Table II. GC retention temperatures of the *S. exempta* olfactory stimulants S(i) and S(ii) and of known *Spodoptera* spp. sex pheromones

Sample	Retention temperature (°C) Column A	Retention temperature (°C) Column C
Tetradecanyl aceate	172.9	133.0
(Z)-9-Tetradecenyl acetate	176.3	137.8
(Z)-9, (E)-12-Tetradecadienyl acetate	183.2	144.9
(Z)-9, (E)-11-Tetradecadienyl acetate	189.0	145.5
Olfactory stimulant S(i)	176.2	137.5
S(ii)	183.4	144.8

Column C: EGSS-X (other conditions as for Column A in Table I).

Table III. Comparison of male moth antennal responses to natural and synthetic olfactory stimulants

Sample	EAG response (mV)	
	Run 1	Run 2
Female tip extract containing S(i) 4 ng	1.0	1.1
S(ii) ca. 0.2 ng	0.4	0.4
(Z)-9-Tetradecenyl acetate, 4 ng	1.0	1.0
(Z)-9, (E)-12-Tetradecadienyl acetate, 0.2 ng	0.6	0.4

All samples were chromatographed on Column C (Table II). Weights quoted are for the amount of sample injected onto the GC column.

(Z)-9, (E)-12-tetradecadienyl acetate (II) co-chromatographed with S(ii) on all 3 columns, and this was the only diene isomer which caused an antennal response.

The potencies of compounds (I) and (II) were shown to be comparable with those of the natural olfactory stimulants by recording EAG responses of the same male moth to female extract and to corresponding amounts of compounds (I) and (II) (Table III). In a laboratory bioassay involving attraction of male moths into a trap[7], a mixture of (I) (50 ng) and (II) (2.5 ng) attracted 40–60% of the test moths. It was concluded that these 2 compounds constitute the female sex pheromone of *S. exempta*, although field trials are needed to confirm this and to determine the exact functions of the 2 components.

Pheromones (I) and (II) are also produced by female *S. eridania*[4] but in a different ratio from that found in *S. exempta*. The diene (II) has been found in *S. litura*[6] in combination with a conjugated diene, and in *S. exigua*[5]; it has also been reported to be attractive to male *S. d olichos*[8]. The mono-ene (I) has been isolated from *S. frugiperda*[3] and forms part of the pheromone complex of *S. littoralis*[2].

Zusammenfassung. Es wurden mit gas-chromatographischer Elektroantennogramm-Methode (Z)-9-tetradecenyl-1-acetat und (Z)-9, (E)-12-tetradecadienyl-1-acetat als die Sexuallockstoffe weiblicher *Spodoptera exempta* (Wlk.) identifiziert.

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[9] Acknowledgment: We are grateful to Mr. C. Rivers, Unit of Insect Virology, University of Oxford Field Station, for supplying insect pupae.

How Homologous are the α and β Subunits of C-Phycocyanin?

In recent years it has been demonstrated that C-phycocyanins are composed of two types of subunits (α and β) which always occur in a 1:1 ratio. The nature of these subunits – they are both single polypeptide chains – was clearly established by analysis of their amino terminals, molecular weights and amino acid composition[1–7]. As soon as the results of the amino acid analyses of those subunits were available, a considerable homology between the two chains was anticipated owing to the fact that their amino acid compositions, though not identical, show remarkable analogies, e.g. the high content of glycine and alanine. This holds for the C-phycocyanins of all types of blue green algae which have so far been investigated in this respect[4–7]. However, a high degree of homology was not found when large chromophore-containing peptides of both chains from

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C-phycocyanin of *Mastigocladus laminosus* were subjected to sequence analysis in our laboratory[8]. Within a span of 13 comparable residues only two homologous ones were identified in addition to the chromophore-binding cysteine which serves as a reference point for aligning the two polypeptide chains. This is just about the minimum of homology necessary to exclude the possibility of chance in a statistical distribution of residues.

In this paper we show that a region of remarkably high homology between the subunits of C-phycocyanin from *Mastigocladus laminosus* exists close enough to the amino terminals that it can be investigated by means of automatic Edman degradation.

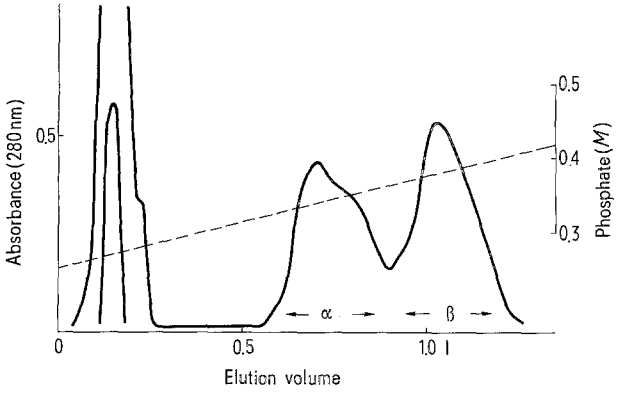

Fig. 1. Separation of *Mastigocladus laminosus* C-phycocyanin (CPC) subunits on hydroxyapatite in presence of SDS. Denatured CPC was applied to a column (40 × 2.5 cm) and eluted with phosphate buffer (pH 6.4) containing 0.1% SDS and 0.25% β-mercaptoethanol. A linear gradient of phosphate was applied (ascending straight line).

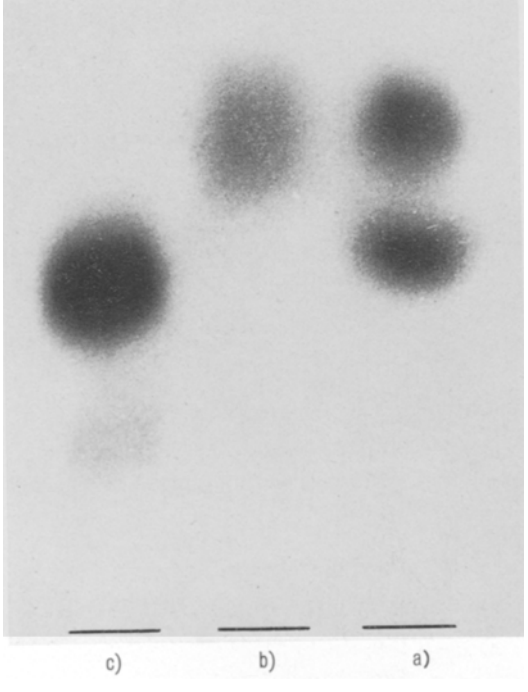

Fig. 2. Cellogel electrophoresis of C-phycocyanin (a), α-chain (b) and β-chain (c). The proteins were urea-treated (9 *M* urea, 2½ h at 37 °C) and submitted to electrophoresis at pH 7.2 (0.2 *M* potassium phosphate, 0.1 m*M* EDTA, 10 m*M* β-mercaptoethanol and 6 *M* urea). The strips were stained as described by HEIL and ZILLIG[12].

Improvements in the methods for chain separation, particularly the switch from preparative gel electrophoresis to a column method, provided us with the amounts of single chains necessary for more detailed sequence studies.

Materials and methods. C-phycocyanin (CPC) from *M. laminosus* was prepared as described earlier[4]. Hydroxyapatite (dry powder) was Biogel HTP from Calbiochem. SDS and trypsin (treated with tosyl-L-phenylalanyl-chloroketone) were purchased from Serva. Sequencer reagents and solvents were Pierce sequenal grade (Zürich) and Beckman (Basle). All other chemicals were analytical grade from either Merck or Fluka.

Chain separation was achieved on an SDS hydroxyapatite column (25 × 40 cm, equilibrated with 0.01 *M* Na-phosphate buffer pH 6.4 containing 0.1% SDS and 0.25% β-mercaptoethanol) following closely the conditions of Moss and ROSENBLUM[9]. CPC (65 mg) was dissolved in 15 ml of the equilibration buffer to which 300 mg SDS and 600 mg β-mercaptoethanol were then added. The solution was heated to 100 °C for 5 min, then diluted with 15 ml of the same buffer and immediately applied to the column. The column was rinsed with approximately 100 ml of the buffer. A linear gradient from 0.25 *M* to 0.5 *M* phosphate, both solutions at pH 6.4 and containing 0.1% SDS and 0.25% β-mercaptoethanol, was then applied. Elution (40 ml/h) was monitored by recording the optical density of the eluent at 280 nm. Fractions containing the α and β subunit respectively were pooled and exhaustively dialyzed against deionized water. Both chains precipitated to a large extent during dialysis. The suspensions were frozen and lyophilized. For total elimination of residual SDS, the method of WEBER and KUTER[10] was used.

Under the usual conditions, tryptic cleavage of either chain is incomplete. Strong intra- and intermolecular hydrophobic interactions, presumably involving the chromophore, call for special denaturation with exclusion of partial renaturation before cleavage has begun. 5 mg of either subunit were suspended in 100 µl 8 *M* urea and heated for 20 min to 50 °C. After cooling 100 µl 1 *M* ammonium hydrogen carbonate, 100 µl DMSO and 10 µl enzyme solution (20 mg trypsin per ml 1 m*M* hydrochloric acid) were added and cleavage was allowed to proceed at room temperature (24 °C). After 2 and 4 h, 10 µl aliquots of fresh enzyme solution was added to each of the digestion mixtures. When an incubation time of 6 h had been reached, digestion was stopped by heating the mixtures for 2 min to 100 °C. The solutions were clarified by centrifugation (small amount of precipitate discarded) and passed through a Sephadex G-15 column (1.5 × 20 cm equilibrated with 5 m*M* ammonia) to separate the peptides from DMSO and urea. The frontal peak fractions (monitored at 215 nm) were pooled and lyophilized.

Peptide mapping was done by two-dimensional high voltage paper electrophoresis (Whatman No. 1 paper, 50 V/cm, Savant tank apparatus). The first dimension was run at pH 6.5 (pyridine-acetic acid-water, 25:1:225 by volume) for 1 h. The paper strip containing the peptides was then cut into 2 pieces, one carrying the basic peptides and the other bearing the acidic and neutral peptides. For the second dimension, both pieces were stitched to separate papers. They were both run at pH 2.0 (formic acid-acetic acid-water, 1:4:45, by volume), the basic peptides for 45 min and the others for 75 min.

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The sequence analyses were performed on the whole chains using a Beckman model 890 C sequencer (Zürich) and a model 890 B (Basle). PTH-amino acids were identified either by means of TLC, GLC and mass spectroscopy (Basle) or, in Zürich, by high speed LC with the systems described previously[11]. Serine residues could only be identified with the latter method.

Results and discussion. The result of the chain separation is represented in Figure 1. The first peak consists of yellow material which is not protein but presumably material derived from the chromophore. The following 2 peaks of blue material, although not symmetrical, are the separated subunits with a maximum cross contamination of 5% (calculated from the first step of the sequenator analyses, assuming that there were no other background amino acids than those which stemmed from such cross contaminations). Figure 2 shows a comparison of the separated subunits with the starting material on cellogel electrophoresis. The minor component seen to accompany the β-subunit on Cellogel-electrophoresis cannot be observed in the CPC before separation of the subunits and the refore seems to be an artefact. Since both chains appear to be homogenous in the sequenator analysis, we assume that the minor component, as well as the shoulder in the α-chain peak from hydroxyapatite, reflects differences in the chromophore parts of the molecules and not in the polypeptide chains.

In the two-dimensional peptide maps (Figure 3) of tryptic peptides from both subunits the total number of peptides, as well as the number of arginine-containing peptides, are in good agreement with the numbers expected from the amino acid compositions. They are slightly smaller than the theoretical numbers (α-chain: 12 instead of 14; β-chain 10 instead of 13 and in both chains one arginine-peptide less). This had to be expected because both digests had contained insoluble peptides which could not be applied to the paper for electrophoresis. However, all but 2 peptides of each chain are located in such positions that they cannot possibly be common to both chains. Furthermore, the 2 peptides of similar location are amongst the neutral peptides for which such coincidence is less significant than for the charged peptides in the system we used. Thus it is quite unlikely that larger regions of identical sequence exist in the α- and β-chains of CPC from *M. laminosus*.

The results of the sequenator analyses are given in Figure 4. Within the first 11 residues, a minimum of 15 base changes (out of 33) is needed to explain how the two chains could have developped from a common ancestor. Thus, in this region the situation hardly differs from that of 2 different proteins with random distribution of amino acid residues, although there is a common threonine. For the next 17 residues, the picture changes greatly to a minimum number of 10 base changes out of 51 bases and 10 identical amino acid residues. From further sequenator results, which are not clear enough to be published in detail, we get the impression that there will be very little, if any, homology for the subsequent 6 residues, and no information is so far available for regions beyond this.

The C-phycocyanins are phylogenetically very old proteins. The observed large differences between the amino acid sequences of the 2 subunits can easily be explained under the assumption that a common ancestor diverged into the 2 chains a sufficiently long time ago. Most plausibly such divergence will have been enhanced by the gain of extra properties of the CPC as a whole, e.g. establishment of cooperativity, assuming the selection of the most successful molecules. On the other hand, the persistence till today of sequences from a common ancestor to both chains can only be understood by means of a strong functional imperative. Quite likely, the 2 subunits retain that degree of homology which is vital for maintenance of those functional properties which they must have in common with each other and with their common ancestor.

In this paper we demonstrate that such minimum homology is still quite apparent within the regions concerned.

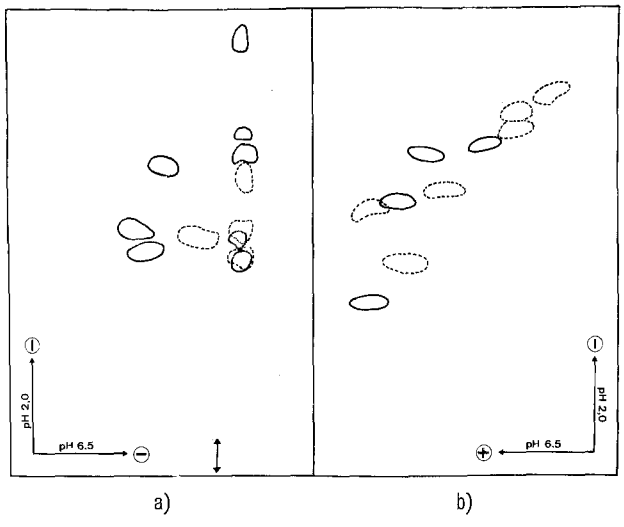

Fig. 3. Two-dimensional peptide maps of tryptic digests of the two CPC subunits. a) acidic and neutral peptides b) basic peptides. Solid outline, peptides derived from α-chain; dotted outline β-chain peptides. Ninhydrin-positive spots are shown.

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α-SUBUNIT	Ala	Tyr	Asp	Val	Phe	Thr	Lys	Val	Val	Ser	Gln	Ala	Asp	Ser	Arg	Gly	Glu	Phe	Leu	Ser	Asn	Glu	Gln	Leu	Asp	Ala	Leu	Ala	Asn
β-SUBUNIT	Val	Lys	Thr	Pro	Ile	Thr	Asp	Ala	Ile	Ala	Ala	Ala	Asp	Thr	Gln	Gly	Arg	Phe	Leu	Ser	Asn	Thr	Glu	Leu	Gln	Ala	Val	Ala	Gly
MINIMUM NO. OF BASE CHANGES	1	2	2	2	1	0	2	1	1	1	2	0	0	1	1	0	2	0	0	0	0	2	1	0	2	0	1	0	2

Fig. 4. Comparison of the first 29 amino acid residues of both subunits of CPC from *M. laminosus*. Minimum number of single spot mutations necessary to derive both chains from a common ancestor is listed in the 3rd line.

Zusammenfassung. N-terminale Sequenzanalyse der an SDS-Hydroxyapatit im präparativen Maßstab getrennten α- und β-Untereinheiten zeigte in den letzten 11 Aminosäureresten geringe, in den folgenden 17 Aminosäureresten aber starke Sequenzhomologie zwischen den beiden Polypeptidketten. Starke Unterschiede bestehen zwischen beiden Untereinheiten in den tryptischen Peptiden.

[13] We thank Miss CH. BECK and Miss E. USTER for their careful technical assistance. This work was supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung (Project No. 3.379.70).

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[15] We are grateful to Messrs. G. OESTERHELT and R. RUEHR, Physical Research Dept., F. Hoffmann-La Roche & Co. Ltd. for performing the GLC and mass-spectrometrical identifications of the PTH-amino acids.

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Increase in Cystathionine β-Synthase Activity in the Liver of Rats Treated with L-Ethionine

Cystathionine β-synthase (EC 4.2.1.21) is a pyridoxal-P-dependent enzyme catalyzing the formation of cystathionine from L-homocysteine and L-serine. Recently it has been found that both the cystathionine β-synthase and L-serine sulfhydrase (EC 4.2.1.22) activities are due to a single enzyme[1,2]. Deficiency, or absence, of cystathionine β-synthase activity caused by a genetic defect, results in a serious inborn disease called homocystinuria[3–6].

Ethionine is known to produce an inhibition of hepatic protein synthesis[7–10] and increase in the concentration of free amino acids in plasma and tissues[11]. This analog of methionine also causes an inhibition of phospholipid biosynthesis[12] as well as a fatty liver[13,14]. Treatment with ethionine induces a rapid fall in the liver ATP concentration[15–19] and causes a depression of RNA synthesis[20–23]. It has been demonstrated that ethionine causes an alteration in hepatic ionic composition[24–26], then a diminution of NAD and NADP levels[27] and a change in the poliamines content[28].

In view of the importance of transsulfuration in mammals, the effect of ethionine on cystathionine β-synthase activity is reported in this paper.

Table I. Specific activities of cystathionine β-synthase measured as L-serine sulfhydrase (in nmoles cysteine/mg protein/h)

Treatment	Cystathionine β-synthase
NaCl	75.8 ± 4
L-Ethionine	371.6 ± 18[a]
Actinomycin D	73.3 ± 9
Actinomycin D + L-Ethionine	359.2 ± 12[a]
Cycloheximide	72.8 ± 6
Cycloheximide + L-Ethionine	354.7 ± 21[a]
DL-Methionine	71.9 ± 8
DL-Methionine + L-Ethionine	89.9 ± 6
DL-Methionine sulfone	76.5 ± 4
DL-Methionine sulfone + L-Ethionine	363.5 ± 11[a]
L-Methionine-DL-sulfoximine	74.6 ± 5
L-Methionine-DL-sulfoximine + L-Ethionine	367.4 ± 15[a]
ATP	77.1 ± 8
ATP + L-Ethionine	366.8 ± 20[a]

Results are expressed as mean ± standard error for 12 rats. [a] Differs from NaCl-group at $p < 0.001$.

Materials and methods. Female and male albino rats weighing 180–220 g were used. L-ethionine, actinomycin D, cycloheximide and ATP were injected i.p. in doses of 400 mg, 1 mg, 20 mg and 200 mg per kg of body weight, respectively. DL-methionine, L-methionine-DL-sulfoximine

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