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Characterization of dihydrolipoamide dehydrogenase from the mitochondria of *Helicoverpa armigera*, a pest resistant to insecticides

S. Md. AKBAR,[1,3] S. K. JAYALAKSHMI,[2] H. C. SHARMA[3] and K. SREERAMULU[1]

1 Department of Biochemistry, Gulbarga University, Gulbarga, India
2 Agriculture Research Station, University of Agricultural Sciences, Gulbarga, India
3 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India

Correspondence
K. Sreeramulu, Department of Biochemistry, Gulbarga University, Gulbarga 585106, Karnataka, India. Email: ksramu@rediffmail.com

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Abstract

Dihydrolipoamide dehydrogenase (DHLDH) was isolated from the mitochondria of *Helicoverpa armigera*, a destructive pest which has developed resistance to commonly used insecticides. The flavoenzyme was purified 17.98-fold to homogeneity with an overall yield of 10.53% by employing ammonium sulfate precipitation, hydroxylapatite chromatography and CM-Sephadex chromatography. The purified enzyme exhibited the specific activity of 18.7 U/mg and was characterized as a dimer with a subunit mass of 66 kDa. The enzyme showed specificity for nicotinamide adenine dinucleotide – hydrogen (NADH) and lipoamide, as substrates, with Michaelis-Menten constants (K_m) of 0.083 mmol/L and 0.4 mmol/L, respectively. The reduction reaction of lipoamide by the enzyme could be explained by ping-pong mechanism. The spectra of DHLDH showed the maximum absorbance at 420 nm, 455 nm and 475 nm. The enzyme activity was strongly inhibited by mercurial and arsenical compounds. The N-terminal sequence of Ha-DHLDH showed homology with those of mammalian and arthropod DHLDH. Since *H. armigera* has developed high levels of resistance to commonly used insecticides, biochemical properties of the metabolic enzymes such as DHLDH, could be helpful to develop insecticidal molecules for the control of *H. armigera*, with a different mode of action.

Key words: arsenical compounds, dihydrolipomide dehydrogenase, *Helicoverpa armigera*, mitochondria.

Introduction

The mitochondrial enzyme, dihydrolipoamide dehydrogenase (DHLDH) (E.C.1.8.1.4) belongs to the group of flavin-containing pyridine nucleotide disulfide oxidoreductases (Huo *et al*. 2010) such as trypanothione reductase, glutathione reductase, thioredoxin reductase and mercuric reductase. DHLDH is a component of multienzyme complexes such as pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (α-KGDH) and branched chain ketoacid dehydrogenase (BCKADH). PDH is involved in irreversibly committing pyruvate from glycolysis to the Krebs cycle, whereas α-KGDH and BCKADH catalyze decarboxylation of α-ketoglutarate and regulate oxidation of branched chain ketoacids, respectively (Yan *et al*. 2008).

These multienzyme complexes consist of three subunits (E_1, E_2 and E_3) with analogous functions. Subunit E_1 is the decorboxylase dehydrogenase, E_2 is transacetylase containing covalently bound lipoic acid, while subunit E_3 is the DHLDH, which reoxidises the dihydrolipoyl moiety linked to a specific lysine side chain of subunit E_2 and regenerates lipoamide for the latter part of the E_1 reaction (Pullikuth &

Gill 1997). Subunits E_1 and E_2 are substrate-specific and differ in each complex, whereas E_3 is identical in all the three complexes, catalyzing the reversible reaction: lipoamide + nicotinamide adenine dinucleotide – hydrogen (NADH)+H^+ ↔ dihydrolipoamide + nicotinamide adenine dinucleotide (oxidized) $(NAD)^+$ (Lohrer & Krauth-Siegel 1990). All DHLDHs are stable homodimers, each monomer possessing a noncovalently but tightly bound flavin adenine dinucleotide (FAD), a transiently bound NADH or NAD^+ molecule, and two redox-active cysteine residues (Brautigam *et al*. 2005). Apart from its role in α-ketoacid dehydrogenase complexes, E_3 exhibits a number of additional functional capacities. DHLDH acts as a diaphorase (Patel *et al*. 1995), is also capable of scavenging nitric oxide (Igamberdiev *et al*. 2004) and can serve as an antioxidant by protecting other proteins against oxidative inactivation (Korotchkina *et al*. 2001). DHLDH can also act as a proteolytic enzyme when the stability of the enzyme is altered (Babady *et al*. 2007).

Previously, DHLDH of human (Babady *et al*. 2007), *Mycobacterium tuberculosis* (Rajashankar *et al*. 2005), silk worm (Huo *et al*. 2010) and *Manduca sexta* (Pullikuth & Gill 1997) were cloned and expressed in *Escherichia coli* for structural and functional studies. However, DHLDH has been characterized only from a limited number of insect species. Despite the similarities in metabolism between vertebrates and arthropods, insects specifically possess some features as additional requisite reactions, superimposed on normal metabolic pathways as a possible adaptive mechanism (Pullikuth & Gill 1997), such as utilization of proline for energy generation during flight, use of trehalose as a major blood sugar and so on. Since insects represent the most abundant and diverse group of organisms in any phylum, their number and diversity far exceeding species in all other phyla combined (Novotny *et al*. 2002), they are interesting candidates to study evolutionary, genetic and biochemical properties of multienzyme complexes.

Helicoverpa armigera (Noctuidae: Lepidoptera), commonly called cotton bollworm or legume pod borer, is one of the major pests of cotton, pulses, cereals, vegetables, fruits and forest trees (Sharma 2005). It has developed high levels of resistance to several insecticides such as endosulfan, methomyl, monocrotophos, quinalphos, chlorpyriphos, fenvalerate and cypermethrin (Chaturvedi 2007), and also to *Bacillus thuringenisis* Cry1Ac toxin (Gunning *et al*. 2005). Because of the amenability of insects to adapt to environments and develop resistance to several insecticides, biochemical properties of the enzymes involved in metabolism, such as DHLDH, could be helpful to develop insecticidal molecules for the control of *H. armigera*, with a different mode of action. In the present study, we report the characterization of purified DHLDH from the mitochondria of *H. armigera*, its N-terminal sequence analysis and inhibition of enzyme activity by arsenical compounds.

Materials and methods

Chemicals

α-Lipoamide, CM-Sephadex, 3[(3-cholamidopropyl) dimethylammonio]-propanesulfonic acid (CHAPS), hydroxylapatite, NADH, sodium arsenite and melarsoprol were purchased from Sigma Aldrich (Mumbai, India). Tris-base, pyruvate and 5,5[1]-dithio-bis(2-nitro benzoic acid) were purchased from Himedia (Mumbai, India) while the other chemicals were of analytical grade.

Insects

Cypermethrin-resistant (60-fold) population of *H. armigera* was supplied by Dr S.S. Udikeri, Agriculture Research Station, UAS, Dharwad, Karnataka, India. The larvae were washed in chilled distilled water, their gut contents removed and body membranes taken for isolation of DHLDH.

Isolation of enzyme

For the isolation of DHLDH, the mitochondria were isolated from body membranes of fourth and fifth instar larvae of *H. armigera* (~23 g) according to the modified method of Yan *et al*. (2008). This entailed gently homogenizing the body membranes of the larvae in isolation buffer containing 0.25 M sucrose, 1 mmol/L ethylendiametetraacetic acid (EDTA) and 50 mmol/L Tris-HCl, pH 7.4. The homogenate was centrifuged at 800 × *g* for 5 min, and the supernatant was centrifuged at 12 000 × *g* for 10 min. The pellet containing mitochondria was suspended in 50 mmol/L Tris-HCl buffer, pH 7.4, containing 1 mmol/L EDTA and 1% CHAPS and kept stirring at 4°C overnight. The extract was centrifuged at 15 000 × *g* for 30 min, and the supernatant was used as a source of DHLDH.

Enzyme assays

The DHLDH activity was assayed spectrophotometrically in 50 mmol/L Tris-HCl buffer, pH 7.4, containing 1 mmol/L EDTA, 1 mmol/L dithioerythritol, 0.025 mmol/L NADH and 0.5 mmol/L lipoamide, in a total reaction volume of 1 mL in presence of purified protein at 30°C (Huo *et al*. 2010). The reaction was started by addition of lipoamide, and NADH oxidation was measured as change in absorbance at 340 nm over time. The PDH complex activity was measured spectrophotometrically at 30°C according to Hein and Steinbüchel (1994). Reduction of NAD was measured at 340 nm and the reaction started with 1.5 mmol/L pyruvate. One unit of enzyme activity was expressed as μmoles of NADH consumed or produced per minute at 30°C. Protein concentration was determined by Lowry's method (1951).

Purification of DHLDH

To purify DHLDH, ammonium sulfate was added to 50 mL of crude extract (1100 mg of protein) to give 40% saturation. The solution was kept for overnight stirring at 4°C, and the precipitate was removed by centrifugation for 30 min at 10 000 × *g*, and the supernatant was brought to 60% ammonium sulfate saturation. After overnight stirring at 4°C, salt-precipitated proteins were collected by centrifugation for 30 min at 10 000 × *g*, and dissolved in buffer A (50 mmol/L Tris-HCl buffer, pH 7.4, containing 1 mmol/L EDTA, 1 mmol/L dithioerythritol), and dialyzed for 18 h against the preceding buffer at 4°C. This pool was applied to a hydroxylapatite column (2 × 10 cm) previously equilibrated with buffer A. After the column was washed with 100 mL of the same buffer, proteins were eluted with a step gradient of 0–1.0 M potassium phosphate buffer, pH 7.4, and the fractions were collected each of 3 mL. DHLDH activity was eluted at 0.4 M and 0.5 M phosphate buffer fractions, and the active fractions were pooled, concentrated and dialyzed against buffer B (50 mmol/L phosphate buffer, pH 7.4, containing 1 mmol/L EDTA and 1 mmol/L dithioerythritol), and applied to a column of CM-Sephadex (2 × 10 cm) previously equilibrated with the preceding buffer. After the column was washed with 40 mL of buffer B, proteins were eluted from the column with a step gradient of 0–1 M NaCl and fractions of 2 mL each were collected. DHLDH activity was eluted at 0.5 M and 0.6 M NaCl. Fractions containing DHLDH activity were pooled, concentrated using Viva cell 250 (molecular weight [MW] 50 000) to a final volume of 2.5 mL, and dialyzed against buffer B. DHLDH in the purified preparation was tested for homogeneity by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970).

Electrophoresis

The molecular weight of the protein under denaturing conditions was determined by 10% SDS-PAGE (Laemmli 1970). Native molecular weight of the protein was determined by native PAGE, in polyacrylamide gel gradient formed from 5% to 15% using Sigma molecular weight markers in the range of 29 to 205 kDa (Margolis & Kenrick 1967). DHLDH activity was detected by incubating the gel in a reaction mixture containing 2 mmol/L lipoamide, 0.4 mmol/L NADH, 0.5 mmol/L 5,5[1]-dithio-bis(2-nitro benzoic acid) and 1 mmol/L EDTA in 50 mmol/L potassium phosphate buffer, pH 7.4, at 37°C, until yellow bands appeared (Dietrichs & Andreesen 1990).

Kinetic studies and substrate specificity

Kinetic studies were carried out in 50 mmol/L phosphate buffer, pH 7.4, containing 1 mmol/L EDTA and 1 mmol/L dithioerythritol in a total reaction volume of 1 mL at 30°C. NADH concentration varied from 0.05 mmol/L to 0.4 mmol/L at 2.5 mmol/L lipoamide, for determination of Michaelis-Menten constants (K_m) of NADH and lipoamide concentration varied from 0.5 mmol/L to 4 mmol/L at 0.25 mmol/L NADH, for determination of K_m of lipoamide. Substrate specificity was tested using both the reduced pyridine nucleotides, NADH and Nicotinamide adenine dinucleotide phosphate – hydrogen (reduced) (NADPH) for the reduction of lipoamide. Double-reciprocal plots were drawn to determine the mechanism of enzyme catalyzed reaction in the direction of oxidation of NADH with varying NADH concentrations at fixed lipoamide concentrations of 1 mmol/L, 1.5 mmol/L and 2 mmol/L.

Influence of pH, temperature and metal ions

Influence of pH on the activity of DHLDH was studied in the above-mentioned reaction mixture at different pH values ranging 4–10. Acetate buffer, Tris-HCl and bicarbonate buffer were used for acidic, neutral and basic pH of the reaction mixture, respectively. The enzyme was incubated at different temperatures ranging 20–80°C for 15 min, to determine the effect of temperature on enzyme activity. Metal ions such as Na, Mg, K, Ca, Mn, Fe, Cd, Ag, Zn, Co, Pb, Cu, Hg, arsenite and melarsoprol at 1 mmol/L concentrations were incubated with the enzyme for 15 min at room temperature to determine the effect of different metal ions on the catalytic activity of the enzyme.

Identification of coenzyme

The purified enzyme was denatured at 100°C in dark to liberate the bound flavin and centrifuged at 15 000 × *g* for 15 min. The released flavin was analyzed by thin layer chromatography (Dietrichs & Andreesen 1990). Protein spectra were obtained by Schimadzu spectrophotometer (Shimane Shimadzu Corporation, Shimane, Japan) keeping 50 mmol/L phosphate buffer, pH 7.4, as reference.

N-terminal sequencing

For determination of N-terminal sequence, purified DHLDH protein was subjected to SDS-PAGE, then the protein was transferred to polyvinylidenedifluoride membrane, and the membrane was stained with Coomassie blue R250. The enzyme was detected in gel by activity staining (Dietrichs & Andreesen 1990); areas of DHLDH from the membrane were excised and sent for sequence analysis using Edman degradation based Procise protein sequencing system at HSC Advanced Protein Technology Center, Department of Structural Biology and Biochemistry, Toronto, Canada. The N-terminal sequence was blasted with mammal and arthropod DHLDH sequences using the NCBI website (http://www.ncbi.nlm.nih.gov).

Results

Purification of DHLDH

Figures 1 and 2 show the elution profile of DHLDH of *H. armigera* from hydroxylapetite and CM-Sephadex, respectively. From 1100 mg of total protein, 4 mg of enzyme was obtained with 17.98-fold-increased specific activity and 10.53% recovery. Specific activity of purified enzyme was 18.7 U/mg of protein (Table 1).

Molecular weight of DHLDH

The subunit molecular weight of DHLDH was estimated to be 66 kDa. The native molecular weight of the protein was determined to be 128 kDa, suggesting that the protein existed as a dimer (Fig. 3).

Kinetic studies and substrate specificity

The velocity of NADH-dependent reduction of lipoamide showed a hyperbolic dependence on the NADH and lipoamide concentration (data not shown). Estimated K_m were 0.083 mmol/L for NADH and 0.4 mmol/L for lipoamide, maximum velocity (V_{max}) of 61.25 U was observed in both the cases, that is, with varying concentrations of lipoamide and NADH. Substrate specificity tested in the direction of oxidation of reduced pyridine nucleotides indicated that the enzyme was highly specific to NADH as a coenzyme, and did not use NADPH. Double reciprocal plots drawn for the enzyme activity yielded a series of parallel lines, which fitted the ping-pong mechanism of catalysis (Fig. 4).

Influence of pH, temperature and metal ions

Enzyme activity showed parabolic dependence on pH, with an optimum pH of 7.4. The enzyme activity was unaffected even at a high temperature of 80°C. Heavy metal ions such as Cu, Cd, Zn, Co, Pb, Ni, Hg, arsenite and melarsoprol completely inhibited enzyme activity (Table 2).

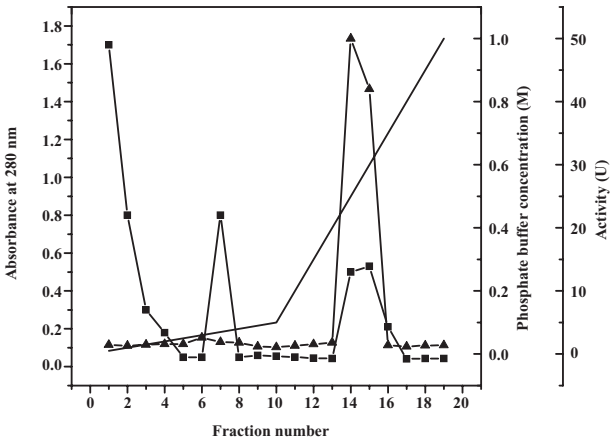

Figure 1 Elution profile for the protein and dihydrolipoamide dehydrogenase activity on hydroxylapatite column. Protein (■), enzyme activity (▲), phosphate buffer concentration (–).

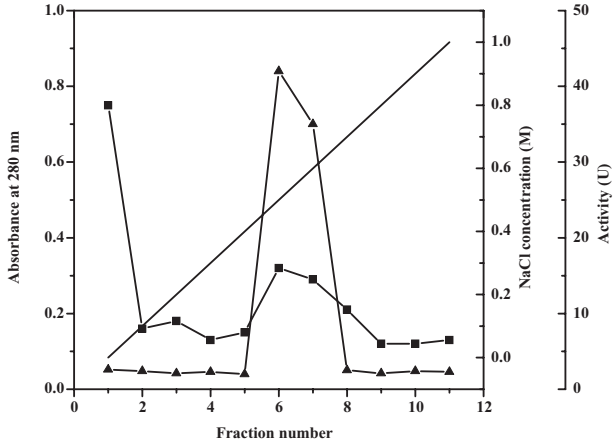

Figure 2 Elution profile for the protein and dihydrolipoamide dehydrogenase activity on CM-Sephadex column. Protein (■), enzyme activity (▲), NaCl concentration (–).

Table 1 Purification of dihydrolipoamide dehydrogenase from mitochondria of *H. armigera*

	Volume (ml)	Total protein (mg)†	Total activity (U)†	Specific activity (U/mg)†	Purification fold	Yield (%)
Crude extract	50	1100 ± 5.25	1146.30 ± 10.12	1.04 ± 0.02	1.00	100.00
NH_4SO_4 precipitation	16	192 ± 2.89	867.84 ± 5.78	4.52 ± 0.43	4.34	75.70
Hydroxylapatite	6	48 ± 1.54	404.16 ± 2.87	8.42 ± 0.77	8.09	35.25
CM-Sephadex	2.5	4 ± 0.19	120.80 ± 1.99	18.70 ± 1.22	17.98	10.53

†Values in the table represents Mean ± SE ($n = 3$).

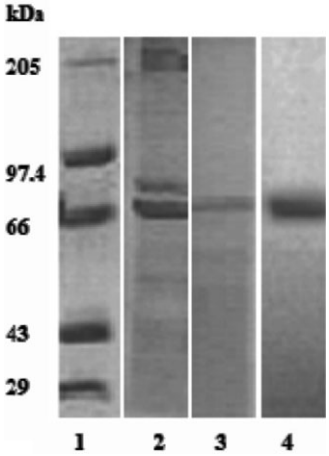

Figure 3 Sodium dodecylsulfate polyacrylamide gel electrophoresis pattern of dihydrolipoamide dehydrogenase of *H. armigera*. The proteins were detected by Coomassie blue. Lane 1, molecular weight markers (29–205 kDa); lane 2, 3[(3-cholamidopropyl) dimethylammonio]-propanesulfonic acid solublized mitochondrial pellet; lane 3, purified enzyme (CM-Sephadex fraction); lane 4, gel stained for the activity of the enzyme (staining procedure mentioned in text).

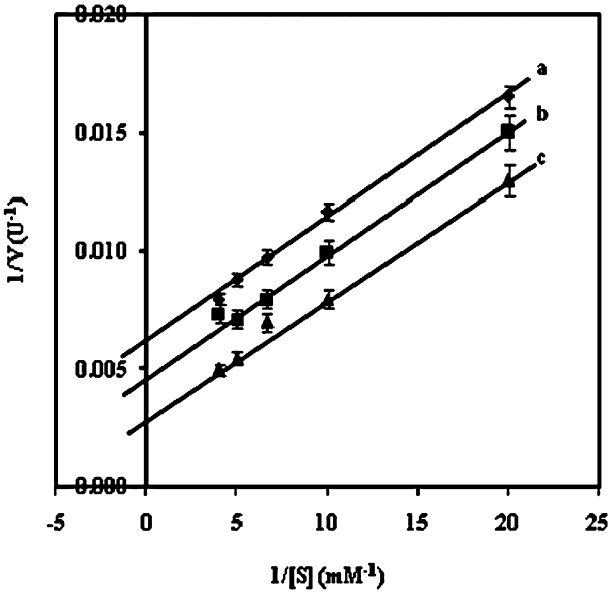

Figure 4 Double reciprocal plots for the initial velocity of the enzyme with varying concentrations of NADH at fixed concentrations of lipoamide, 1 mmol/L (a), 1.5 mmol/L (b) and 2 mmol/L (c), in 50 mmol/L phosphate buffer, pH 7.4, containing 1 mmol/L ethylendiametetraacetic acid and 1 mmol/L dithioerythritol.

Identification of coenzyme

The spectra of DHLDH showed absorption maxima at 420 nm, 455 nm and 475 nm (Fig. 5). The absorbance at 455 nm decreased when the enzyme was reduced with

Table 2 Effect of metal ions on dihydrolipoamide dehydrogenase activity of *H. armigera*

Metal (1 mmol/L)	Activity (%)[†]
Control	100.00
$CaCl_2$	156.67
KCl	153.34
NaCl	113.33
$MgCl_2$	106.67
$MnCl_2$	106.67
$FeSO_4$	90.00
$AgNO_3$	33.33
$FeCl_3$	20.00
$CuSo_4$	3.76
$PbSO_4$	3.79
$ZnSo_4$	3.72
$CdCl_2$	3.72
$HgCl_2$	4.09
$CoCl_2$	3.76
Sodium arsenite	3.79
Melarsoprol	3.45

[†]The values are means of three different assays.

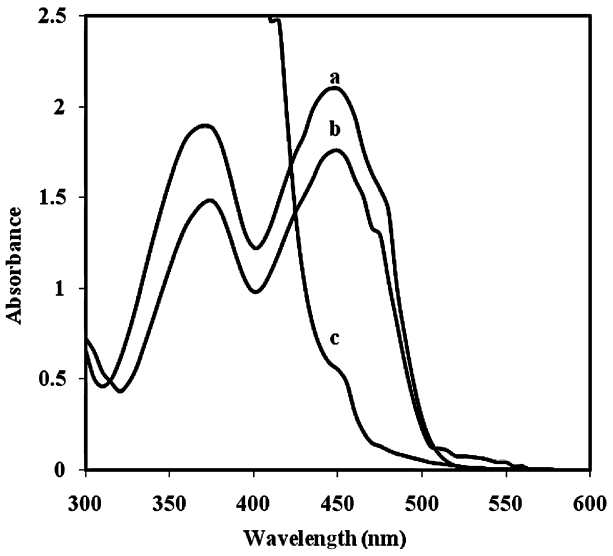

Figure 5 Absorption spectra of dihydrolipoamide dehydrogenase of *H. armigera*. The oxidized enzyme in 50 mmol/L phosphate buffer, pH 7.4 (a) was reduced with 2 mmol/L NADH (b) and excess sodium dithionite (c).

2 mmol/L NADH. Addition of sodium dithionite resulted in complete decrease of absorbance at 455 nm. These types of spectra are typical of flavoproteins (Lohrer & Krauth-Siegel 1990).

N-terminal sequence of DHLDH

Figure 6 shows the alignment of N-terminal sequence of Ha-DHLDH with different organisms, and about ten

1MQKFDVVVIGAGPGGY16……*H. armigera*

39 DIVVIGSGPGGY50…… *D. melanogaster* (GI: 7293932)

32 DLVVIGAGPGGY43……*M. sexta* (GI: 2267131)

33 DLVVIGSGPGGY44……..*B. mori* (GI: 112983096)

13 DVVVIGGGPGGY24……*T. cruzi* (GI: 6166121)

43 DVTVIGSGPGGY54……..Human (GI: 1339989)

43 DVTVIGSGPGGY54……. Bovine (GI: 296488519)

43 DVTVIGSGPGGY54……..Pig (GI: 262072947)

7 DVVII GGGPAGY16…….Yeast (GI: 15826395)

1MQKFDVVVLGAGPGGY16……*M. mycoides* (GI: 256385283)

Figure 6 Alignment of the N-terminal sequence of *H. armigera* dihydrolipoamide dehydrogenase (DHLDH) with DHLDHs of *Drosophila melanogaster, Manduca sexta, Bombyx mori, Trypanosoma cruzi*, human, bovine, pig, yeast, and *Mycoplasma mycoides*.

positions were found to be identical with the sequence of mammalian and arthropod DHLDH. The sequence of DHLDH of *H. armigera* showed homology with the oxidoreductase region of mammal's DHLDH (Otulakowski & Robinson 1987) and the stretch of sequence GAGPGG represented the binding site for the pyrophosphate group of FAD (Lohrer & Krauth-Siegel 1990).

Discussion

The present study describes the isolation of DHLDH from the mitochondria of *H. armigera*. Apart from its role in metabolism in association with ketoacids dehydrogenase complexes, DHLDH is also involved in reducing intracellular thiol groups. It has also been reported that, in mitochondria, dihydrolipoic acid plays an important role in maintaining the Adenosine triphosphate (ATP) content in the cell, that is, they enhance the ATP synthase activity and prevent ATPase activation (Zimmer *et al*. 1991) and also maintain the membrane proteins in proper redox state (Serrano 1992).

DHLDH from *H. armigera* was found to be specific to NADH and no lipoamide-dependent NADPH oxidation was detected. Based on substrate specificity, Dietrichs and Andreesen (1990) classified DHLDH into four types: one utilizing NADH alone, the second utilizing both NADH and NADPH, the third utilizing NADPH alone and the fourth utilizing both NADH and NADPH with preference for NADPH. The DHLDH isolated from *H. armigera* thus represents the classical type of enzyme utilizing only NADH as its substrate, having a relative molecular weight (Mr) of 66 kDa per subunit. The native molecular weight of the protein was found to be 128 kDa, suggesting that the protein existed as a homodimer in association with ketoacid dehydrogenase complexes. The molecular weight of the enzyme reported from *Trypanosoma cruzi* (Lohrer & Krauth-Siegel 1990), *Manduca sexta* (Pullikuth & Gill 1997) and human liver (Ide *et al*. 1967), have a subunit Mr of 55 kDa, 51 kDa and 138 kDa (native molecular weight), respectively, which are around the Mr of DHLDH of *H. armigera*. The enzyme also reduces 2,6-dichloroindophenol and potassium ferricyanide using NADH as a physiological electron donor. DHLDH is known for its broader specificity for artificial electron acceptors (Youn *et al*. 1998).

The enzyme showed a parabolic dependence on pH of the medium, with an optimum pH of 7.4. In alkaline conditions, the enzyme activity gradually decreased due to denaturation of the enzyme. Optimum pH for DHLDH from *T. cruzi* is 6.75, and human liver 6.5, which is around neutral pH. The enzyme was resistant to thermal inactivation and enzyme activity was not affected even at high temperatures up to 80°C. According to Schmincke-ott and Bisswanger (1981), the transition state activation energy is 43 kJ/mol for the complex bound form and 74.8 kJ/mol for the individual E3 component in *E. coli* K12 strain. This reflects the stability of the component and its ability to withstand such high temperatures to overcome the transition state barrier.

K_m for the substrates, lipoamide and NADH were 0.4 mmol/L and 0.083 mmol/L, respectively. These values are similar for the substrates from other organisms, for example, K_m for NADH with human liver (Ide *et al*. 1967) and *T. cruzi* (Lohrer & Krauth-Siegel 1990) DHLDH is 0.023 mmol/L and 1.33 mmol/L, respectively, while a high K_m of 5 mmol/L has been reported for lipoamide with DHLDH of *T. cruzi* (Lohrer & Krauth-Siegel 1990) and bovine liver mitochondria (Lusty 1963). A series of parallel lines were obtained when a double reciprocal plot was drawn (Fig. 4). Such behavior is exhibited by enzymes operated by a ping-pong mechanism, as proposed in some bacteria (Youn *et al*. 1998) and in eukaryotes (Massey *et al*. 1960; Reed 1973). This also implied that NADH and disulfide substrate have different binding sites on the enzyme. The spectral properties found with DHLDH of *H. armigera* are in accordance with those of DHLDH of *T. cruzi* (Lohrer & Krauth-Siegel 1990) and pig (Massey *et al*. 1960). Metal ions such as Cu, Cd, Zn, Co, Pb, Ni, Hg, arsenite and melarsoprol completely inhibited enzyme activity, whereas Ca and K enhanced enzyme activity up to 50% (Table 2).

The N-terminal sequence of Ha-DHLDH was identical to bacterial, arthropod and mammalian DHLDH. The sequence lies in FAD binding domain of the enzyme, which is highly conserved, as observed in the E3 component of *M. sexta* (Pullikuth & Gill 1997) and *T. cruzi* (Lohrer & Krauth-Siegel 1990). The enzyme also showed homology to the conserved

sequence of human erythrocyte glutathione reductase (Thieme *et al*. 1981), and *Pseudomonas aeruginosa* mercury (II) reductase (Brown *et al*. 1983). Evidence has shown that DHLDH present in cytoplasmic membranes of *T. brucei* (Danson *et al*. 1987) and *lpd* mutant *E. coli* (Richarme 1989) are not a component of ketoacid dehydrogenase but are meant for uptake of monosaccharides in the cell. Such a cytoplasmic localization of the enzyme in *H. armigera* was not observed in the present study. Biochemical documentation of the catalytic properties and the behavior of the multienzyme complexes are necessary to understand their role in metabolism. These studies will help to link evolutionary relationships. Arsenical drugs such as melarsoprol (Friedheim 1994) and melarsen oxide (Fairlamb *et al*. 1992; Milord *et al*. 1992) have been used for the treatment of African trypanosomiasis caused by trypanosome. These drugs mainly target oxidoreductase enzymes such as trypanothione reductase and lipoamide dehydrogenase. In the present study we observed that some of the arsenical compounds are potent inhibitors of Ha-DHLDH (Table 2). Therefore, it will be interesting to synthesize similar arsenical compounds targeting metabolic enzymes for the control of phytophagous insect pests such as *H. armigera*, since this insect has developed a high level of resistance to commonly used insecticides.

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