The Use of Mass Spectrometry in the Structural Elucidation of Scotophobin—A Specific Behaviour-inducing Brain Peptide

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Summary High resolution mass spectrometry elucidated the sequence of scotophobin, a pentadecapeptide shown to induce a specific behaviour in untrained mice.

MASS spectrometry has been used with increasing frequency in the structural elucidation of biological compounds such as biologically important peptides.[1] We report the sequencing of a pentadecapeptide shown to be responsible for transfer of a learned behaviour from one animal to another. The original training, testing, and extraction procedures have been published.[2] This substance has been named scotophobin.

Amino-acid analysis of scotophobin gave the following: 4 Glx, 3 Asx, 3 Gly, 2 Ser, Ala, Lys, and Tyr. Hydrolysis of scotophobin with trypsin produced two fragments, T_1 and T_2. Qualitative amino-acid analysis by microdansylation of the two fragments yielded the following: T_1 contained Asx, Glx, Gly, Lys, and Ser, and T_2 contained Ala, Glx, Gly, Ser, and Tyr. By microdansylation, we determined that the N-terminus of scotophobin, T_1 and T_2 was Ser.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Ser	Asx	Asn	Asn	Glx	Gln	Gly	Lys	Ser	Ala	Glx	Gln	Gly	Gly	$TyrNH_2$
			Asn	Asn	Glx	Gln	Gly	Lys	Ser	Ala	Glx	Gln	Gly	Gly	$TyrNH_2$
							Gly					Gln	Gly	Gly	$TyrNH_2$
(A)	Ser	Asx	Asn	Asn	Glx	Gln	Gly	Lys	Ser	Ala	Glx	Gln	Gly	Gly	$TyrNH_2$
(B)	Ser	Asp	Asn	Asn	Glu	Gln	Gly	Lys	Ser	Ala	Gln	Gln	Gly	Gly	$TyrNH_2$
(C)	Ser	Asn	Asn	Asn	Gln	Gln	Gly	Lys	Ser	Ala	Gln	Gln	Gly	Gly	$TyrNH_2$
(D)	Ser	Asp	Asn	Asn	Gln	Gln	Gly	Lys	Ser	Ala	Gln	Gln	Gly	Gly	$TyrNH_2$
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

FIGURE. *Sequence assignment*

About 300 μg of scotophobin were extracted originally. After the amino-acid analyses, about 100 μg remained. This was too small a sample for wet chemical methods of peptide sequencing such as Edman degradation or microdansylation, and mass spectrometry was used as it yields more sequence information per μg than any other method

20 μg of peptide were dissolved in 10 μl of water. The solution was treated with diazomethane. (At this time, the carboxy-end was presumed to be free.) The reaction product was treated with pyridine–trifluoroacetic anhydride (3 : 1). After being kept overnight, solvent was removed under vacuum, and the residue was used as the mass spectrometric sample in the capillary. Derivatives of the natural scotophobin, T_1 and T_2, were prepared in this manner. High resolution mass spectral data were obtained with CEC 21-110B (Lot 9) mass spectrometer using photographic ion-detection. The direct-introduction probe temperature was 220—240 °C. Accurate masses were obtained to within four millimass units. This accuracy permitted the assignment of a unique elemental composition to most fragments.

Unfortunately, no molecular ion was found in any spectrum. These spectra showed that the oligopeptides had been pyrolytically fragmented. Fortunately, di- and tri-peptide fragments were produced (Figure) that overlapped sufficiently to permit us to assign sequence A to the peptide. Ambiguities at positions 2, 5, and 11 were found, due to the possible loss of ammonia from asparagine and glutamine.

Sequence B was synthesized by Dr. B. Weinstein (Univ. of Washington) and had 2% biological activity. Sequences C and D were synthesized by Dr. W. Parr (Univ. of Houston). Sequence C had 10% and sequence D had 100% biological activity.[3] Thus, the first member of a family of substances that code information in the brain has been isolated, sequenced, and synthesized.

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