthionine was not as efficient as cysteine. Partial sparing of dietary methionine by lanthionine, a non-member of the cystathionine pathway, indicated the possibility of sulphur amino acid metabolism by routes other than the cystathionine pathway.

The sulphur amino acid methionine is required in the diet of almost all insects studied and cannot usually be replaced by other sulphur amino acids[3]. Nutritional investigations on *Argyrotaenia velutinana* (Walker) have shown that methionine is essential[4] but that approximately 75% of methionine requirement could be spared by cyst(e)ine[5] or other members of the cystathionine pathway (cystathionine and homocysteine)[6]. Lanthionine, a non-member of the cystathionine pathway has been found to spare the dietary cyst(eine) requirement of rats[7]. Lanthionine has been isolated from the acid hydrolysate of locust wing muscle[8], and the haemolymph of *Bombyx mori*[9, 10] and *Antheraea pernyi*[9]. The present investigation was undertaken to investigate the possibility of partial sparing of dietary methionine by lanthionine, a non-member of cystathionine pathway in *A. velutinana* larvae.

The composition of the control diet containing 17 amino acids, in which methionine at 100 mg/100 g diet was the only sulphur amino acid, was similar to that described by Sharma et al.[6]. In the cysteine supplemented diet 241 mg/100 g diet of cysteine was provided to supplement approximately 75% dietary methionine requirement. The sulphur level was kept constant in all diets, and the amount of supplemented lanthionine provided the quantity of sulphur present in 241 mg cysteine. The non-availability of L-isomer necessitated the use of DL-

[1] Dept. of Entom., Univ. Coll. Agric., Udaipur 313001 (Raj.) India.
[2] Dept. of Entom., P.O. Box 5215, N.C. State Univ., Raleigh, N.C., USA.
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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.

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

G. Voss and J. SPEICH

Agrochemicals Division, CIBA-GEIGY Ltd., CH-4002 Basel (Switzerland), 18 June 1976.

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

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