blood glucose, as well as lowering growth hormone levels, the results being qualitatively similar to those observed with somatostatin.

In the gastric secretion studies it was noted that Id maintained the same inhibitory effect for about 30 min after the infusion had stopped, whereas the inhibitory effect of somatostatin and of Ic was lost shortly after the infusion had been discontinued. This prolonged duration of the action of Id may be a result of the elimination of those structural features which would lead to metabolism by aminopeptidases, carboxypeptidases, and reduction.

We conclude from the high biological activities of Ic and Id that the cyclic form of somatostatin can be active, that the disulfide bridge does not play a functional role, and that the presence of a terminal carboxyl group is not required for these activities.

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(7) Unless otherwise designated all chiral amino acids are of the L-configuration. Abbreviations used: iNoc = isonicotinyloxycarbonyl; OMe = methyl ester; O-*t*-Bu = *tert*-butyl ester; DCCI = dicyclohexylcarbodiimide; TFA = trifluoroacetic acid; Tcp = trichlorophenyl; Asu = α-aminosuberic acid; Aha = ω-aminoheptanoic acid.
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X-Ray Photoelectron Spectroscopic Studies of the Thermal Stability of Chlorophyll a Monohydrate

Sir:

The x-ray photoelectron spectrum (XPS) of porphyrins and related molecules has been of current interest due to the important structural details inherent in the N 1s, C 1s, and metal spectra.[1] An extension of XPS determinations to a characterization of chlorophyll (Chl) a-H_2O interactions appears to be desirable. Recently it has been inferred from optical and water titration data that the driest Chl a prepared from existing procedures (heating under vacuum up to 80 °C for a prolonged period) occurs as a monohydrate.[2] The intimate relationship between Chl a and H_2O lies at the heart of the photosynthesis problem.[3] It is believed that the primary molecular adduct P700[4] in photosystem I is a symmetrical dimeric aggregate of Chl a monohydrate.[3a,5]

The objective of the present work is twofold: (1) By XPS determination of the O 1s spectrum, we hope to make a direct experimental observation of the chlorophyll monohydrate. (2) Using XPS as a monitor, we hope to chart the course of the dehydration of the tightly bound[2] water as the temperature is incrementally raised above 80 °C, the temperature commonly employed as the upper limit in most drying procedures.[2,6]

The Chl a was extracted from spinach and purified in the usual manner.[2] Film preparation was accomplished using the sample preparation equipment available on the modified Hewlett-Packard 5950A ESCA spectrometer.[8] An atomically clean gold surface was transferred from the sample preparation chamber with a residual pressure 5×10^{-9} Torr into the attached N_2 atmosphere box. About 10^{15} Chl a molecules cm^{-2} were deposited on this gold surface by allowing 2 μl of a 3×10^{-4} M solution of Chl a in highly purified butyronitrite[9] × evaporate. The "dry" film was then inserted into the analyzer section of the ESCA instrument which has a residual pressure of 2×10^{-9} Torr. The film was thick enough so that no gold peaks were visible in the XPS spectrum. Slight charging effects (~1-2 eV) were partially compensated by using an electron gun[8] that floods the sample with low energy (<1 eV) electrons. Measurement of accurate peak positions is not possible using this approach, but the spectral distributions can be obtained with high accuracy. The gold blank was spectroscopically examined (Figure 1h) in order to exclude the possible contamination of the sample spectrum from an adventitious source.

The 30 °C O 1s spectrum of Chl a, given in Figure 1a, is referenced to the corresponding C 1s binding energy (284.3 eV at 30 °C) to offset the charging effects that are, within experimental errors, the same for both the C 1s and O 1s spectra in the temperature range 30-125 °C. We assign the high binding energy shoulder (533.1 eV at 30 °C) to the oxygen of the Chl a water of hydration. The value 533.1 eV for the O 1s binding energy appears to be indistinguishable from that found for condensed H_2O,[10] or H_2O present in various types of hydrated samples.[11] The main band centered at 531.8 eV appears to be an overlap of spectral contributions attributable to the five oxygen atoms of the Chl a C7 propionic ester, C10 carbomethoxy, and C9 keto groups. This value agrees well with the O 1s value of 531.4 eV reported for the oxygens in sodium benzoate.[12]

The attribution of the high binding energy shoulder at 533.1 eV to the presence of water of hydration is supported by the sequence of XPS Chl a O 1s spectra measured at different temperatures in the 30-250 °C range. No discernible changes are observed as the sample temperature is varied from 30 to 120 °C (Figure 1a-c). At temperatures exceeding 120 °C the high binding energy shoulder begins to diminish and, at 250 °C, this shoulder appears to have vanished quantitatively (Figure 1d-g).

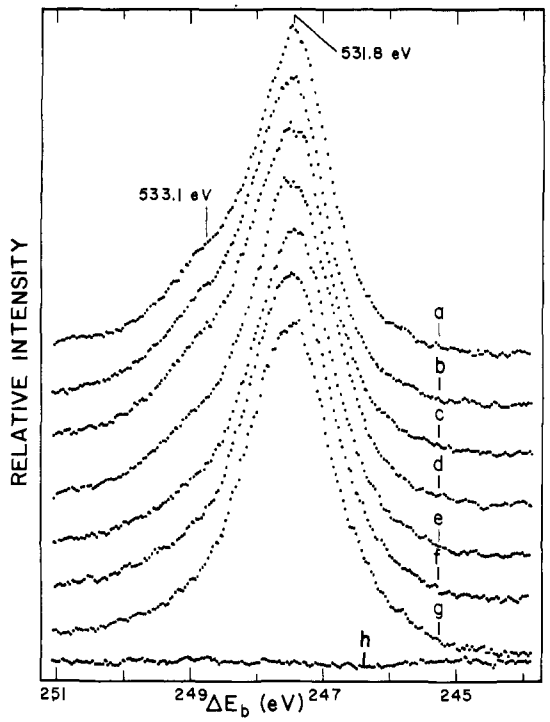

Figure 1. Oxygen 1s spectrum of Chl a. The ΔE_b is defined as the difference between the binding energy of the O 1s peak and the C 1s peak: (a) 30 °C, (b) 90 °C, (c) 120 °C, (d) 150 °C, (e) 180 °C, (f) 210 °C, (g) 250 °C, (h) gold blank.

A computer deconvolution of the O 1s spectrum (30–120 °C) reveals an approximate 5:1 area ratio for the main band and the high binding energy shoulder, consistent with the monohydrate stoichiometry of Chl a·H_2O.[2,13,14] The sample chlorophyll after heat treatment at 250 °C was redissolved in diethyl ether. The absorption spectrum of this solution had maxima at 428.2 and 660.2 nm reproducing those expected of pure Chl a solutions in ether.[2a] However, a blue-red peak absorbancy ratio of 3.2 (instead of the corresponding value 1.29 ± 0.01 observed[2a] for the sample prior to the heat treatment) was obtained. In addition, an onset at 380 nm of a pronounced absorption edge (absent in the case of pure Chl a) into the near-uv region was also found. These optical measurements are indicative of probable Chl a degradation as a result of the dehydration of the tightly bound[2] water of hydration.[6,7] We believe the present determination to be the first direct spectroscopic observation of the water of hydration bound to Chl a. Earlier conclusions of the stoichiometric formulas for Chl a-H_2O complexes had been derived from optical measurements interpreted in terms of an equilibration of multiple Chl a-Chl a and Chl a-H_2O aggregates.[2,15]

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(6) The "tightly bound" water refers to the remaining water of hydration as the dihydrate Chl a·2H_2O loses one H_2O molecule to yield the corresponding monohydrate.[2] It appears reasonable to suppose that the tightly bound water molecule plays a role in the stabilization of the five-coordinated Mg atom in Chl a·H_2O, and that the removal of this water may be concomitant to degradation of the chlorophyll molecule.[7]
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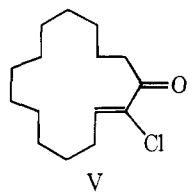
Additions and Corrections

A Synthesis of *d,l*-Muscone from Cyclododecanone [*J. Am. Chem. Soc.*, **97**, 1264 (1975)]. By GILBERT STORK* and T. L. MACDONALD, Department of Chemistry, Columbia University, New York, New York 10027.

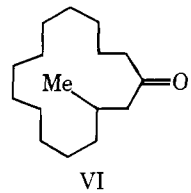
On page 1264, line 2, instead of muscone (V), read muscone (VI).

On page 1265, line 9, instead of δ 4.42 quintet, J = 7.0 Hz, 1 H, read NMR δ 4.35 t, J = 7.0 Hz, 0.8 H (cis isomer), 4.58 t, J = 7.0 Hz, 0.2 H (trans isomer).

Structures V and VI should be:



V



VI

Reference 2: Instead of 6.18 (m, 1 H), read 6.18 (d, J = 15.5 Hz, 1 H). Instead of "the β,γ isomer", read the (cis and trans) β,γ isomer.