Summary

Photosynthesis is the conversion of light energy into chemical energy, followed by CO_2-fixation. In plants photosynthesis takes place in the chloroplast. The light is absorbed by two photosystems (I and II), located in the thylakoid membranes, which are embedded in the chloroplast. When much more light is absorbed than can be used for CO_2-fixation, inactivation of photosynthesis occurs. This inactivation is called photoinhibition.

The primary site of photoinhibition is the multi-protein complex photosystem II (PS II). Illumination of PS II by excess light results into inactivation of its electron transport activity and damage to one of the proteins (the D_1-protein) involved in stabilization of important redox components within PS II.

In this thesis several aspects of photoinhibition have been studied. Photoinhibition of PS II was studied, both on a basic (biophysical and biochemical) level and on a more integrated (eco)physiological level. The results of the different approaches were integrated and discussed with respect to the mechanism of photoinhibition of the leaf under light stress. Depending on the questions raised, isolated PS II particles, thylakoids, chloroplasts, protoplasts and intact leaves of field lettuce (Valerianella locusta) and/or spinach (Spinacia oleracea) have been used. A variety of techniques has been applied, varying from electron spin resonance at low temperatures (5-15 K), measurements of electron transport in vitro, room temperature chlorophyll a fluorescence measurements both in vivo and in vitro, and gas exchange measurements in vivo.

In **Chapter 2** the basic mechanism of photoinhibition of PS II was studied, using isolated PS II-particles. In the absence of efficient electron acceptors, strong illumination of PS II led to a stepwise inactivation of different electron transport components within PS II. The sequence of inactivation steps was explained by 'overreduction' of the acceptor side of PS II. Degradation of the D_1-protein was shown to be a secondary event and it was not accompanied by further loss of active electron transport components.

In **Chapter 3**, the influence of O_2 on photoinhibition of PS II and overall photosynthesis was studied in isolated protoplasts and intact leaves of field-lettuce. Under the experimental conditions, where energy-turnover by photorespiration was low, photoinhibition of PS II was promoted by oxygen. No oxygen dependence of

photoinhibition of gross maximal photosynthesis was observed.

The PS II population is heterogeneous with respect to both antennae size and functionality of the acceptor side. The hypothesis that this heterogeneity plays a physiological role in recovery of PS II, with different PS II populations representing different steps in a repair-cycle of photoinhibited PS II, was tested in **Chapter 4**. No evidence for such a repair cycle was found, but new results regarding the sensitivity and inactivation state of the different PS II populations were obtained.

In **Chapter 5** methodological aspects of the application of chlorophyll fluorescence to calculate electron transport rates in intact leaves were evaluated.

In **Chapter 6**, the mechanism of photoinhibition of PS II was further analyzed in vivo. The metabolic demand for ATP and NADPH was manipulated by temperature and CO_2- and O_2-concentrations at different light levels. Photoinhibition became only significant when the PS II population was nearly completely down-regulated by the light-induced acidification of the intrathylakoid space. These results were discussed with regard to the mechanism of photoinhibition of PS II in vivo.

In **Chapter 7**, the recovery from photoinhibition of PS II in vivo was analyzed. Different recovery phases were detected revealing different sensitivities for temperature and streptomycin (an inhibitor of chloroplast translation). Hypotheses explaining the different recovery phases were connected to the stepwise nature of photoinhibition of PS II and breakdown of the D_1-reaction center protein.

The proposal, that long-term cold acclimation of plants should lead to increased resistance against photoinhibition at low temperatures, was tested for field-lettuce (**Chapter 3**) and spinach (**Chapters 6,7**). Cold-acclimation of spinach did indeed lead to diminished photoinhibition at low temperatures. This diminished sensitivity was subscribed to small changes of the pigment composition of PS II and increased maximum photosynthesis rates at these low temperatures. Full recovery from photoinhibition was reached more quickly in the cold-acclimated spinach leaves and it was attributed to diminished levels of photoinhibition. No significant alteration of the O_2-sensitivity during high light stress at low temperature was found in cold-acclimated field lettuce.

In **Chapter 8** the experimental results of the various chapters are integrated and discussed with respect to the physiological situation of the plant under light stress.