DICHROISM IN ENZYME-TREATED CHLOROPLASTS

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SUMMARY

Lipase-treated spinach chloroplasts show a decreased dichroism. Treatment with protease yields rather scattering results, be it that in most cases protease causes the dichroic effect to increase. The results are discussed in terms of an earlier suggested hypothetical model of a quantasome.

According to an earlier proposed hypothetical model of a quantasome, it has been suggested that the chlorophyll molecules occur in the photosynthetic unit at the surface of lipoprotein particles which are partly embedded in a lipid layer (THOMAS *c.s.* 1967). The reason for proposing such a chlorophyll arrangement was derived from dichroism measurements which indicated that the chlorophyll-*a* form absorbing around 680 nm, C*a*680, is mainly or solely responsible for the observed dichroic effect in 0.18 M sucrose containing media. In this connection it seemed worth while to study the response of the dichroic effect to removal of either protein or lipid by enzymic digestion. BAMBERGER & PARK (1966) investigated the action of a protease and a lipase on spinach chloroplasts, and concluded that chlorophyll is likely to be associated with particles consisting of both protein and lipid as well as with their embedding matrix, rather than with an underlying galactoplipid layer.

Because of the poor degree of orientation dichroism, it has been suggested in the above-mentioned model that chlorophyll is confined to these particles proper, whereas the partly embedding lipid layer – as arranged according to the model proposed by MÜHLETHALER *c.s.* (1965) – is believed to be chlorophyll-free.

Chloroplast suspensions were prepared from spinach, some purchased and some obtained from the botanic gardens, using a 0.02 M phosphate buffer, pH 7.3. The chloroplasts were oriented and dichroism was measured as described earlier (THOMAS 1967). Because of the considerably increased scattering of results with chloroplasts in sucrose-containing media (THOMAS *c.s.* 1967), this sugar was omitted in the present experiments. The measured dichroic effects therefore, represent a combination of structural and intrinsic dichroism. The hydrolytic enzymes used are hog pancreas lipase (steapsin) type II and *Streptomyces griseus* protease type VI, both purchased from Sigma Chemical Co. All enzyme solutions were freshly prepared. 8 mg protease were dissolved in 2 ml phosphate buffer, pH 7.3., and added to 15 ml chloroplast suspension of

such a density that the absorption in the red maximum amounted to 40%. After incubation at room temperature, 20°C, for 1 hr this suspension as well as a blank were placed on ice, and measured when cold. With lipase, 125 mg of the crude enzyme were added to 5 ml of the mentioned buffer, homogenized, and centrifuged at 10.000 × *g* for 10 minutes. The supernatant was mixed with 20 ml chloroplast preparation of the above concentration, incubated, together with a blank, at room temperature for 2 hrs, subsequently placed on ice, and used when cooled down. For reasons derived from fluorescence experiments (BRIL *c.s.*, to be published), the incubation period with lipase was chosen twice that with protease. Each series consisted of 10 experiments. The results are summarized in table 1.

Since, at room temperature, dichroism in isolated chloroplasts declines with time (THOMAS *c.s.* 1967), whereas the rate of this decline may vary for different samples, the values of the blanks are lower and scatter more than when measured immediately upon preparation. As the blanks had to be kept under the temperature conditions of the enzyme containing samples during incubation, this disadvantage could not be circumvented. The fact that, on the whole, the blanks for protease activity are lower than those of the lipase experiments is due to seasonal variations (THOMAS *c.s.* 1967). For most of the latter experiments and for those with protease the used spinach was grown in the fall and in early spring respectively.

Except for one case, lipase treatment resulted in decrease of dichroism, whereas with protease the results scattered considerably more, as expressed in the standard deviation. In most cases increase of the dichroic effect was observed with protease. The experiments, *table 1*, are given in sequence of increasing dichroism of the blanks. Such is done in order to show that there might be a relation between

Table 1. Effect of incubation with lipase, 2 hours, and protease, 1 hour, on dichroism in spinach chloroplasts. Dichroism expressed in % of total chlorophyll *a* absorption. Experiments performed in sequence according to experiment number, and presented in sequence of increasing dichroism of the blanks.

expt. nr.	dichroism, %			lipase effect, %	expt. nr.	dichroism, %			protease effect, %
	− lipase a	+ lipase b	b−a	$\frac{b-a}{a} \times 100$		− protease c	+ protease d	d−c	$\frac{d-c}{c} \times 100$
2	2.6	0.0	−2.6	−100	I	0.9	6.3	+5.4	+600
5	3.5	3.8	+0.3	+ 9	III	1.0	7.8	+6.8	+680
1	4.6	0.0	−4.6	−100	X	1.9	8.8	+6.9	+363
6	6.9	3.4	−3.5	− 51	V	2.0	3.5	+1.5	+ 75
8	6.9	1.9	−5.0	− 72	VII	4.9	6.3	+1.4	+ 29
10	7.0	3.2	−3.8	− 54	IV	5.1	9.8	+4.7	+ 92
3	7.1	0.9	−6.2	− 87	VI	5.1	6.2	+1.1	+ 21
9	9.5	7.3	−2.2	− 23	IX	5.2	3.1	−2.1	− 40
4	11.1	10.0	−1.1	− 10	II	6.0	4.1	−1.9	− 32
7	11.7	6.7	−5.0	− 43	VIII	6.9	4.7	−2.2	− 32
mean lipase effect				− 53 ± 12*				protease	+ 176 ± 86*

* standard deviation of the mean.

original dichroism and enzyme effect. In particular with protease, it might be that the lower the dichroism of the blank, the higher the dichroic effect upon incubation. In this respect it may be worth mentioning that three additional experiments on the time course of enzyme-induced dichroic changes were made. With incubation times of 30, 60 and 120 minutes, lipase caused a decline of dichroism for all periods, whereas protease induced an initial drop, then a rise, and finally, a drop again. However, notwithstanding the latter drop, the dichroism remained enhanced when compared with the blanks.

As a rule, the location of the dichroic maximum for the enzyme-treated preparations and the red absorption maximum of the same non-treated suspension differed slightly, about 4 nm, in two cases 10 nm. As these variations were at random, they are not indicative of a chlorophyll form other than C_a680 becoming oriented due to enzyme action.

In terms of the above mentioned hypothetical model of a quantasome, the effect of enzymic digestion can be explained as follows. Lipase is likely to attack the free lipids in the lipid layer, partly embedding the lipoprotein particles, earlier than the bound lipids in these particles. As a result, these particles may be entirely or partly set free, and, consequently, become liable to displacement into random directions. As a consequence, the overall dichroism should decline. In addition, the enzymic digestion of the lipid layer will result in a decline of structural dichroism. Protease, on the other hand, leaves the lipid layer intact, but causes destruction of the partly embedded particles. This process most probably implies changes in shape of the particles which, according to the suggestions of MÜHLETHALER *c.s.* (1965), are assumed to be spherical. The same holds in case of somewhat flattened particles as proposed by BAMBERGER & PARK (1966). As it is indicated by the fact that the measured values scatter considerably for the protease experiments, such a destruction may proceed in a more or less irregular way. The fact that, on the whole, dichroism is increased after a one-hour incubation with protease could be explained by assuming that the lipoprotein particles tend to flatten as a result of destruction. This flattening might cause increased orientation parallel to the plain of the thylakoid, and thus enhance the dichroic effect. According to the model, this enhancement is likely to be confined to C_a680, C_a670 being randomly oriented and protected from enzymic action by the surrounding lipid layer, whereas C_a is arranged in such a way that changes of the particle shape need not affect the absence of dichroism for this chlorophyll. The observation that no appreciable shift of the dichroic band maximum was observed, is in keeping with this explicatory way.

Contrary to MÜHLETHALER *c.s.* (1965) who proposed a model for the chloroplast lamellae in which lipids occur as a continuous layer partly embedding the quantasomes, BRANTON & PARK (1967) suggested that these particles are associated with a matrix consisting of densily packed protein subunits. Though the present experiments do not allow to discriminate between both models, the above results can be more readily explained in terms of Mühlethalers' concept.

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