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POSITIVE AND NEGATIVE ION FAST ATOM BOMBARDMENT MASS SPECTROMETRIC STUDIES ON CHLOROPHYLLS : STRUCTURE OF 4-VINYL-4-DESETHYL CHLOROPHYLL B

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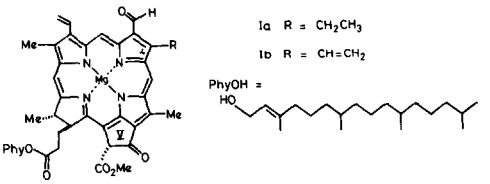
Summary : *Positive and negative ion Fast Atom Bombardment mass spectra have been used to study chlorophylls at pmol levels in a matrix of thiodiglycol, and the molecular ion is used to distinguish reliably the 2 mass unit shift between chlorophyll b and 4-vinyl-4-desethyl chlorophyll b.*

Good mass spectra of chlorophylls are very hard to obtain because, first, chlorophylls readily decompose to pheophytins (loss of Mg) and allomers (oxygen incorporation into ring V) under only very gentle conditions; second, chlorophylls are very involatile. The interpretation of the spectra is further complicated because Mg has significant proportions of heavy isotope. We originally overcame the problem of volatilisation by using "in beam" electron impact (EI/D) mass spectra[1] from a quartz probe tip; another group used a similar method with a gold probe tip[2]; however problems of pheophytinisation were great. We have used[3,4] Fast Atom Bombardment (FAB), a technique hitherto mainly used for polar molecules such as peptides[5,6,7] and successfully recorded the spectra of 4-vinyl-4-desethyl chlorophyll a and bacteriochlorophyll a using a matrix of chloroform/trigol. Significant $M+H^+$ peaks were observed.

The matrix of chloroform/trigol is too volatile for general use, and we now report improved FAB mass spectra using thiodiglycol (2,2'-thio-*bis*-ethanol) as a matrix. Chlorophyll b (**Ia**) was extracted from

Zea mays L. and dried for several days under vacuum over silica gel prior to use[8]. A sample was dissolved in 1 - 5 μl of either AR chloroform (which had previously been passed over a silica column to remove traces of HCl which would otherwise pheophytinise the chlorophyll) or alternatively AR acetone and was added directly to 1 - 2 μl of thiodiglycol on the probe tip of a Kratos MS-50 mass spectrometer. The sample was bombarded with a 7-9 KeV Xe beam, and spectra were recorded.



Structures of chlorophyll b (Ia) and 4-vinyl-4-desethyl chlorophyll b (Ib)

We were able to obtain good signal to noise ratios on 100 pmol of chlorophylls (as determined by extinction coefficients), and in all cases we used less than 1 nmol quantities. With optimum spectrometer tuning it is likely that mass spectra of chlorophylls from quantities of only a few pmol could be recorded.

In the positive ion mass spectra the most intense peak was at 907 mass units corresponding to a mixture of the "plus one" heavy isotope peak of $M^{\cdot +}$ (which is normally intense in chlorophylls as the natural abundance of ^{24}Mg is only 78.7%), and the even electron species $M+H^+$. The relative intensities (measured by background corrected peak heights) were averaged for 4 spectra, and the theoretical intensities for $M^{\cdot +}$ and $M+H^+$ were computed. Using constrained minimisation[9] we can fit the isotopic cluster to a mixture of $M^{\cdot +}$: $M+H^+$ of 0.94 : 1 (Table). We performed a similar experiment in the negative ion mass spectra, and find that the ratio of $M^{\cdot -}$: $M-H^-$ is 3.4 : 1 . Because of the problems of background subtraction the exact ratios should be interpreted with caution.

These data are in contrast to those from peptides, in which the predominant species observed are the even electron $M+H^+$ and $M-H^-$ ions in the positive and negative ion spectra repectively. The marked difference in behaviour can be attributed, at least partially, to the ease of radical formation of chlorophylls due to the delocalised π system of the chlorin macrocycle, which is consistent with their well known biological role. Both radical cations[10] and radical anions[11] can be formed in solution and studied by nmr very readily. However, the radical anions of chlorophylls

are generally less stable in solution than the corresponding cations. The apparent relative intensities of the paramagnetic species in the mass spectra are due to the stabilities of the diamagnetic $M+H^+$ and $M-H^-$ compared to $M\cdot^+$ and $M\cdot^-$ respectively rather than the inherent stabilities of the paramagnetic species. When phenol or hydroquinone was added as a radical quencher to the matrix, no significant change in the mass spectrum was observed. This could mean that the chlorophylls are ionised in the gas phase or if the radical ions are produced in the liquid matrix, then they must be expelled faster than they can be quenched.

	Calculated spectra			Positive FAB		Negative FAB	
Mass	CHL b	CHL b + H	Chl b - H	OBS	PRED	OBS	PRED
905	-	-	100.0			30	27
906	100.0	-	77.3	56	56	100	114
907	77.3	100.0	44.1	100	102	85	84
908	44.1	77.3	16.9	66	70	52	46
909	16.9	44.1	4.6	38	35	26	17
910	4.6	17.0	1.0	14	12		
911	1.0	4.6	0.2				

Table : Calculated relative intensities (base peak = 100) for chlorophyll b, chlorophyll b + H and chlorophyll b - H, measured positive and negative ion FAB intensities, and predicted intensities using curve fitting described in the text.

The practical importance of these observations can be demonstrated when using mass spectra to determine the molecular weight of a new chlorophyll (Ib). The structure 4-vinyl-4-desethyl chlorophyll b was previously predicted from absorption and fluorescence studies,[12,13] and differs in only 2 mass units from chlorophyll b. Using most techniques it is impossible to reliably determine such a small difference. However as illustrated (Figure) both positive and negative ion FAB mass spectra reflect this 2 unit shift. Peaks due to loss of phytyl (not illustrated) are also consistent with the two unit mass difference.

In conclusion FAB is a useful technique for molecular weight determination of novel chlorophylls. However, in view of the possible production of both even electron and odd electron ions, it is important to use both positive ion and negative ion FAB mass spectra.

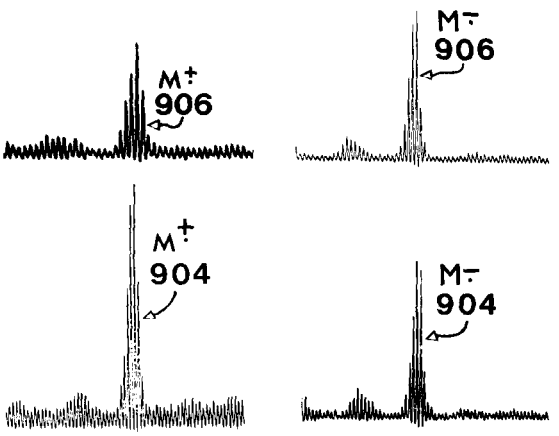


Figure : positive (left) and negative ion (right) FAB mass spectra of the molecular ion cluster of chlorophyll b (top) and 4-vinyl-4-desethyl chlorophyll b (bottom).

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