properties of TM_A and myosin and certain features of their amino-acid composition have suggested[7,8] the possibility that vertebrate muscle contains TM_A as an integral part of the myosin molecule and not in a free form. If so, one would expect to obtain a 'tryptic fragment' similar to that obtained from TM_A, after tryptic hydrolysis of myosin. Experiments designed to test such a hypothesis were performed and it was found that although some TCA-insoluble material was obtained after digestion for 24 h, its solubility properties in ammonium sulphate were quite different from the TM_A 'tryptic fragment'. No material was precipitated at ammonium sulphate saturation exceeding 50 per cent (TM_A 'tryptic fragment' precipitates between 38–50 per cent saturation), suggesting that it is composed of smaller peptides.

The fact that the 'tryptic fragment' has been found in TM_A of three different species but not in TM_B suggests a structural characteristic which differentiates these two molecules. Whether this chemical characteristic is related to its functional properties remains to be seen.

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[1] Bailey, K., *Pubbl. Staz. Zool. Napoli*, **29**, 96 (1956).
[2] Bailey, K., and Milstein, C. P. de, *Biochim. Biophys. Acta*, **90**, 492 (1964).
[3] Bailey, K., Milstein, C. P. de, Kay, C. M., and Smillie, L. B., *Biochim. Biophys. Acta*, **90**, 503 (1964).
[4] Milstein, C. P. de., thesis, Univ. Buenos Aires.
[5] Bailey, K., and Rüegg, J. C., *Biochim. Biophys. Acta*, **38**, 239 (1960).
[6] Kominz, D. R., Saad, F., and Laki, K., *Conference on the Chemistry of Muscular Contraction*, 66 (Igaku Shoin Ltd., Tokyo, 1957).
[7] Bailey, K., *Biochem. J.*, **43**, 271 (1948).
[8] Laki, K., *Nature*, **193**, 269 (1962).

Distribution of Chlorophylls in Chloroplast Fragments

THE molecular arrangement of the lipid-protein layers in chloroplasts, chloroplast grana, and smaller sub-units, is unknown but is vital to understanding the photophysical and photochemical events that occur there.

Recently, Boardman and Anderson[1] fragmented spinach chloroplasts with digitonin and showed a changing ratio of chlorophyll *a* to chlorophyll *b* in fractions separated by differential high-speed centrifugation. A fraction sedimenting between 50,000 and 144,000*g* had an *a*/*b* ratio of 6 and was interpreted to represent pigment system 1, the long wave-length system of photosynthesis. Fractions sedimenting at 10,000*g* or less had an *a*/*b* ratio of 2 and were interpreted to represent pigment system 2, the short wave-length system.

Murakami *et al.*[2] have also extracted spinach chloroplasts with digitonin and found a progressively increasing chlorophyll *a*/*b* ratio from 2·15 in whole chloroplasts to 12·0 in the supernatant after centrifugation at 105,000*g*. Their investigations showed reduced Hill activity and triphosphopyridine nucleotide reduction in the high-speed fractions suggesting that light-dependent reaction 2 was lost as particle size decreased. This was interpreted to mean that there was loss of an essential component(s), which, they point out, is supported by the concomitantly increasing chlorophyll *a*/*b* ratio, thus signifying loss of chlorophyll *b*.

We have mechanically fragmented spinach chloroplasts by sonication in an aqueous suspension rather than by using digitonin; fragment suspensions were then fractionated by differential centrifugation. The experimental conditions and centrifugation times have been published in detail[3–5]. The fractions (CF) are designated by subscripts indicating the centrifugal force range used to sediment the particular fraction. For example, CF_{1-20} is the fraction that pelleted between 1,000 and 20,000*g*.

Chlorophyll content was assayed in 80 per cent acetone and computed according to Arnon's equations[6]. Boardman and Anderson used Vernon's equations[7]. The method used by Murakami *et al.* is not reported. Thus the differences in absolute values between our results (Table 1) and theirs[1,2] may be due to the method of calculation or perhaps to inherent differences in the spinach. The highest *a*/*b* ratio occurred in CF_{70-145}, which corresponds to Boardman and Anderson's 50,000–144,000*g* fraction. The *a*/*b* ratio of 3 in the lower-speed pellet, CF_{1-20}, containing large fragments, corresponds fairly well to Boardman and Anderson's 1,000–10,000*g* fraction. The 145,000*g* supernatant ($CF_{145\ spt}$) shows a decreased *a*/*b* ratio relative to the CF_{70-145} fraction, which also agrees with the studies of the Australian investigators.

Table 1. CHLOROPHYLL CONTENT OF FRACTIONS ISOLATED FROM SPINACH CHLOROPLASTS

Fraction	Chlorophyll (mg)	Absorbancy ratio†	*a*/*b*
Chloroplast suspension	60·5*	2·56	2·74
CF_{0-1}	3·1		
CF_{1-20}	33·4	2·66	3·01
CF_{20-50}	16·4	2·73	3·25
CF_{50-70}	2·8		
CF_{70-145}	2·3	3·02	4·00
$CF_{145\ pts}$	1·5	2·82	3·32

* Average chlorophyll yield from 800 to 1,000 g wet weight of de-ribbed spinach leaves.
† Ratio of 663 mμ/645 mμ.

Preliminary investigations on small chloroplast particles sedimenting at 173,000*g* from $CF_{145\ spt}$ also showed a differential distribution of chlorophyll content. These small fragments, referred to as polyquantasomes, are photoactive[5,8].

Although the significance of these results is not clear, Boardman and Anderson's assignment of their 50,000–144,000*g* fraction to system 1 and of the remaining fractions to system 2 cannot be accepted unequivocally. The fraction sedimenting at 1,000–10,000*g* (system 2) is probably composed of particles greater than 1500 Å in diameter with an average molecular weight greater than 1×10^8 (refs. 5, 8). The fraction sedimenting at 50,000–144,000*g* is probably composed of particles 600–700 Å in diameter with an average molecular weight of 1×10^7 (refs. 5, 8). The sizes of all these particles are immense in comparison with quantasomes[9] or polyquantasomes[5], and the fractions are unquestionably composed of a considerable number of such sub-units. It is apparent that additional study of small-particle fractions is essential to establish firmly that the chlorophyll of spinach chloroplasts is associated with two different particles representing the two pigment systems.

Nevertheless, regardless of the fragmentation method, the changing chlorophyll ratios when correlated with the size of particles in the centrifuged fractions suggest that natural fault lines exist in the chloroplast lamellar structure along which rupture is most likely to occur when the organelle is subjected to chemical or physical stress.

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[1] Boardman, N. K., and Anderson, J. M., *Nature*, **203**, 166 (1964).
[2] Murakami, S., Katoh, S., and Takamiya, A., in *Proc. First Intern. Symp. Cellular Chem.*, Ohtsu, Japan (1963). *Intracellular Membranous Structure*, 173 (Chugoku Press, Japan, 1965).
[3] Becker, M. J., Shefner, A. M., and Gross, J. A., *Nature*, **193**, 92 (1962).
[4] Becker, M. J., Gross, J. A., and Shefner, A. M., *Biochim. Biophys. Acta*, **64**, 579 (1962).
[5] Becker, M. J., Shefner, A. M., and Gross, J. A., *Plant Physiol.*, **40**, 243 (1965).
[6] Arnon, D. I., *Plant Physiol.*, **24**, 1 (1949).
[7] Vernon, L. P., *Analyt. Chem.*, **32**, 1144 (1960).
[8] Gross, J. A., Becker, M. J., and Shefner, A. M., *Nature*, **203**, 1263 (1964).
[9] Park, R. B., and Biggins, J., *Science*, **144**, 1009 (1964).