Two processes for the breakdown of the Q_B protein of chloroplasts

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The behaviour of the 32 kDa Q_B protein of the chloroplast thylakoid membrane was investigated in senescing leaves of *Festuca pratensis*. Bf 993, a non-yellowing *Festuca* mutant, is known to be unable to degrade at least some of its thylakoid proteins during senescence. A high rate of Q_B protein turnover under illumination was observed in both Bf 993 and a normally yellowing cultivar. In the dark, however, the Q_B protein of Bf 993 exhibited the unusual stability of other thylakoid proteins that have been investigated in this genotype, whereas the normal cultivar was able to degrade its Q_B protein at an appreciable rate. These observations suggest that two independent processes exist for the degradation of the Q_B protein, one of which is impaired in mutant genotype Bf 993.

(Festuca pratensis) *Senescence* *Q_B protein* *Herbicide-binding protein*

1. INTRODUCTION

A polypeptide of 32–34 kDa has been identified as the binding site of the triazine and urea herbicides, which act on the photosynthetic membrane of the chloroplast [1,2]. It has been suggested that this protein, variously known as Q_B protein, D1 protein, herbicide-binding protein or the 32 kDa protein, has a functional role within photosystem II (PS II), and may be associated with the secondary quinone acceptor, 'B'. This protein has been the focus of considerable attention, not least because in the light it appears to be subject to a rate of turnover (synthesis and degradation) which normally far outstrips that of any other protein component of the membrane [3,4]. This light-dependent turnover has been extensively characterised (reviews [3,5]); the rate of turnover is proportional to light intensity and may reflect a peculiar vulnerability of the Q_B protein to light-induced damage, presumably as a consequence of its role within PS II. In the absence of light the Q_B protein is generally considered to be stable. During leaf senescence, however, there occurs a general controlled dismantling of the photosynthetic apparatus of the leaf, often with a redistribution of material to other parts of the plant [6]. We describe here the behaviour of Q_B protein in non-yellowing senescence mutant Bf 993, a genotype of *Festuca pratensis* with an impaired ability to degrade its thylakoid membrane proteins [7,8].

2. MATERIALS AND METHODS

Plants of *F. pratensis* cv. Rossa and Bf 993 were grown as in [9]. Fully expanded third leaves were excised above the ligule, and placed in 350 μl of distilled water containing 22.75 $\mu Ci \cdot ml^{-1}$ of L-[^{35}S]methionine (new England Nuclear) for 24 h at 20°C under illumination of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Following labelling, leaves were either processed immediately or transferred to distilled water at 20°C for a 4 day chase period in the light or in the dark. Proteins were extracted by homogenisation of 5 cm of leaf in 600 μl ice-cold 50 mM Tris-HCl buffer, pH 7.5, containing 0.04% (v/v)

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2-mercaptoethanol, followed by centrifugation at 12000 × *g* at 5°C. The washed pellet was resuspended in 400 μl of a similar buffer to which had been added 1.0% (w/v) SDS, and incubated at 40°C for 1 h. Electrophoresis and fluorography of the solubilised proteins were carried out by the methods in [10]. Equal amounts of radioactivity were applied to each track. Radiolabelling using L-[U-^{14}C]lysine (Amersham) was carried out in a similar manner.

Thylakoids for atrazine-binding studies were prepared from leaves of both genotypes by homogenisation in grinding medium [9] for 30 s, filtration through Miracloth and centrifugation at 6000 × *g*. The pellet was washed in 50 mM Tricine (pH 7) containing 700 mM sucrose and 1 mM $MgCl_2$ and resuspended in a small volume of this medium. Following assay of chlorophyll [9] thylakoids were diluted to 50 μg chlorophyll/ml in 25 mM Tricine (pH 8), containing 50 mM NaCl and 5 mM $MgCl_2$; [*ethyl*-^{14}C]atrazine (Amersham) was added in ethanol, in a range of concentrations between 0.025 and 1.0 $\mu g \cdot ml^{-1}$. The ethanol concentration was constant at 0.114% (v/v). For each genotype, four replicates of 1 ml volume were employed at each atrazine concentration, each replicate being accompanied by a control tube to which no thylakoid preparation was added. Tubes were vortex-mixed and centrifuged at 12000 × *g* for 5 min; radioactivity in the supernatant from each tube was measured in triplicate by liquid scintillation counting [10]. Results were analysed by non-linear regression analysis using the MLP program running on a VAX 11/750 computer, for atrazine concentrations of 0.5 $\mu g \cdot ml^{-1}$ and lower.

3 RESULTS AND DISCUSSION

Since Q_B protein undergoes very rapid synthesis in the light and yet remains a minor component of thylakoid membranes in mass terms, the protein must be subject to a correspondingly high rate of breakdown. Q_B protein is an intrinsic membrane polypeptide; the mutant genotype Bf 993 of *Festuca pratensis* is unable to remove many of the proteins of this type from its thylakoid membranes [8,11]. It was nevertheless observed (fig.1a) that leaves of Bf 993, when incubated with [^{35}S]methionine in the light, rapidly accumulated radiolabel in a species with an apparent molecular

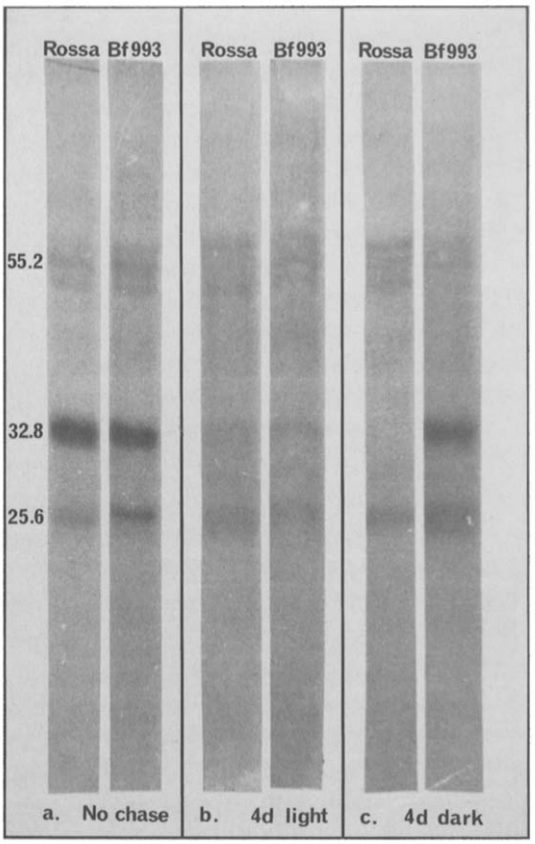

Fig.1. Fluorogram of lamellar proteins from normal and non-yellowing mutant of *Festuca pratensis*, following labelling with L-[^{35}S]methionine. Molecular masses are shown in kDa. (a) No chase, (b) 4 days chase in the light, (c) 4 days chase in the dark.

mass of 32–33 kDa, characteristic of the Q_B protein. The extent of incorporation was indistinguishable from that seen in the normal cultivar, Rossa. Incorporation of radiolabel into this polypeptide did not occur in the dark, nor when the incubation medium was supplemented with 0.15 mM chloramphenicol. The identification of the radiolabelled protein as Q_B protein was confirmed using the approach of Leto et al. [12]. Leaves labelled in the presence of L-[U-^{14}C]lysine in place of L-[^{35}S]methionine failed to accumulate radiolabel in the protein band in question (fig.2). This is consistent with the identification since Q_B protein contains no lysine.

If the breakdown of Q_B protein were impaired in Bf 993, its continued synthesis should lead to an accumulation of this protein in the mutant's

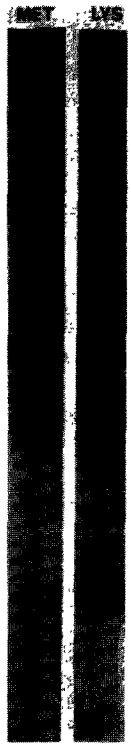

Fig.2. Lamellar proteins of *Festuca pratensis* cv. Rossa, radiolabelled with either L-[^{35}S]methionine (MET) or L-[U-^{14}C]lysine (LYS).

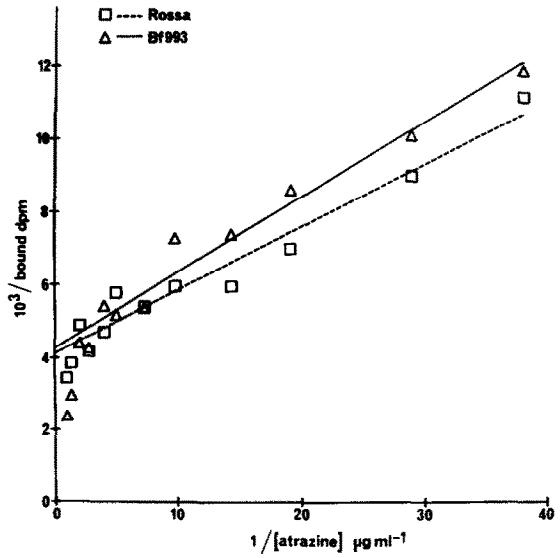

Fig.3. Atrazine-binding study: double-reciprocal plot for the binding of [^{14}C]atrazine to thylakoids of mutant and normal genotypes of *Festuca pratensis*. Deviations from linearity observed in both genotypes at higher inhibitor concentrations are evidence of biphasic or nonspecific binding [13]. The ratios of atrazine-binding sites to chlorophyll molecules were calculated to be 1:283 and 1:292 for normal and mutant genotypes, respectively.

chloroplasts. Radiolabelled atrazine was used to measure the absolute concentrations of Q_B protein in normal and mutant tissue; from the double-reciprocal plot [13] of fig.3 the concentrations of atrazine-binding sites in mutant and wild type were found to be virtually identical. Since Q_B protein did not accumulate abnormally in the thylakoids of Bf 993, it follows that breakdown in the light is unimpaired in the mutant.

Pulse-chase radiolabelling studies were employed to probe the behaviour or Q_B protein in mutant and wild type: after feeding [^{35}S]methionine in the light, leaves were removed from radiolabel and incubated in light or darkness. During a 4 day chase period in the light (fig.1b), the rapid rate of turnover of Q_B resulted in complete removal of radiolabel from this protein in the normal genotype Rossa, in accordance with other reports [14], and in Bf 993, confirming that the mutant does not differ from the wild type in its ability to degrade this protein in the light. During a chase period in the dark (fig.1c), the radioactivity associated with this protein in Rossa declined. Since the synthesis of protein is light-dependent this must be the result of degradation unmatched by synthesis; the photosynthetic activity of PS II is falling during this time [9]. In the mutant Bf 993, on the other hand, following a 4 day chase period in the dark, virtually no radiolabel was lost from Q_B protein, which clearly now enjoyed the enhanced stability seen in other intrinsic proteins in this genotype. Measurements of the activity of PS II during senescence in the dark in Bf 993 [9] had suggested that there was some retention of PS II activity, for which Q_B protein would presumably be required.

It has been suggested that the rapid turnover rate of Q_B protein following illumination is a consequence of its hazardous occupation in the electron transport system, and reflects the rate at which the protein becomes 'damaged' and needs to be replaced. The separation of this phenomenon from

the slower rate of degradation that occurs in the dark during senescence and possibly also in non-senescent tissue remained to be demonstrated, and indeed no explicit demonstration of degradation of the Q_B protein during senescence had previously been made. Here normal and non-yellowing mutant genotypes of *F. pratensis* have been employed to these ends; Bf 993 shares wild the wild type the capacity for rapid light-dependent turnover of Q_B protein, but has an impaired or absent capacity for its degradation in the dark. We therefore propose that two systems exist for the degradation of the Q_B protein of PS II; a rapid light-dependent or light-driven process, unimpaired in mutant Bf 993, and a light-independent pathway which is missing or impaired in this mutant.

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