Comparison of the effects of trypsin and chymotrypsin on thylakoid membrane function

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The components involved in the light reactions of photosynthesis are non-covalently bound to proteins which are specifically arranged anisotropically across the lipid bilayer of the thylakoid membrane. There is a strict structure-to-function relationship in the membrane. Using proteolytic enzymes it is possible to investigate functional components associated with proteins which are close to or protruding from the outside of the thylakoid, e.g. Renger (1976) proposed the existence of a proteinaceous component associated with Photosystem II which has since been identified as a 32 kDa herbicide-binding protein (Mullet & Arntzen, 1981). In addition, by using enzymes which have specificity for particular amino acid bonds it may be possible to identify the bonds within the protein which are of functional significance. In this study, a comparison was made between the effects of trypsin (specificity for bonds which involve lysine and arginine residues) and chymotrypsin (specificity for tryptophan, tyrosine and phenylalanine). The effects of these enzymes on the function of the membrane were studied using the flash-induced electrochromic band shift at 515 nm (ΔΔA₄₃₃) (Junge & Jackson, 1982).

Measurements of ΔΔA₄₃₃ were made after incubating chloroplasts (25 µg/ml) with trypsin (5 µg/ml) or chymotrypsin (15 µg/ml). Two alterations in ΔΔA₄₃₃ were seen. (a) The rate of decay was increased, with trypsin having a more marked effect than chymotrypsin. This effect has been correlated with changes in the function of the ATPase complex due to partial digestion of coupling factor 1 (Raines & Hipkins, 1984). (b) The extent was reduced by 80% in the case of trypsin (10 min), but only by 20% with chymotrypsin (10 min). The effects of trypsin and chymotrypsin on the amplitude of ΔΔA₄₃₃, in chloroplasts suspended in low- or high-salt, are shown in Fig. 1. The figure shows that a difference existed between high- and low-salt controls; this could be due to a direct reduction in efficiency of Photosystem II (Bose & Arntzen, 1978) or to a redistribution of energy in favour of Photosystem I. The data indicated that trypsin inhibited this increase, while chymotrypsin had no effect. These results support the suggestion that the large reduction in ΔΔA₄₃₃ induced by trypsin, in chloroplasts suspended in high-salt, was in part due to the removal of a part of the LHCP leading to a reduced excitation of Photosystem II, and enhanced excitation of Photosystem I.

However, this cannot account for the reduction in amplitude induced by trypsin in low-salt, or by chymotrypsin. Measurements of the flash-yield of oxygen as an effect of flash intensity suggest that chymotrypsin had no effect on the light-harvesting pigments associated with Photosystem II. Two possible explanations for the decrease in amplitude remain: (a) it could be due to a loss of ΔΔA₄₃₃-sensing pigments, as occurs in photosynthetic bacteria (Webster et al., 1980), or (b) a direct effect on the reaction centre polypeptides.


Abbreviation used: LHCP, light-harvesting chlorophyll a/b protein

Fig. 1. Effect of trypsin (a) and chymotrypsin (b) on the amplitude of ΔΔA₄₃₃ in chloroplasts resuspended in high-salt (+2 mM divalent cations, ▲) or low-salt (no divalent cations: ■). Chloroplasts (25 µg/ml) were incubated with trypsin (1.5 µg/ml) or chymotrypsin (5 µg/ml). The medium contained Tricine (20 mM), KCl (10 mM) ± 2 mM-MgCl₂, pH 7.4.

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