EOSIN-SCN, A TRIPLET PROBE FOR CONFORMATIONAL CHANGES OF CF1.
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The relatively long lived triplet state of eosin-isothiocyanate, which covalently binds to CF1, can be used to follow conformational changes of CF1 spectrophotometrically in either of two ways: 1.) Changes of the triplet lifetime of bound dye reflect conformational changes of the "opening-closing type". 2.) Changes of the rotational diffusion of the labeled enzyme reflect the alteration of the hydrodynamic shape.

These two indicators (triplet lifetime and rotational diffusion) were used to study conformational changes of CF1 both when bound to the Thylakoid membrane and, when isolated, in solution.

Binding of eosin-SCN to membrane bound CF1 depends critically on the presence of a proton-motive force. In its absence, the label has access predominantly to a first class of binding sites ("outside sites") which are only moderately shielded from oxygen and which do not much interfere with any of the catalytic activities of CF1. Another class of sites ("inside sites"), at maximum 3 per CF1, becomes accessible additionally after the generation of a proton-motive force. When bound to one of these sites, eosin-SCN inhibits ATP-synthesis (and the $Mg^{2+}$-ATPase) and it is by a factor of 750 less accessible to oxygen than in solution.

Chloroplasts, which are specifically labeled at CF1 and only there were prepared by reconstitution of labeled and purified CF1 into unlabeled and depleted membranes. With this material we observed the following effects: 1.) With eosin-SCN bound to the "inner sites" the very long lifetime of the triplet state is shortened drastically, when a proton-motive force is generated across the membrane. This indicates the opening of the previously closed CF1-structure. 2.) With eosin-SCN bound to the "outer sites" (note full activity) the rotational correllation time is shortened in the presence of nucleotides and furthermore in the presence of proton-motive force. This indicates an alteration of the interaction of CF1 with the membrane. The apparent microviscosity of the thylakoid membrane is very high (45 Poise).

With "outside labeled" CF1, which was isolated, purified and resuspended in buffer we observed the following: The five-subunit enzyme appears hydrodynamically as a rather excentric ellipsoid of revolution (axial ratio between 2 and 3) although we cannot discriminate between oblate and prolate geometry. With "inside labeled" CF1 we observed changes in the access of oxygen to eosin-SCN during heat treatment and under activating procedures.