Photoaffinity Labeling of Spinach Thylakoids and Cytochrome b_6/f-Complex by the Hydrophobic Reagent 3-(Trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)-diazirine

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In isolated spinach thylakoids the hydrophobic photoreactive probe 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl-diazirine almost exclusively labels the photosystem I reaction center and the light-harvesting chlorophyll *a*/*b* protein. In isolated cytochrome b_6/f-complex, all four components of the complex get labeled, but to a different extent. The amount of labeling in the protein components is correlated to the number of polypeptide segments embedded in the membrane system.

Introduction

The carbene-generating photoreactive probe [^{125}I] TID was recently introduced for the identification of lipid embedded protein segments. Due to its hydrophobic character TID preferentially partitions into the lipid phase of a membrane system and almost exclusively labels integral membrane proteins at the lipid-protein boundary [1–3]. As to photosynthetic systems, TID-labeling so far has only been applied to chromatophores and reaction centers of photosynthetic bacteria, where it was shown to have a high affinity to pigment-polypeptides [4, 5].

We wish to report here that TID in spinach thylakoids only labels the photosystem I reaction center and the light-harvesting chlorophyll *a*/*b*-protein. In isolated thylakoid cytochrome b_6/f-complex, all 4 subunits are labeled by TID, but to a different degree.

Abbreviations: Chl, chlorophyll; CPI, photosystem I reaction center, DNP-INT, 2-iodo-2′,4,4′-trinitro-2-methyl-6-isopropyldiphenylether; LHCP, light-harvesting chlorophyll *a*/*b* protein complex; LDS, lithium dodecylsulfate; TID, 3-(trifluoromethyl)-3-(*m*-iodophenyl)diazirine; Tricine, N-[tris(hydroxymethyl)-methyl]-glycine.

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Materials and Methods

Thylakoids from spinach were prepared according to [6] and cytochrome b_6/f-complex according to [7].

[^{125}I] TID (spec. activity 10 Ci/mmol) was purchased from Amersham, Braunschweig. For binding experiments, thylakoids corresponding to 100 µg Chl were incubated with TID for 5 min in a medium containing 20 mM Tricine, pH 8.0, and 20 mM $MgCl_2$. Thylakoids were pelleted at 10000 × *g* and pellet and aliquots of the supernatant assayed for radioactivity in a γ-counter.

For photoaffinity labeling experiments, samples were illuminated for 2 min at 0 °C in a nitrogen atmosphere. They were subsequently subjected to LDS polyacrylamide gel electrophoresis (10–15%). For assay of radioactivity the gel after staining was cut into 1 mm pieces, which were counted in a γ-counter. Alternatively, the gel was exposed to a Kodak X-Omat AR X-ray film at −80 °C for 52 h.

Results and Discussion

As evident from the binding experiment (Fig. 1), TID efficiently partitions into the thylakoid membrane. At each concentration applied, 84% of TID are found in the thylakoid membrane and only 16% in the aqueous phase. Binding of TID to the thylakoid is completely linear to the total concentration of TID applied (Fig. 1). No specific, *i.e.* high affinity binding to a component of the thylakoid can be detected. Contrary, specific binding is observed for in-

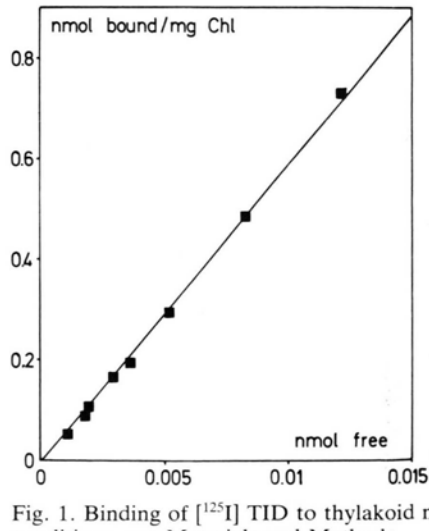

Fig. 1. Binding of [^{125}I] TID to thylakoid membranes. For conditions, see Materials and Methods.

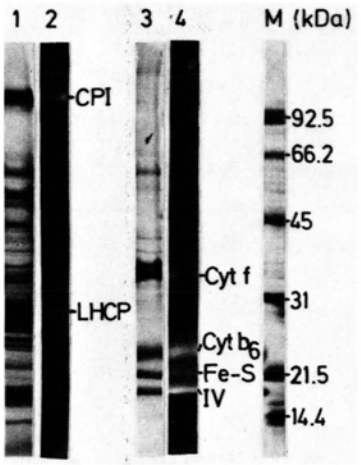

Fig. 2. Photographs (1, 3) and autoradiograms (2, 4) of LDS-polyacrylamide gels (10–15%) of thylakoids (1, 2) labeled by 7.5 nmol/mg Chl and cytochrome b_6/f-complex (3, 4) labeled by 1.2 nmol/nmol cytochrome f [^{125}I] TID. M = marker proteins.

hibitors of photosynthetic electron transport and photoaffinity labels derived thereof (for review see [8]).

However, after covalent attachment of TID to thylakoids by UV-illumination, radioactivity is only found in two major proteins, migrating at approximately 110 kDa and 26–29 kDa, referred to as the photosystem I reaction center (CPI) and the Light-harvesting chlorophyll *a*/*b* protein complex (LHCP), respectively [9] (Fig. 2). The radioactivity found in front of the gel is probably due to labeled free lipids and pigments. To establish whether besides CPI and LHCP other proteins within the thylakoid membrane have been labeled by [^{125}I] TID, a gel was cut into 1 mm pieces and each piece assayed for radioactivity. The result is shown in Fig. 3. As can be seen some minor labeling is also found in two photosystem II proteins of 43 and 47 kDa [10] and in some proteins in the 15–20 kDa molecular weight range. However, the labeling of these proteins is small as compared to CPI and LHCP. There are two reasons for the intense labeling of the latter two proteins: (i) they are integral membrane proteins, and (ii) they are highly abundant within the thylakoid membrane (compare their staining intensities in relation to other thylakoid proteins; Lane 1, Fig. 2).

In addition to thylakoids the labeling pattern of [^{125}I] TID in isolated cytochrome b_6/f-complex has been investigated. The cytochrome b_6/f-complex consists out of 4 major peptides: cytochrome f (double band at 33, 34 kDa), cytochrome b_6 (23.5 kDa), the Rieske Fe-S protein (20 kDa), and subunit IV (17.5 kDa) [7] (Fig. 2, lane 3). As is evident from the autoradiogram (Fig. 2, lane 4), all four proteins are tagged by TID, but to a different extent. It should be noted that preincubation of the complex with the inhibitor DNP-INT, which prevents plastohydroquinone oxidation at the complex [11], prior to addition of TID decreases labeling of all four proteins to about the same extent (data not shown).

A densitometric scan of the autoradiogram (Fig. 2, lane 4) and integration yields the following quantitative radioactivity distribution within the four proteins of the complex: cytochrome f, 10%; cytochrome b_6, 43%; Fe-S protein, 17%; subunit IV, 30%. Except for the Rieske Fe-S protein, the amino acid sequences of the cytochrome b_6/f-complex proteins are known [12–14]. An amino acid sequence for the Rieske Fe-S protein from *Neurospora* mitochondria has recently been reported [15]. Because the homologies between the cytochromes in the mitochondrial b/c_1- and the spinach b_6/f-complex are very high [12], a similar situation might exist for the

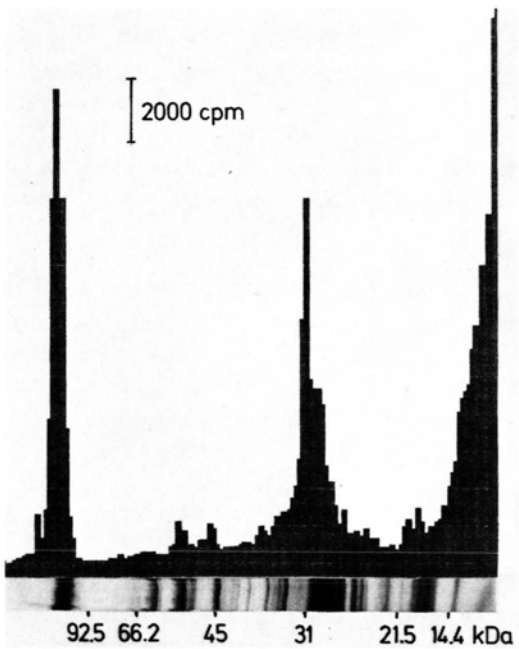

Fig. 3. Photograph and radioactivity scan of a LDS polyacrylamide gel (10–15%) of thylakoids labeled by 7.5 nmol/mg Chl [^{125}I] TID. The molecular weight numbers indicate the positions of the marker proteins.

Fe-S protein. Hydropathy analysis for the cytochrome b_6 has provided evidence that this protein is arranged in five helical spans through the thylakoid membrane [12, 13]. Three helical spans have been predicted for subunit IV, which is functionally related to cytochrome b_6 [12, 13]. Contrary, only one helical span has been predicted for either cytochrome *f* [14] or the Fe-S protein (from *Neurospora*) [15]. Thus, the amount of labeling within the four proteins of the cytochrome b_6/f-complex correlates well with the percentage of membrane intrinsic parts of the respective protein.

In conclusion, TID proves to be an efficient tool for the evaluation of intramembrane parts of integral membrane proteins.

Acknowledgement

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