General characteristics of normal and stress-enhanced protein degradation in *Lemna minor* (duckweed)

Robert J. COOKE and David D. DAVIES

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

(Received 13 March 1980/Accepted 21 July 1980)

The general features of protein degradation in *Lemna minor* were studied by using a double-isotope technique. In common with several animal systems, there are correlations between the relative rate of protein degradation on the one hand and molecular weight, charge and carbohydrate content on the other. Large proteins, acidic proteins and non-glycosylated proteins are degraded relatively more rapidly than small or basic proteins, or glycoproteins. The correlations with size and carbohydrate content are explicable on the basis of differential susceptibility to Pronase, whereas the charge correlation cannot be explained on this basis. In addition, acidic proteins are not generally of higher molecular weight than neutral or basic proteins. The correlations are found in fronds growing in normal complete medium and in fronds transferred to medium lacking nitrate or made 50% (v/v) with respect to deuterium oxide, both of which are conditions that induce a large increase in protein breakdown in *Lemna*. Thus basal protein degradation and enhanced degradation do not appear to differ fundamentally in their general characteristics. The results are discussed in relation to the reported features of normal and enhanced proteolysis in animal tissues and to the possible mechanism of protein degradation in *Lemna*.

Protein turnover is now generally recognized as an important means of regulation in biological systems. Increasingly, attention is being paid to the molecular mechanisms involved in the control of protein degradation. The view that the selectivity of rates of protein degradation in animal tissues is due to the physical properties of individual proteins is supported by several observations indicating that large proteins tend to be degraded more rapidly than small ones (Dehlinger & Schimke, 1970, 1971; Dice & Schimke, 1972; Dice *et al.*, 1973) and that acidic proteins tend to be degraded more rapidly than neutral or basic ones (Dice & Goldberg, 1975; Dice *et al.*, 1979). In addition, glycoproteins appear to be degraded more quickly than non-glycosylated proteins (Gurd & Evans, 1973; Kalish *et al.*, 1979), and there are reports of a correlation between protein hydrophobicity and rates of breakdown (Dean, 1975; Segal *et al.*, 1976). It has furthermore been argued that these correlations are independent of one another (Dice *et al.*, 1979) and are generally explicable on the basis that the rates of hydrolysis of cytosol proteins by various proteolytic enzymes *in vitro* correlate well with their rates of degradation *in vivo* (Dice *et al.*, 1973; Goldberg & Dice, 1974; Segal *et al.*, 1974, 1976).

There has been little corresponding work with plants. Dice *et al.* (1973) reported a correlation between protein molecular weight and relative rates of degradation in pea stems, and Acton & Gupta (1979), using density labelling to measure the half-lives of five plant enzymes, confirmed the correlation between size and degradation and found a weak correlation between isoelectric point and rate of breakdown. Therefore we have examined the relationship between relative rates of protein degradation on the one hand and protein molecular weight, charge and carbohydrate content on the other in *Lemna minor*, growing either normally or under two conditions of stress. We have previously used this organism to investigate various aspects of the mechanism and control of protein turnover in plants (Cooke *et al.*, 1979a,b, 1980a,b).

Materials and methods

Plant material and growth conditions

Lemna minor grown in complete sterile culture medium as previously described (Trewavas, 1970) was used for all of the experiments. Where appropriate, the medium was modified to provide the following stress conditions: (a) isotopic stress, in

which the water in the complete medium is replaced by 50% (v/v) $^2\text{H}_2\text{O}$ (Prochem Ltd., British Oxygen Co., London SW19 3UF, U.K.); (b) nitrate deprivation, in which the KNO_3 and $\text{Ca}(\text{NO}_3)_2$ of the complete medium are replaced by equivalent concentrations of KCl and CaCl_2 .

Double-isotope labelling

The relative rates of protein degradation were measured by a modification of the double-isotope technique of Arias *et al.* (1969). The modification is similar to that described by Dice *et al.* (1973) for pea-stem sections and can be used in either steady-state conditions or in circumstances where physiological changes are occurring (Dice *et al.*, 1978).

Normal growth

In experiments estimating relative rates of degradation in complete medium, under conditions of no stress, two samples of *Lemna* fronds (approx. 3 g fresh wt.) were grown in complete medium containing either 100 μCi of $[2,3\text{-}^3\text{H}]\text{alanine}$ or 50 μCi of $[\text{U-}^{14}\text{C}]\text{alanine}$ (both from The Radiochemical Centre, Amersham, Bucks., U.K.) for 24 h. The ^{14}C -labelled plants were washed and killed immediately after the cessation of the labelling period, whereas the ^3H -labelled plants were allowed to grow for 6 days in unlabelled complete medium before being harvested. This time allows for the degradation of approximately half of the original protein (Cooke *et al.*, 1979b). A control experiment was performed in which ^{14}C - and ^3H -labelled plants were both allowed to grow for 6 days after labelling.

Stress conditions

In experiments carried out under stress conditions, a further modification was used. After labelling with $[\text{H}^3]$ - or $[\text{C}^{14}]$ -alanine in complete medium, the ^{14}C -labelled plants were transferred to unlabelled complete medium, whereas the ^3H -labelled plants were transferred to unlabelled medium containing 50% (v/v) $^2\text{H}_2\text{O}$ or to unlabelled medium lacking nitrate. Both ^{14}C - and ^3H -labelled fronds were harvested after 8 h ($^2\text{H}_2\text{O}$ experiment) or 3 days ($-\text{NO}_3^-$ experiment), again these being the times required for half of the original protein to be broken down (Cooke *et al.*, 1979a,b). In this instance, the control experiment was to transfer both ^{14}C - and ^3H -labelled plants to the appropriate unlabelled stress medium for the same time before harvesting.

The design of these experiments is such that a determination of the $^{14}\text{C}/^3\text{H}$ ratio of isolated protein fractions provides a measure of the relative rates of degradation *in vivo*. As no attempt was made to decrease re-cycling of the labelled alanine, no absolute rates of degradation can be accurately

obtained. Furthermore, the methodology means that radioisotope ratios between different treatments or experiments are not comparable. However, differences in the radioisotope ratios within a given experiment will reflect differences in relative rates of breakdown, such that proteins that are being broken down quickly will have higher $^{14}\text{C}/^3\text{H}$ ratios than those that are being slowly degraded.

Isolation of total soluble protein

Frozen *Lemna* fronds were ground to a fine powder, allowed to thaw and extracted in 100 mM-Tris/HCl buffer, pH 7.3. Extracts were centrifuged at 20000 g for 30 min, and the supernatants were used as a source of total soluble protein.

Fractionation of total soluble protein

Gel filtration. Gel filtration was used to determine the effect of molecular weight on relative degradative rates. Samples of ^{14}C - and ^3H -labelled protein were thoroughly mixed and separated on a 50 cm $\times$ 1.5 cm column of Sephadex G-200 at 4°C, the column being eluted with 100 mM-Tris/HCl buffer, pH 7.3, at a flow rate of 8.0 ml/h. Fractions (3 ml) were collected and the protein was precipitated with 10% (w/v) trichloroacetic acid. Samples of the washed protein pellets were removed for protein determination by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, and for the determination of the $^{14}\text{C}/^3\text{H}$ ratio.

DEAE-cellulose fractionation. DEAE-cellulose was used to fractionate proteins according to net charge (Duncan & Bond, 1977; Duncan *et al.*, 1980). Mixed samples of ^{14}C - and ^3H -labelled protein were desalted by passage through Sephadex G-50 and applied to a 4.0 cm $\times$ 3.5 cm column of Whatman DE-52 DEAE-cellulose. The protein was separated into three fractions by elution as follows: (a) 30 ml of 10 mM-Tris/HCl buffer, pH 8.0 (basic fraction); (b) 30 ml of 100 mM-NaCl in the above buffer (neutral fraction); (c) 30 ml of 300 mM-NaCl in the above buffer (acidic fraction). Each fraction was freeze-dried and resuspended in 3 ml of 100 mM-Tris/HCl buffer, pH 7.3. Samples were removed for determination of the $^{14}\text{C}/^3\text{H}$ ratio.

Affinity chromatography. Affinity chromatography was used to fractionate glycoproteins and non-glycosylated proteins. Double-labelled proteins were desalted by passage through Sephadex G-50 and applied to a 3 ml column of packed Glycosylex A (Miles Laboratories) (Kalish *et al.*, 1979). Glycosylex A consists of concanavalin A linked to agarose. Proteins that did not bind to the column (non-glycosylated proteins) were eluted with 30 ml of salt solution (75 mM-NaCl/1 mM- CaCl_2 /1 mM- MgCl_2). The glycoproteins were then eluted with 20 ml of 5% (w/v) α -methyl D-mannoside in the

above salt solution. The protein fractions were freeze-dried, resuspended in 3 ml of 100 mM-Tris/HCl, pH 7.3, and samples were removed for the determination of $^{14}\text{C}/^3\text{H}$ ratio.

Measurement of $^{14}\text{C}/^3\text{H}$

In all experiments, the double-labelled protein fractions were oxidized in a Biological Materials Oxidiser (R. J. Harvey Instrument Corp., Hilldale, NJ, U.S.A.), and the $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ thus formed were measured separately in an Inter technique SL30 Scintillation Spectrometer.

Results

The design of the experiments described in this paper is such that a comparison of the $^{14}\text{C}/^3\text{H}$ ratios of the isolated protein fractions provides a measure of the relative rates of degradation *in vivo*. A high $^{14}\text{C}/^3\text{H}$ ratio is indicative of a relatively more rapid rate of breakdown than a low $^{14}\text{C}/^3\text{H}$ ratio.

Correlation between rate of degradation and molecular weight

(a) *Normal growth.* The correlation between degradative rate and protein molecular weight in *Lemna* fronds growing in normal complete medium is shown in Fig. 1(a). In normal growth conditions, the high-molecular-weight proteins eluted first from the Sephadex G-200 column have significantly higher $^{14}\text{C}/^3\text{H}$ ratios than do proteins eluted later from the column, and hence have been degraded relatively more quickly. The control experiments (open circles in Fig. 1) show no evidence for systematic variations in $^{14}\text{C}/^3\text{H}$ ratio.

(b) *Stress conditions.* Figs. 1(b) and 1(c) show the relationship between rate of protein breakdown and molecular weight in *Lemna* fronds subjected to stress conditions. Again the higher-molecular-weight proteins are degraded relatively more rapidly than are the low-molecular-weight ones.

Proteolytic susceptibility of proteins of different molecular weight

Dice *et al.* (1973) reported that one explanation for the correlation between protein size and relative degradation rate was that high-molecular-weight proteins were more susceptible to attack by proteolytic enzymes. We have examined this possibility in *Lemna* growing in complete medium. Protein labelled *in vivo* with $50\ \mu\text{Ci}$ of $[\text{U-}^{14}\text{C}]$ alanine was isolated and fractionated on Sephadex G-200. The protein profile obtained is shown in Fig. 2(a), which also shows the position of markers of known molecular weight. The eluate from the column was divided into three fractions, according to molecular weight: I, mol.wt. $> 350\,000$ (fractions 20–35); II, mol.wt. $130\,000$ – $340\,000$ (fractions 36–50); III,

Fig. 1. Relative degradative rates of soluble proteins from stressed or unstressed *Lemna minor* fronds, separated by gel filtration on Sephadex G-200

Double-labelled proteins from *Lemna* fronds growing in complete medium (a), $-\text{NO}_3^-$ medium (b) or 50% (v/v) $^2\text{H}_2\text{O}$ medium (c) were isolated and fractionated on Sephadex G-200. The $^{14}\text{C}/^3\text{H}$ ratio in each fraction was measured. For details see the Materials and methods section. O, Control experiments. The results shown are from one experiment; each experiment was repeated at least twice with qualitatively similar results. r = regression coefficient at 1% level of probability ($P < 0.01$).

mol.wt. $< 130\,000$ (fractions 51–66). Each fraction was adjusted to the same protein concentration (1–2 mg/ml), and Pronase (B grade; Calbiochem) was added to a final concentration of $50\ \mu\text{g}/\text{ml}$. The release of trichloroacetic acid-soluble ^{14}C -labelled material was determined after incubation at 25°C for various periods of time. The results (Fig. 2b) show that high-molecular-weight proteins are hydrolysed by Pronase more quickly and more extensively than are either of the other two fractions.

Fig. 2. Pronase-susceptibility of different molecular-weight classes of soluble proteins isolated from *Lemna* minor and fractionated on Sephadex G-200

(a) Separation of proteins from *Lemna* fronds on Sephadex G-200. The position of various marker proteins is also shown [RCase, ribulose 1,5-bisphosphate carboxylase from spinach leaves (mol.wt. 550 000); Cat, catalase from bovine liver (mol.wt. 240 000); Ald, aldolase from rabbit muscle (mol.wt. 138 000); Cyt. c, cytochrome c from horse heart (mol.wt. 12 500)]. (b) On the basis of this fractionation, ^{14}C -labelled protein was divided into three classes: Class I (mol.wt. > 350 000); Class II (mol.wt. 130 000–340 000); class III (mol.wt. < 130 000). See the text for details. The concentration of each fraction was adjusted to the same value (1–2 mg/ml), and Pronase was added to each to a final concentration of 50 $\mu\text{g}/\text{ml}$. Each fraction was incubated at 25°C, and at intervals samples were removed and added to an equal volume of cold 10% (w/v) trichloroacetic acid. The precipitated protein was pelleted by centrifugation and the acid-soluble ^{14}C -labelled material released was measured by scintillation counting of the radioactivity. □, Class-I proteins; ○, class-II proteins; ●, class-III proteins. The initial specific radioactivities of the fractions were: class I, 8500 c.p.m./mg; class II, 7800 c.p.m./mg; class III, 7500 c.p.m./mg.

Correlation between rate of degradation and net protein charge

(a) *Normal growth.* Double-labelled proteins isolated from *Lemna* fronds growing in complete medium were desalted and fractionated on DEAE-cellulose. Three fractions were obtained, containing basic, neutral or acidic proteins, corresponding to the fractions obtained by Duncan *et al.* (1980). The $^{14}\text{C}/^3\text{H}$ ratios in these fractions were determined, typical results being given in Table 1. There is a strong correlation between charge and relative degradative rate in that acidic proteins are degraded more rapidly than are neutral or basic proteins.

(b) *Stress conditions.* Table 1 also shows the $^{14}\text{C}/^3\text{H}$ ratios of the differently charged protein fractions in *Lemna* subjected to stress conditions. Fronds transferred to $-\text{NO}_3^-$ medium show a strong correlation between protein charge and degradation, whereas this correlation is somewhat diminished in fronds subjected to isotopic stress. The control experiments show no significant variations in $^{14}\text{C}/^3\text{H}$ ratio.

Proteolytic susceptibility of differently charged protein fractions

The possibility that the correlation between degradation and charge is due to the enhanced susceptibility of acidic proteins to proteolytic enzymes was investigated. *Lemna* fronds growing on complete medium were labelled with 50 μCi of [^{14}C]alanine, and the labelled proteins were isolated, desalted and fractionated on DEAE-cellulose. Each protein fraction was freeze-dried, resuspended in 100 mM-Tris/HCl buffer, pH 7.3, adjusted to the same protein concentration (1–2 mg/ml) and desalted by passage through Sephadex G-50. Pronase was added to a final concentration of 50 $\mu\text{g}/\text{ml}$. The release of trichloroacetic acid-soluble ^{14}C -labelled material was determined after incubation at 25°C. The results (Fig. 3) show that the acidic proteins, which are degraded more rapidly *in vivo* (Table 1), are not more susceptible to Pronase *in vitro*.

Molecular-weight distribution of differently charged protein fractions

Another possibility to account for the relatively higher rate of degradation of acidic proteins would be that this fraction contains more proteins of high molecular weight than do the neutral or basic fractions. As we have demonstrated a close correlation between molecular weight and rates of degradation (Fig. 1), such a size distribution of the differently charged fractions could explain the results given in Table 1. Protein from fronds growing in complete medium and labelled with 50 μCi of [^{14}C]alanine was isolated, desalted by passage

Table 1. Correlation between rate of protein degradation and net charge in *Lemna minor* in complete growth medium or subjected to stress

Double-labelled proteins from *Lemna* fronds growing in complete medium or transferred to stress conditions were isolated, desalted and fractionated on DEAE-cellulose into acid, neutral and basic fractions. The $^{14}\text{C}/^3\text{H}$ ratio in each fraction was measured. For details see the Materials and methods section. The results shown are each from one experiment. Each experiment was repeated two or three times, with essentially similar results. Values in parentheses indicate $^{14}\text{C}/^3\text{H}$ ratios expressed as percentages of the values for the acidic fractions.

Growth conditions	Protein fraction	^{14}C (d.p.m.)/ ^3H (d.p.m.) ratio	Control ^{14}C (d.p.m.)/ ^3H (d.p.m.) ratio
Complete medium	Acidic	4.24 (100)	0.44 (100)
Complete medium	Neutral	2.65 (63)	0.48 (109)
Complete medium	Basic	0.98 (23)	0.52 (118)
$-\text{NO}_3^-$ medium	Acidic	2.68 (100)	0.25 (100)
$-\text{NO}_3^-$ medium	Neutral	1.65 (62)	0.30 (120)
$-\text{NO}_3^-$ medium	Basic	1.21 (45)	0.25 (98)
$^2\text{H}_2\text{O}$ medium	Acidic	1.55 (100)	0.40 (100)
$^2\text{H}_2\text{O}$ medium	Neutral	2.44 (158)	0.41 (103)
$^2\text{H}_2\text{O}$ medium	Basic	1.02 (66)	0.39 (98)

Fig. 3. Pronase-susceptibility of differently charged fractions of soluble proteins from *Lemna minor*

^{14}C -labelled protein from *Lemna* fronds growing on complete medium was isolated and separated on DEAE-cellulose into acidic, neutral and basic fractions. See the text for experimental details. Each protein fraction was adjusted to the same concentration (1–2 mg/ml), and Pronase was added to a final concentration of 50 $\mu\text{g}/\text{ml}$. The fractions were incubated at 25°C, and at intervals samples were removed and added to an equal volume of cold 10% (w/v) trichloroacetic acid. The precipitated protein was pelleted by centrifugation, and the amount of acid-soluble ^{14}C -labelled material released was measured by scintillation counting of the radioactivity. □, Basic protein fraction; ○, neutral protein fraction; ●, acidic protein fraction. The initial specific radioactivities of the fractions were: basic, 5400 c.p.m./mg; neutral, 7400 c.p.m./mg; acidic, 6300 c.p.m./mg.

Table 2. Molecular-weight distribution of differently charged protein fractions in *Lemna minor* growing in complete medium

^{14}C -labelled soluble protein was isolated from *Lemna* fronds growing in complete medium and fractionated on DEAE-cellulose, and each fraction was passed through Sephadex G-200. For experimental details see the text. The results given indicate the percentages of labelled protein eluted from Sephadex G-200 as peak I (mol.wt. > 350 000), peak II (mol.wt. 130 000–340 000) and peak III (mol.wt. < 130 000).

Fraction from DEAE-cellulose	Percentage of labelled proteins eluted as		
	Peak I	Peak II	Peak III
Acidic	33	31	36
Neutral	30	24	46
Basic	28	40	32

through Sephadex G-200 and separated into the molecular-weight ranges shown in Fig. 2(a). The percentage of the labelled protein in each fraction is shown in Table 2. There is no evidence that the acidic fraction has a higher percentage of high-molecular-weight proteins.

Correlation between rate of degradation and carbohydrate content

To confirm that the fraction eluted from the Glycosylex A column with α -methyl D-mannoside (see the Materials and methods section) does contain glycoproteins, soluble protein was isolated from *Lemna* fronds growing in complete medium, desalted and fractionated into non-glycosylated proteins and glycoproteins. Half of each fraction was hydrolysed with 2M-HCl for 6h. Both the hydrolysed and non-hydrolysed samples were assayed for hexose content by a modified orcinol/ H_2SO_4 procedure

through Sephadex G-50 and fractionated on DEAE-cellulose into basic, neutral and acidic fractions. Each of these fractions in turn was then passed

(François *et al.*, 1962). Both sets of results, when expressed per mg of protein, indicated that the glycoprotein fraction contained at least 10 times the hexose content of the non-glycosylated protein fraction, confirming that this fraction is enriched for glycoproteins.

(a) *Normal growth.* Double-labelled proteins from normal growing *Lemna* fronds were desalted and separated on Glycosylex A into glycoproteins and non-glycosylated proteins. The $^{14}\text{C}/^3\text{H}$ ratios of these fractions are given in Table 3, along with the results of the appropriate control experiments. The non-glycosylated protein fraction (which constituted 92–95% of the total soluble protein) has a higher $^{14}\text{C}/^3\text{H}$ ratio than does the glycoprotein fraction, and thus has been degraded more quickly *in vivo*. The controls showed no systematic variations in $^{14}\text{C}/^3\text{H}$ ratio.

(b) *Stress conditions.* Table 3 also shows the $^{14}\text{C}/^3\text{H}$ ratios of the non-glycosylated protein and glycoprotein fractions isolated from *Lemna* fronds transferred to stress conditions. Again the non-glycosylated protein fraction appears to have been degraded relatively more rapidly than the glycoprotein fraction.

Proteolytic susceptibility of non-glycosylated proteins and glycoproteins

The susceptibility of the two fractions to hydrolysis by Pronase was investigated. *Lemna* fronds were grown in complete medium and labelled with $50\mu\text{Ci}$ of $[\text{U-}^{14}\text{C}]\text{alanine}$. The soluble protein was isolated, desalted by passage through Sephadex G-50 and then separated into glycoprotein and non-glycosylated protein fractions. Each fraction was freeze-dried, resuspended in 100 mM-Tris/HCl buffer, pH 7.3, its concentration adjusted to 1–2 mg/ml and desalted again. The release of trichloroacetic acid-soluble ^{14}C -labelled material was measured after the addition of Pronase ($50\mu\text{g}/\text{ml}$ final concentration) and incubation at 25°C . Fig. 4

shows that non-glycosylated proteins, which are degraded more quickly *in vivo*, are hydrolysed more rapidly by Pronase than are glycoproteins.

Fig. 4. Pronase-susceptibility of glycoproteins and non-glycosylated proteins isolated from *Lemna minor*

^{14}C -labelled protein was isolated from *Lemna* fronds growing on complete medium, and the glycoprotein and non-glycosylated protein fractions were separated by affinity chromatography on Glycosylex A. See the text for experimental details. The concentration of each of the two fractions was adjusted to 1–2 mg/ml, Pronase was added to a final concentration of $50\mu\text{g}/\text{ml}$ and the fractions were incubated at 25°C . At intervals samples were removed and added to an equal volume of cold 10% (w/v) trichloroacetic acid. The precipitated protein was pelleted by centrifugation and the acid-soluble ^{14}C -labelled material was measured by scintillation counting of the radioactivity. ●, Non-glycosylated protein fraction; ○, glycoprotein fraction. The initial specific activities of the fractions were: non-glycosylated proteins, 10300 c.p.m./mg; glycoproteins, 9700 c.p.m./mg.

Table 3. Relative rates of degradation of the glycoprotein and non-glycosylated protein fraction from stressed or non-stressed *Lemna minor*

Double-labelled proteins isolated from *Lemna* fronds growing in complete medium or transferred to stress conditions were isolated, desalted and fractionated on Glycosylex A. The $^{14}\text{C}/^3\text{H}$ ratio in each fraction was measured. For details see the Materials and methods section. The results shown are each from one typical experiment; each experiment was repeated at least twice. The values in parentheses indicate the $^{14}\text{C}/^3\text{H}$ ratios expressed as percentages of the values of the non-glycosylated protein fraction.

Growth conditions	Fraction	^{14}C (d.p.m.)/ ^3H (d.p.m.) ratio	Control ^{14}C (d.p.m.)/ ^3H (d.p.m.) ratio
Complete medium	Non-glycosylated protein	3.62 (100)	0.39 (100)
	Glycoprotein	0.97 (27)	0.33 (85)
–NO ₃ [–] medium	Non-glycosylated protein	2.24 (100)	0.30 (100)
	Glycoprotein	0.74 (33)	0.35 (117)
² H ₂ O medium	Non-glycosylated protein	5.27 (100)	0.24 (100)
	Glycoprotein	2.68 (51)	0.24 (100)

Discussion

The rather simple view that the differential rate of degradation of proteins in animals is due to certain physical properties and to differences in proteolytic sensitivity (see the introduction) has become less clear as investigations have extended to a wider range of organisms and tissues. Thus, although Brinster *et al.* (1979) found a tendency for large and acidic proteins in mouse blastocysts to be degraded more rapidly, the regressions of these parameters on protein half-life were not statistically significant. Walker *et al.* (1978) found no correlation between size and degradation for any rat liver submitochondrial fraction. Dice & Goldberg (1976) also found no correlation between molecular weight and rate of degradation in rat serum proteins. The correlation between charge and degradation does not appear to apply to rat liver membrane proteins solubilized by Triton X-100 (Dice *et al.*, 1979). Neither size nor charge correlates with protein half-life in Chinese-hamster ovary-cell plasma membranes (Horst & Roberts, 1979).

The independence of the relationships between size and degradative rate and between charge and degradative rate has been asserted by Dice *et al.* (1979), who reported that the acidic proteins of rat liver and muscle are not significantly larger than the neutral or basic proteins. On the other hand, Duncan *et al.* (1980) concluded that in mouse and rat liver acidic proteins are generally of higher molecular weight, thus implying that the correlations are not independent.

The correlation between rates of degradation *in vivo* and susceptibility to proteinases has been observed with a number of different proteolytic enzymes (see Goldberg & Dice, 1974). However, Bond (1971) reported that, although the half-lives of five rat liver enzymes correlated with their relative sensitivities to inactivation by trypsin and chymotrypsin, there was no relationship with Pronase or subtilisin. Again, although larger proteins are in general more susceptible to hydrolysis *in vitro*, Dice *et al.* (1979) concluded that a wide range of proteolytic enzymes did not attack acidic rat liver proteins more rapidly than neutral or basic ones. Duncan *et al.* (1980) have reached the opposite conclusion. The more rapid catabolism of glycoproteins is not thought to be due to increased sensitivity to proteolytic attack (Kalish *et al.*, 1979).

This somewhat confused situation is further complicated by the report that the enhanced protein degradation observed in diabetic or starved animals is fundamentally different from normal degradation. The correlations between relative degradative rates and size, charge and carbohydrate content are absent or markedly diminished in the liver and muscle of diabetic or starved rats, but are retained in brain tissue (Dice *et al.*, 1978).

The present work was carried out to illustrate some of the features of the protein-degradation system in plants, both under normal growth conditions and under conditions of enhanced protein breakdown. *Lemna* growing in complete growth medium (normal conditions) shows a strong correlation between the rate of protein degradation and molecular weight (Fig. 1a), net protein charge (Table 1) and protein carbohydrate content (Table 3). Thus the more rapidly degraded proteins in *Lemna* tend to be of high molecular weight, have acidic isoelectric points and are not glycosylated. The first two of these correlations agree with the general findings reported in animal cells (see Goldberg & Dice, 1974; Goldberg & St. John, 1976), whereas the decreased catabolism of glycoproteins is the reverse of the situation in animal systems (see, e.g., Kalish *et al.*, 1979). The correlations with size and carbohydrate content are explicable on the basis of increased proteolytic sensitivity of large proteins and non-glycosylated proteins (Figs. 2b and 4). However, acidic proteins do not seem to be more susceptible to Pronase than are other fractions (Fig. 3), nor do they have generally higher molecular weights (Table 2). The explanation for the rapid degradation of acidic proteins is thus unclear. Acidic proteins may require certain specific conditions before exhibiting increased proteolytic susceptibility, and these conditions may well be absent in the present study. Alternatively, it is possible that it is not net charge that is the critical property determining rates of breakdown, but that a related property such as charge density (Momany *et al.*, 1976) is more important. Again, acidic proteins may tend to become concentrated in regions of high proteolytic activity or be preferentially absorbed by the degradative apparatus, presumed to be the lysosome in animals (Segal, 1976) or the vacuole in plants (Matile, 1978; Cooke *et al.*, 1980a,b).

Lemna fronds subjected to nitrate starvation or isotopic stress also demonstrate the correlations between protein degradative rate and molecular weight (Figs. 1b and 1c), charge (Table 1) and carbohydrate content (Table 3). We have previously shown that these stress conditions induce a large increase in protein degradation (Cooke *et al.*, 1979a,b). Thus these results represent a major difference between our study with *Lemna* and corresponding work on animals, since the characteristics of enhanced protein breakdown in various rat tissues are reported as being fundamentally different from those of basal proteolysis (Dice *et al.*, 1978). As one interpretation of their results, Dice *et al.* (1978) suggested the existence of dual catabolic pathways, a hypothesis originally advanced by Knowles & Ballard (1976). These pathways are envisaged as (i) the normal degradation machinery, a poorly understood but specific process that may or

may not involve the lysosomes (see, e.g., Dean, 1979), and (ii) lysosomal autophagy, a rather indiscriminate process responsible for enhanced protein breakdown. The results of experiments with inhibitors of protein synthesis have led various authors to reach similar conclusions (for discussion see Amenta *et al.*, 1977). Compounds such as puromycin or cycloheximide have little or no effect on basal proteolysis, whereas they will inhibit the increased degradation brought about by starvation or other physiological conditions. We have previously reported that, although cycloheximide decreases the stress-induced increase in protein breakdown in *Lemna*, it also lowers the basal rate of protein catabolism, thus casting doubt on the presence of dual pathways for protein degradation in this organism (Cooke *et al.*, 1980b). The present results, showing that the correlations between rate of degradation and physical properties of proteins are retained in stress situations, also offer no support for the dual-pathway hypothesis in *Lemna*, and can be explained on the basis of stress increasing the accessibility of proteins to the degradation apparatus, perhaps by affecting vacuolar membrane permeability properties (Cooke *et al.*, 1980a,b).

Thus our results are consistent with the view that the rate of degradation of a protein is determined by certain of its physical and structural properties. However, it is clear from the work with animals that the situation is rather more complex than was initially believed, and there are many exceptions to the general hypothesis. Further examination of different plant species and tissues, under both normal and stress conditions, and of individual enzymes is required to establish more fully the status of the hypothesis in plants.

We thank Dr. Stefano Grego for carrying out initial experiments on the correlation between rate of degradation and molecular weight. The able technical assistance of Ms. Jane Oliver is also acknowledged. This work was financed by a grant (no. AG 83/14) from the Agricultural Research Council.

References

- Acton, G. J. & Gupta, S. (1979) *Biochem. J.* **184**, 367–377
- Amenta, J. S., Sargus, M. J. & Baccino, F. M. (1977) *Biochem. J.* **168**, 223–227
- Arias, I. M., Doyle, D. & Schimke, R. T. (1969) *J. Biol. Chem.* **244**, 3303–3315
- Bond, J. S. (1971) *Biochem. Biophys. Res. Commun.* **43**, 333–339
- Brinster, R. L., Brunner, S., Joseph, X. & Levey, I. L. (1979) *J. Biol. Chem.* **254**, 1927–1931
- Cooke, R. J., Grego, S., Oliver, J. & Davies, D. D. (1979a) *Planta* **146**, 229–236
- Cooke, R. J., Oliver, J. & Davies, D. D. (1979b) *Plant Physiol.* **64**, 1109–1113
- Cooke, R. J., Grego, S., Roberts, K. & Davies, D. D. (1980a) *Planta* **148**, 374–380
- Cooke, R. J., Roberts, K. & Davies, D. D. (1980b) *Plant Physiol.* in the press
- Dean, R. T. (1975) *Biochem. Biophys. Res. Commun.* **67**, 604–609
- Dean, R. T. (1979) *Biochem. J.* **180**, 339–345
- Dehlinger, P. J. & Schimke, R. T. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1473–1480
- Dehlinger, P. J. & Schimke, R. T. (1971) *J. Biol. Chem.* **246**, 2574–2583
- Dice, J. F. & Goldberg, A. L. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3893–3897
- Dice, J. F. & Goldberg, A. L. (1976) *Nature (London)* **262**, 514–516
- Dice, J. F. & Schimke, R. T. (1972) *J. Biol. Chem.* **247**, 98–111
- Dice, J. F., Dehlinger, P. J. & Schimke, R. T. (1973) *J. Biol. Chem.* **248**, 4220–4228
- Dice, J. F., Walker, C. D., Byrne, B. & Cardiel, A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2093–2097
- Dice, J. F., Hess, E. J. & Goldberg, A. L. (1979) *Biochem. J.* **178**, 305–312
- Duncan, W. E. & Bond, J. S. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 918
- Duncan, W. E., Offermann, M. K. & Bond, J. S. (1980) *Arch. Biochem. Biophys.* **199**, 331–341
- François, C., Marshall, R. D. & Neuberger, A. (1962) *Biochem. J.* **83**, 335–341
- Goldberg, A. L. & Dice, J. F. (1974) *Annu. Rev. Biochem.* **43**, 835–869
- Goldberg, A. L. & St. John, A. (1976) *Annu. Rev. Biochem.* **45**, 747–803
- Gurd, J. W. & Evans, W. H. (1973) *Eur. J. Biochem.* **36**, 273–279
- Horst, M. N. & Roberts, R. M. (1979) *J. Biol. Chem.* **254**, 5000–5007
- Kalish, F., Chovick, N. & Dice, J. F. (1979) *J. Biol. Chem.* **254**, 4475–4481
- Knowles, S. E. & Ballard, F. J. (1976) *Biochem. J.* **156**, 609–617
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Matile, P. (1978) *Annu. Rev. Plant Physiol.* **29**, 193–213
- Momany, F. A., Aguanno, J. J. & Larrabee, A. R. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3093–3097
- Segal, H. L. (1976) *Curr. Top. Cell. Regul.* **11**, 183–201
- Segal, H. L., Winkler, J. R. & Miyagi, M. P. (1974) *J. Biol. Chem.* **249**, 6364–6365
- Segal, H. L., Rothstein, D. M. & Winkler, J. R. (1976) *Biochem. Biophys. Res. Commun.* **73**, 79–84
- Trewavas, A. (1970) *Plant Physiol.* **45**, 742–751
- Walker, J. H., Burgess, R. J. & Mayer, R. J. (1978) *Biochem. J.* **176**, 927–932