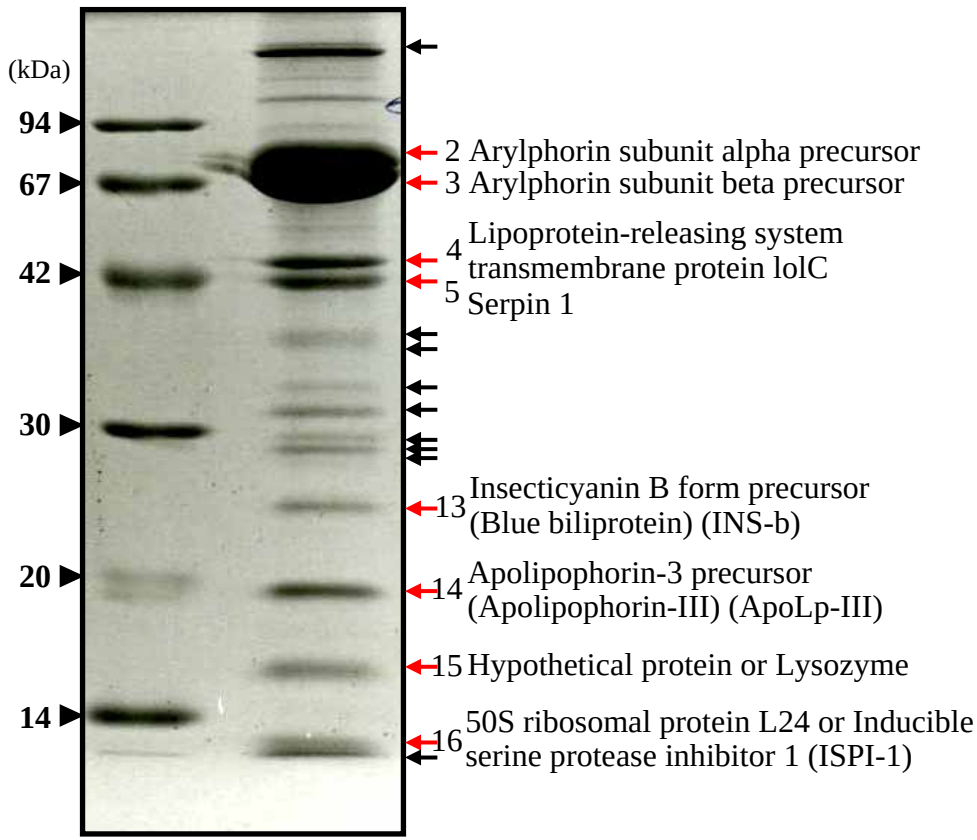
A

Hemolymph
↓
Dissolved in DW
& SDS-sample buffer
↓
SDS-PAGE
↓
CBB stain
↓
N-terminal amino acid
sequence
↓
BLAST/FASTA
Protein identification

B



1-D gel profile of *Manduca sexta* hemolymph proteins. (A) Proteins were extracted from distilled water diluted hemolymph powder by solubilization with SDS-sample buffer and separated by 12.5% SDS-PAGE. **(B)** Proteins were visualized with CBB R-250. Identified proteins are indicated on the right-hand side and low molecular weight markers on the left-hand side. The electroblotted proteins onto PVDF membrane were sequenced on an Applied Biosystems 494 protein sequencer (Perkin Elmer-ABI, Foster City, CA, USA). The obtained sequences were used to interrogate databases with Web accessible search programs like FastaA and Fasta3 (available online from EMBL Outstation of the European Bioinformatics Institute), to identify homology to proteins already present in the protein and nucleic acid databases. The identified proteins are shown in Table II.

Table II

M. sexta hemolymph proteins separated by 1-DGE, and their identification with *N*-terminal amino acid sequencing

Band No	Protein Name	Amino Acid Sequence	pI / MW (Da) Theoretical	Acc. No.	Length	Identity %	Similarity %	Functional category	Species
2	Arylphorin subunit alpha precursor	savpvkhyqhhykvs	6.10 / 83866.31	P14296	702	73.333	86.667	Storage	*Manduca sexta*
3	Arylphorin subunit beta precursor	skvpvkhsfkvkdydanfye	5.95 / 83848.83	P14297	703	80	85	Storage	*M. sexta*
4	Lipoprotein-releasing system transmembrane protein lolC	tpasv-mrxlliqyas	7.04 / 45234.25	Q87EF5	413	50	91.667	Lipid Transport and Metabolism	*Xylella fastidiosa* (strain Temecula1 / ATCC 700964)
5	Serpin 1	qetdlqkilresndqftaqm	5.01 / 44380.96	Q25494	398	100	100	Serine Protease Inhibitor	*M. sexta*
13	Insecticyanin B form precursor (Blue biliprotein) (INS-b)	gdifypgyxpdvkpvddfdl	6.29 / 23182.27	Q00630	206	95	95	Lipid Transport and Metabolism	*M. sexta*
14	Apolipophorin-3 precursor (Apolipophorin-III) (ApoLp-III)	dapaggnafeemekhakefq	6.98 / 20793.37	P13276	189	100	100	Lipid Transport and Metabolism	*M. sexta*
15	Hypothetical protein	eekyteenddldieqvikda	4.93 / 13847.87	Q3LB46	121	95	95	Unclassified	*M. sexta*
	Lysozyme	khfsrxllvhelrwggfpen	8.91 / 16087.31	Q26363	138	80	80	Lysozyme	*M. sexta*
16	50S ribosomal protein L24	dlvpgsnyvkqhprgspiaq	10.28 / 11404.26	Q2L282	106	50	77.778	Ribosomal protein	*Bordetella avium* (strain 197N)
	Inducible serine protease inhibitor 1 (ISPI-1) (Fragment)	dlvxgsnyxkqhpxgspiaq	cannot be computed	P81905	50	57.895	78.947	Serine Protease Inhibitor	*Galleria mellonella* (Wax moth)