Fluorescence Spectra of Chlorophyll Excited by a Continuous Gas Laser

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THE fluorescence spectra of both chlorophyll-a and chlorophyll-b in diethyl ether have been reported by Zscheile and Harris.[1] These workers, using mercury-arc excitation, showed that spectra observed were very dependent on the amount of reabsorption present. Their results for chlorophyll-b indicate that for a very thin layer, when reabsorption is small, the emission maximum is at 648·0 nm. with a high energy cut off at about 620·0 nm.

We have measured the emission spectra of chlorophyll using a Spectra Physics Model 125 He–Ne gas laser emitting approximately 50 milliwatts at 632·8 nm. The spectra were recorded photoelectrically using a Spex 1400-II double monochrometer and photon-counting techniques.

We were surprised to observe emission out to 550 nm., 83 nm. (2400 cm.$^{-1}$) to the high-energy side of the laser emission. This high-energy emission seems to be more or less independent of solvent, the spectra of 2×10^{-5} M-chlorophyll-b in both ether and benzene being shown in Figure 1. (All spectra have been normalised to an intensity of 10^5 at the emission peak.) Similar emission was observed from chlorophyll-a and chlorophyll-b, although the intensity was somewhat greater for chlorophyll-b.

The possibility that the observed high-energy emission arises from some mechanism other than fluorescence has been investigated.

All the spectra shown are for solutions in which no attempt was made to exclude oxygen. However, exclusion of oxygen by sample handling on a high-vacuum system did not affect the observed emission. Furthermore, the effect of β-carotene, a good triplet quencher, was investigated, and again there was no reduction in intensity of emission. Thus the possibility that the high-energy emission occurs *via* triplet states, possibly triplet–triplet annihilation, appears to be excluded.

The possibility that two-photon absorption is occuring, to raise the chlorophyll molecule to some higher energy level from which it emits, has also been considered. This process can be excluded as a plot of the logarithm of the emission intensity against the logarithm of the laser intensity gives a straight line with unit gradient over a 100-fold range of incident intensity.

In an attempt to confirm that the whole emission is due to fluorescence, the effect of nitrobenzene, an efficient singlet quencher, was determined. The emission from a benzene solution of 5×10^{-5} M-chlorophyll-b and 10^{-2} M-nitrobenzene is shown in Figure 1, where the curve has been normalised as explained. It can be seen that the emission on the high-energy side is virtually identical to that observed with no nitrobenzene present. The overall intensity was reduced by a factor of about 2, as would be expected for this concentration of nitrobenzene.[2]

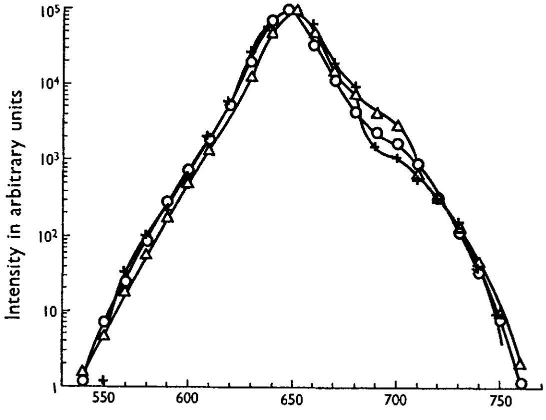

FIGURE 1

Emission from chlorophyll-b at 290° K.

○ 2×10^{-5} M-*Chlorophyll-b in ethyl ether.*
△ 2×10^{-5} M-*Chlorophyll-b in benzene.*
+ 2×10^{-5} M-*Chlorophyll-b plus* 2×10^{-2} M-*nitrobenzene in benzene.*

While this confirms that the emission is entirely fluorescence, it does not explain why the emission extends to some 2400 cm.$^{-1}$ to the high-energy side of the exciting frequency.

One possible, but unlikely, explanation is that at room temperature, before excitation, chlorophyll molecules exist in an energy level at least 7 kcal. (2400 cm.$^{-1}$) above the ground state, but after excitation to the first excited state (zero vibrational level, by internal conversion if necessary), the molecules decay to levels between this hypothetical level and the ground state.

A more acceptable, but nevertheless rather surprising, explanation is as follows. At room temperature, molecules exist in the lowest energy state, S_0, with a Boltzmann distribution in the higher vibrational states of the ground electronic state. Excitation occurs from all these levels to vibrationally excited states of the first excited electronic singlet state, S_1. Fluorescence must then occur from these vibrationally excited states of S_1 to the zero vibrational state of S_0.

While the idea that emission occurs from high vibrational states of a given electronic state is novel, the intensity of the emission we observe on the high-energy side of the laser frequency corresponds approximately to a Boltzmann distribution in S_0 at room temperature.

In an attempt to verify this explanation, the effect of lowering the temperature of the chlorophyll solution was investigated.

The temperature effect on fluorescence emission is very complex due to the superposition of several effects such as changes in solution concentration, refractive index, and extinction coefficient, and also to possible shifts in the molecular energy levels. The effect of temperature on the fluorescence spectrum of chlorophyll has been shown to move the emission maximum to longer wavelength as the temperature is lowered.[1]

If our postulate regarding thermal excitation is correct, we would expect a reduction of intensity on the high-energy side relative to the peak emission as the temperature is lowered, accompanied by a shift of the peak to lower energy. We have obtained the emission spectrum of 2×10^{-5} M-chlorophyll-b in ether at $-95°$, and the spectrum is shown in Figure 2, together with a spectrum of the same solution taken at room temperature. There is a shift of 10·4 nm. to longer wavelength as expected. But we also observe a reduction in the high-energy emission relative to the low-energy emission even after correcting for the overall shift of the emission.

Our results are consistent with the postulate that the extra energy comes from thermal population of vibrational levels of S_0, and that emission is occurring from high vibrational states of S_1.

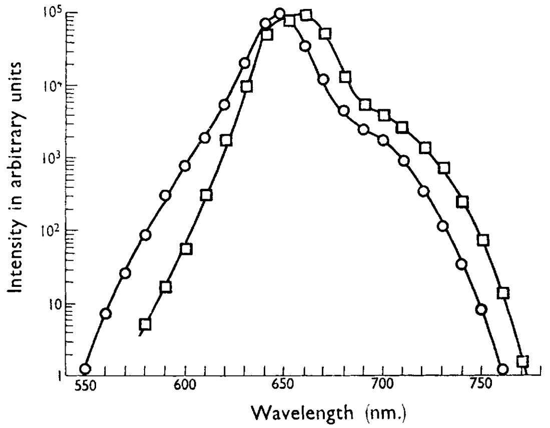

FIG. 2. *Emission from* 2 × 10^{-5} M-*Chlorophyll-b in ethyl ether.*

○ *at* 290° K. △ *at* 178° K.

Recently Kobyshev *et al.*[3] have reported high-energy emission from chlorophyll excited by a ruby laser. Their main peak was at 480 nm., very different from our emission, and it is felt that the two effects are unrelated, the 480 nm. emission probably arising from two-photon effects.

Possibly another indication of the correctness of our postulate is the shoulder at 640 nm. appearing on the −95° spectrum. It may well be that this corresponds to the zero vibration $S_1 \rightarrow S_0$ emission which we would expect to increase in intensity at the lower temperature as the higher vibration states of S_0 are depopulated in favour of the zero-vibration state.

It is intended to extend these measurements to lower temperatures and conditions of less self absorption.

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