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Studies on the Relationship between the Degradative Rates of Proteins *in vivo* and their Isoelectric Points

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Acidic proteins tend to be degraded more rapidly than neutral or basic proteins in rat liver, skeletal muscle, kidney and brain and in mouse liver and skeletal muscle. We now report a similar relationship among soluble proteins from rat lung, heart and testes, and from human fibroblasts and mouse-embryo cells grown in culture. These findings indicate that the correlation between protein net charge and degradative rate is a general characteristic of intracellular protein degradation in mammals. This relationship between isoelectric point and half-life appears to be distinct from the previously reported correlation between subunit molecular weight and protein half-lives. The more rapid degradation of acidic proteins does not result from their being of larger molecular weight than neutral or basic proteins. Furthermore, proteins within specific isoelectric point ranges still exhibit a relationship between subunit size and half-life. Finally, a group of membrane or organelle-associated proteins that are insoluble in phosphate-buffered saline and water but soluble in 1% Triton X-100 exhibit a correlation between size and half-life, but not between net charge and half-life. The biochemical reasons for the relationship between protein isoelectric point and half-life are unclear, although several possible explanations are presented. It is not due to a greater sensitivity of acidic proteins to proteolytic attack since experiments with a variety of endoproteinases, including trypsin, chymotrypsin, Pronase, papain, chymopapain, *Staphylococcus aureus* V8 proteinase, pepsin and lysosomal cathepsins from rat liver, have failed to demonstrate more rapid digestion of acidic proteins.

Proteins within animal, plant and bacterial cells vary widely in their rates of degradation (Schimke, 1970; Goldberg & Dice, 1974; Goldberg & St. John, 1976). One of the major unsolved problems in this area is the biochemical basis for these differences in protein half-lives (Schimke, 1970; Goldberg & Dice, 1974; Goldberg & St. John, 1976). It now appears certain that aspects of polypeptide structure can influence degradative rates, since proteins whose conformations have been altered by genetic mutations, incorporation of amino acid analogues or errors in protein synthesis tend to be degraded more rapidly than are normal proteins (Pine, 1967; Goldberg, 1972*a*; Capecchi *et al.*, 1974; Knowles & Ballard, 1976; Goldberg & Dice, 1974; Goldberg & St. John, 1976). Similarly, the binding of ligands to proteins can influence their conformations and thereby alter their half-lives (Schimke *et al.*, 1965*a*,*b*; Drysdale & Munro, 1966; Schimke, 1970; Goldberg & Dice, 1974).

Certain structural features, including molecular weight (Dehlinger & Schimke, 1970; Dice *et al.*, 1973; Dice & Goldberg, 1975; Goldberg & Dice, 1974; Naya *et al.*, 1977) and net charge (Dice & Goldberg, 1975), have also been reported to influence protein degradative rates. Large proteins tend to be degraded more rapidly than small proteins, and acidic proteins tend to be degraded more rapidly than neutral or basic proteins.

The biochemical explanations for these effects are still uncertain. Perhaps the simplest explanation would be that the proteins with shorter half-lives *in vivo* are inherently more susceptible to attack by proteolytic enzymes. In fact, abnormal proteins, which are rapidly degraded within cells, are also more sensitive to a variety of endoproteinases *in vitro* (Goldberg, 1972*b*; Goldberg & Dice, 1974). Also, in several cases where a ligand is known to influence an enzyme's half-life *in vivo*, it also influences the protein's susceptibility to proteinases *in vitro* (Schimke *et al.*, 1965*a*,*b*; Goldberg & Dice, 1974). Finally, the correlation between protein size and intracellular degradative rate may result from greater proteinase-sensitivity of the large polypeptides (Dice *et al.*, 1973; Goldberg & Dice, 1974).

Understanding the basis for the heterogeneity in

Abbreviation used: SDS, sodium dodecyl sulphate.

degradative rates should help illuminate the mechanisms of protein catabolism. We have therefore examined further the relationship between protein net charge and catabolic rate. The present experiments investigated whether the correlation between isoelectric points and degradative rates (*a*) is a general feature of the degradation of soluable proteins in mammalian cells, (*b*) is independent of the relationship between protein size and half-life, and (*c*) results from a greater sensitivity of the acidic proteins to proteolytic attack.

Materials

Albino male rats weighing 160–180g were from Simonsen Laboratories, Gilroy, CA 95020, U.S.A. [^{3}H]Leucine (6Ci/mmol) and [^{14}C]leucine (309μCi/mmol) were from Schwarz/Mann, Orangeburg, NY 10962, U.S.A. Materials used in isoelectric focusing and SDS/polyacrylamide-gel electrophoresis were from sources listed previously (Dice *et al.*, 1973; Dice & Goldberg, 1975) except the ampholytes which were from Brinkman, Westbury, NY 11540, U.S.A. All proteinases were from Worthington Biochemicals, Freehold, NJ 07728, U.S.A., except *Staphylococcus aureus* V8 proteinase which was from Miles Biochemicals, Elkhart, IN 46515, U.S.A., and rat liver cathepsins, which were prepared as described by Penn (1960). Phosphate-buffered saline refers to the buffer used in most experiments (72mM-Na_2HPO_4/28mM-NaH_2PO_4/26mM-NaCl, pH7.2).

Methods

Double-isotope labelling, preparation of soluble proteins, isoelectric focusing, SDS/polyacrylamide-gel electrophoresis and determination of radioactivity were carried out as described previously (Dice *et al.*, 1973; Dice & Goldberg, 1975) except for radioactive determinations, where the tissue solubilizer was Protosol (New England Nuclear, Boston, MA 02118, U.S.A.), the scintillation 'cocktail' was 3a70B (Research Products International, Elk Grove Village, IL 60007, U.S.A.) and the liquid-scintillation counter was a Beckman model LS 230. Detailed procedures for each experiment are described in the Figure legends.

Results

Generality of the charge correlation

To compare relative degradative rates of proteins, we have used the double-isotope technique originally described by Arias *et al.* (1969). In this technique two isotopic forms of an amino acid, usually [^{14}C]- and [^{3}H]-leucine, are administered at different times to the same animal. Two time points are thus established on the curve describing degradation of the protein. In our experiments a rat was injected initially with [^{14}C]leucine, followed 4 days later by [^{3}H]leucine; 4h later the animal was killed. The proteins that remain soluble after sequential centrifugation at 10000*g* for 10min and 100000*g* for 1h were isolated from the lung, heart and testes. The proteins were then separated into different charge classes by isoelectric focusing. Fractions were

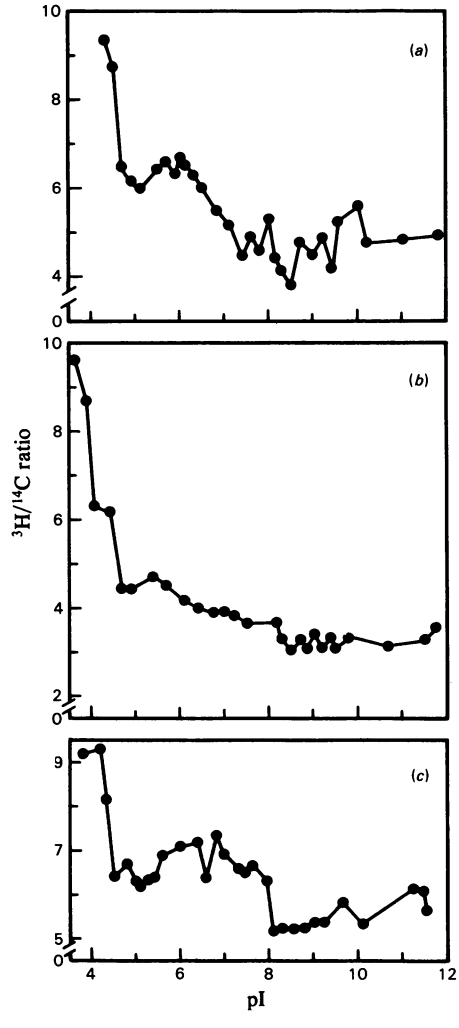

Fig. 1. *Relative degradative rates of soluble proteins from rat lung, heart and testes separated by isoelectric focusing*
A 180g rat was given intraperitoneally 100μCi of [^{14}C]leucine. After 4 days the same animal received 500μCi of [^{3}H]leucine and was then killed 4h later. Soluble proteins (10–30mg) from lung, heart and testes were separated by isoelectric focusing, and ^{3}H/^{14}C ratios of proteins in the different fractions were determined. (*a*) Lung; (*b*) heart; (*c*) testes.

collected, and the pH of each fraction was determined. For any protein fraction the amount of the first isotope remaining (^{14}C) relative to the amount of the isotope most recently incorporated (^{3}H) reflects the amount of degradation during the time interval between isotope administrations. Therefore proteins that are synthesized and degraded rapidly will have high $^{3}H/^{14}C$ ratios. This same approach was used previously to show that acidic proteins were degraded more rapidly than neutral or basic proteins in rat liver, kidney, muscle and brain (Dice & Goldberg, 1975) and in mouse liver (Dice & Goldberg, 1975) and mouse skeletal muscles (Goldberg *et al.*, 1977).

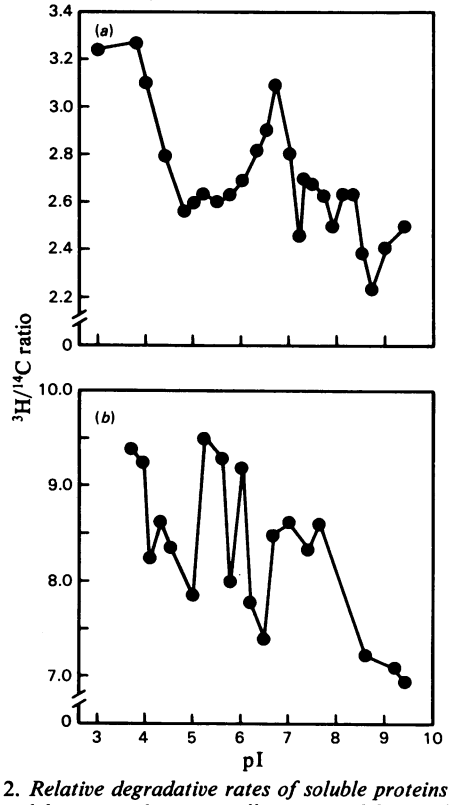

Fig. 2. *Relative degradative rates of soluble proteins from cultured human and mouse cells separated by isoelectric focusing*

Human fibroblasts (WI 38) and mouse-embryo cells (Meek *et al.*, 1977) were grown to confluency in Eagle's minimal essential medium supplemented with 10% foetal calf serum. Cells were labelled with [^{14}C]leucine (0.5μCi/ml) for 3 days in fresh leucine-deficient medium supplemented with 5% foetal calf serum. The cells were then washed with medium containing unlabelled 2mM-leucine, and placed in medium containing unlabelled 2mM-leucine for 2 days. Next the cells were labelled for 30min with [^{3}H]leucine (20μCi/ml) in leucine-deficient medium containing 5% foetal calf serum before they were harvested with a 'rubber policeman'. The cells were collected by centrifugation at 1500*g* for 10min, and 10vol. of phosphate-buffered saline was added before homogenization on ice with ten strokes of a Teflon pestle in a Potter–Elvehem homogenizer. Proteins that remained soluble after sequential centrifugation at 10000*g* for 10min and 100000*g* for 1h were collected and dialysed against phosphate-buffered saline to remove free amino acids. (*a*) WI 38 human fibroblasts; (*b*) mouse-embryo cells.

Fig. 1 shows that, in rat lung, heart and testes, acidic proteins tend to have higher $^{3}H/^{14}C$ ratios than the neutral or basic proteins. Thus on the average the acidic polypeptides are degraded more rapidly in these tissues. The correlation coefficients and levels of statistical significance for the relationships between pI and $^{3}H/^{14}C$ ratios are as follows: lung, $r=-0.72$, $P<0.01$; heart, $r=-0.77$, $P<0.01$; testes, $r=-0.68$, $P<0.01$.

Fig. 2 illustrates the results of similar experiments with mouse-embryo cells and human fibroblasts during growth in cell culture. A modification of the double-isotope technique was used, but high $^{3}H/^{14}C$ ratios once again reflect relatively rapid rates of protein degradation. Acidic proteins tend to turn over more rapidly in both types of cultured cells (human fibroblasts, WI 38, $r=-0.69$, $P<0.01$; mouse embryo cells, $r=-0.65$, $P<0.01$).

Charge correlation versus size correlation

To determine whether acidic proteins might have shorter half-lives *in vivo* simply because they tend to be of larger average molecular weight, soluble proteins from liver or muscle were fractionated by isoelectric focusing, and proteins whose isoelectric points were within a given range were pooled. These proteins were then separated according to subunit molecular weight by SDS/polyacrylamide-gel electrophoresis. The gels were stained for protein, and the average molecular weights determined. Table 1 shows that acidic proteins tend to be of slightly larger subunit size than neutral or basic proteins in liver. However, this difference in size is much too small to explain the observed difference in the $^{3}H/^{14}C$ ratios of these fractions.

This conclusion is based on results of 12 separate experiments (J. F. Dice, unpublished work) that compared the subunit size of liver proteins and their degradative rates in a manner analogous to the experiment presented in Fig. 3(*a*). Graphs of $^{3}H/^{14}C$ ratios versus migration distance into the gel were prepared and the best straight lines calculated by linear-regression analysis. $^{3}H/^{14}C$ ratios of proteins with subunit mol.wt. of 44000 were compiled from the separate experiments and were found to have ratios 1.15 ± 0.05 times those of proteins with subunit mol.wt. of 32000. However, in eight separate experiments comparing isoelectric points of proteins

Table 1. *Average polypeptide molecular weights of different charge classes of proteins from liver and muscle*

Soluble proteins were prepared from liver and skeletal muscle and separated by isoelectric focusing. Various fractions were pooled and precipitated with trichloroacetic acid added to a final concentration of 10%. The precipitates were washed twice with 5% trichloroacetic acid and once with ethanol/diethyl ether (1:1, v/v). Electrophoresis was carried out in small-diameter (5mm) SDS/polyacrylamide gels as described by Dice *et al.* (1973). The gels were stained with 1% (w/v) Amido Schwarz in 10% (v/v) acetic acid, destained in 10% acetic acid, and scanned at 560nm in a Gilford spectrophotometer model 250 with a gel-scanning attachment. The average molecular weight of the proteins from each gel was determined by tracing the gel scan and then determining the position along the gel where the tracing could be cut into two halves of equal weight. The results presented are the average values for three separate experiments for liver and two for muscle. The results of separate experiments agreed within 10%.

Isoelectric-point fraction	Mean apparent subunit mol.wt.	
	Liver	Muscle
4–12 (total)	44000	45000
4.0–4.9	44000	45000
5.0–5.9	44000	46000
6.0–6.9	40000	28000
7.0–7.9	32000	45000
8.0–8.9	34000	47000
9.0–12.0	32000	44000

and their degradative rates (J. F. Dice, unpublished work), the liver proteins with pI values between 4 and 5 have $^3H/^{14}C$ ratios 1.90±0.08 times higher than the proteins with pI values between 7 and 12. In other words, if the observed difference in the $^3H/^{14}C$ ratios between the acidic proteins (pI 4–5) and the basic proteins (pI 7–12) were attributable to size rather than charge, their subunit mol.wts. should be 110000 and 32000 respectively.

Among the soluble proteins from muscle, unlike those from liver, no relationship was observed between isoelectric point and subunit molecular weight. The acidic proteins (pI 4–6) showed $^3H/^{14}C$ ratios twice those of basic proteins (pI 7–9), even though on SDS/polyacrylamide gels the average subunit molecular weights of the two fractions were similar (Table 1).

Also in support of the conclusion that isoelectric point and molecular weight are distinct determinants of protein degradative rates is the finding that soluble proteins with a similar net charge still exhibit a correlation between subunit size and degradative rate. Fig. 3 demonstrates that among total soluble proteins as well as among acidic proteins (pI 5–6) and basic proteins (pI 7–9) from liver, the larger

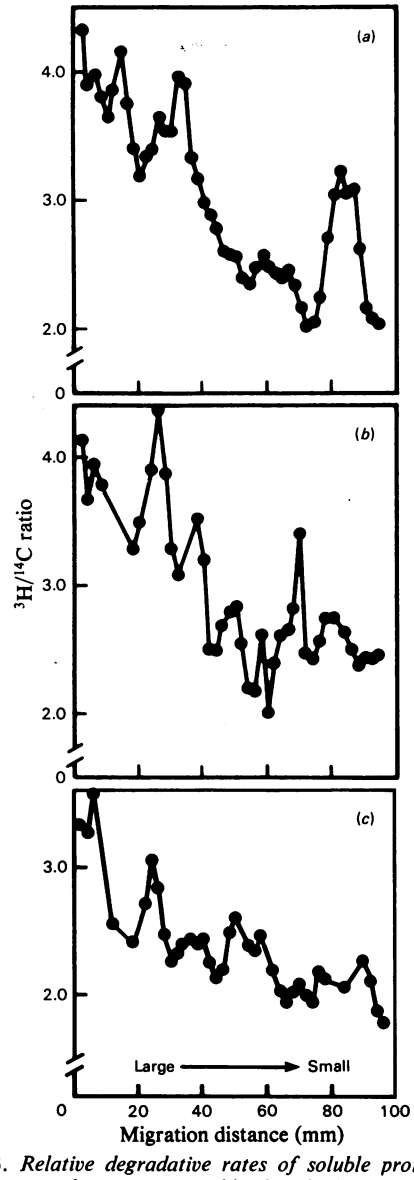

Fig. 3. *Relative degradative rates of soluble proteins of different net charges separated by SDS/polyacrylamide-gel electrophoresis*

A 170g rat was injected intraperitoneally with 150μCi of [^{14}C]leucine. After 4 days the same animal was given 500μCi of [^{3}H]leucine and killed 4h later. Soluble proteins from liver were prepared and separated by isoelectric focusing. After pooling of the various fractions, the proteins were precipitated in 10% (w/v) trichloroacetic acid, washed twice with 5% trichloroacetic acid and once with ethanol/diethyl ether (1:1, v/v). Electrophoresis was carried out in large-diameter (19mm) SDS/polyacrylamide gels as described previously (Dice *et al.*, 1973). (*a*) Total soluble proteins from liver; (*b*) proteins of pI 5–6; (*c*) proteins of pI 7–9.

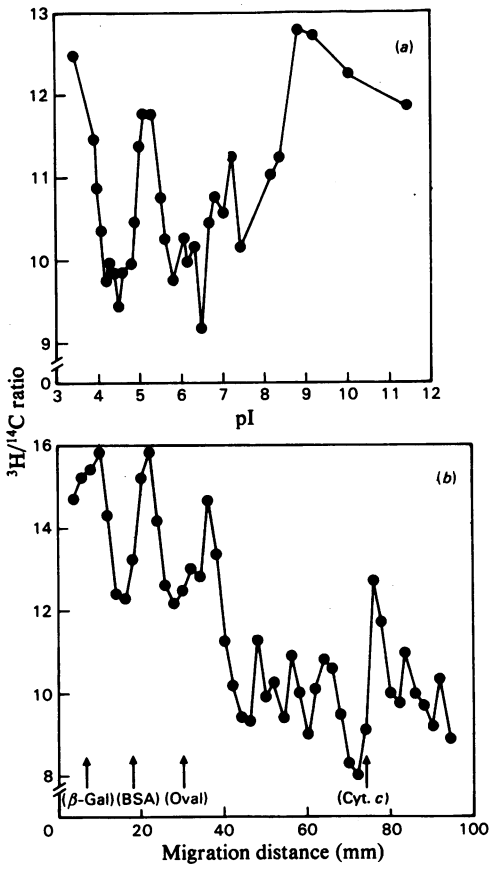

Fig. 4. *Relative degradative rates of rat liver proteins solubilized with Triton X-100 and separated by isoelectric focusing (a) or SDS/polyacrylamide-gel electrophoresis (b)*

A rat was treated as described in the legend of Fig. 1. The liver homogenate was centrifuged at 10000*g* for 10min and 10vol. (v/w) of water was added to the pellet. The pellets were then sonicated at 0°C for 15s at a power-output setting of 7 on a Branson Sonifier. This procedure was repeated four times with a 15s interval between each burst. After centrifugation at 100000*g* for 1h, the pellet was once again resuspended by sonication in 10vol. (v/w) of water. This procedure was repeated twice more before the final pellet was resuspended in 5ml of 1% Triton X-100 and sonicated for 15s. Approx. 50% of the protein remained soluble after centrifugation at 100000*g* for 1h and was analysed by isoelectric focusing (*a*) and by SDS/polyacrylamide-gel electrophoresis (*b*). The isoelectric focusing was carried out in the presence of 1% Triton X-100. Abbreviations used: BSA, bovine serum albumin, mol.wt. 67000; Cyt *c*, cytochrome *c*, mol.wt. 13000; β-Gal, β-galactosidase, mol.wt. 135000; Oval, ovalbumin, mol.wt. 45000.

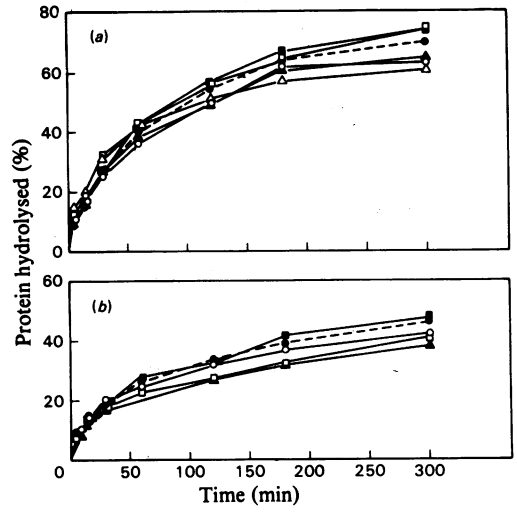

Fig. 5. *Digestion of various charge classes of rat liver soluble proteins by trypsin (a) and chymotrypsin (b)*

Two 160g rats were injected intraperitoneally with 100μCi of [^{14}C]leucine. Two other rats received 625μCi of [^{3}H]leucine. After 4h, the animals were killed and soluble proteins were prepared. ^{3}H-labelled proteins were fractionated by isoelectric focusing as described previously (Dice & Goldberg, 1975), except that the time was decreased to 16h by use of an LKB model 2103 constant power supply. Fractions were collected, their pH values determined, and the ampholytes and sucrose removed by dialysis overnight against phosphate-buffered saline. All fractions were adjusted to the same protein concentration (0.2mg/ml), and to these fractions were added unfractionated ^{14}C-labelled proteins (0.2mg/ml). Proteins in each range of isoelectric points were digested with proteinase (50μg/ml) at 22°C. Portions (1ml) were removed at various times and mixed with 0.25ml of 50% trichloroacetic acid in 1.4ml Beckman Microfuge tubes that were then centrifuged for 3min at 9000*g* in a Beckman model B Microfuge. The upper 1ml of the acid-soluble fraction was removed, and the trichloroacetic acid was extracted with 4ml of diethyl ether. Extracted portions were added to 10ml of scintillation fluor, and the radioactivities determined. The rate of digestion of the ^{3}H-labelled proteins was normalized to the rate of digestion of the added ^{14}C-labelled proteins in each fraction. In this manner, differences in the amount of proteinase added or in amounts of endogenous proteinase or proteinase inhibitors in the isoelectric-point fractions could be corrected. Results for proteins of the following isoelectric points are shown: ●, unfractionated proteins; ○, pI4–5; ■, pI5–6; ▲, pI6–7; □, pI7–9; △, pI 9–12.

subunits tend to have higher $^3H/^{14}C$ ratios than the smaller subunits [$r = -0.78$, $P < 0.01$; $r = -0.75$, $P < 0.01$; $r = -0.81$, $P < 0.01$; for (*a*), (*b*) and (*c*) respectively]. Thus, when one variable, e.g. net charge, is controlled, there is still a relationship between subunit size and degradative rate. The reciprocal

experiment was not done since the use of SDS to separate proteins into distinct classes by subunit size altered the apparent isoelectric points of the proteins even after extensive dialysis (J. F. Dice & A. L. Goldberg, unpublished work).

Finally, among certain groups of proteins it is possible to demonstrate a size correlation, but not a charge correlation. We have found (Fig. 4) that membrane- or organelle-associated proteins that are insoluble in phosphate-buffered saline and water, but soluble in 1% Triton X-100, exhibit the typical relationship between protein subunit size and half-life ($r=-0.74$, $P<0.01$), but not between protein net charge and half-life ($r=+0.44$, $P>0.01$). Together, these results indicate that net charge and subunit size are two distinct factors that influence protein degradative rates.

Proteinase-sensitivity of proteins of various isoelectric points

The relative rates at which proteins are degraded *in vivo* may be due to inherent differences in their susceptibilities to proteolytic digestion (Goldberg, 1972*b*; Dice *et al.*, 1973; Goldberg & Dice, 1974). To test whether proteins of various isoelectric points differ in their proteinase sensitivities, we measured their susceptibility to digestion *in vitro* by trypsin and chymotrypsin.

Rats were injected with [^{3}H]leucine as described in the legend to Fig. 5. Soluble proteins were then separated by isoelectric focusing, and fractions containing proteins with pI values in specified ranges were dialysed to remove ampholytes and sucrose and were adjusted to equal protein concentrations. Unfractionated ^{14}C-labelled soluble proteins were added to each isoelectric-point fraction to serve as an internal standard. The proteinase solution was added to each fraction, and the rate at which ^{3}H and ^{14}C radioactivities appeared in acid-soluble form was measured. This experimental design allowed the digestion of ^{3}H-labelled proteins of each range of pI values to be compared with digestion of unfractionated ^{14}C-labelled proteins within the same digestion mixture.

Previous studies of this type gave inconsistent results, owing to partial denaturation of proteins during the focusing and subsequent dialysis (Dice & Goldberg, 1975). By conducting the focusing more rapidly with a power supply providing constant power, it was subsequently found that recombined ^{3}H-labelled proteins that had been focused and dialysed were no more proteinase-sensitive than were untreated ^{14}C-labelled proteins. This result indicates that little, if any, protein denaturation occurred with these procedures.

Fig. 5 shows that the acidic proteins with rapid degradative rates *in vivo* (pI4–5 and 5–6) were not digested preferentially by either chymotrypsin or trypsin. In fact, there were no consistent differences in proteolytic susceptibility of any particular charge fraction compared with that of unfractionated soluble proteins represented by the 'control' in Fig. 5. In other experiments, no preferential digestion of acidic proteins was observed with chymotrypsin when (*a*) the digestion was done at 40°C instead of 22°C, (*b*) the proteins were dissolved in nutrient-rich culture medium 199 instead of phosphate-buffered saline, or (*c*) thiol groups of proteins were reduced with 100mM-dithiothreitol before proteolysis (results not shown).

Similar experiments were performed with a wide variety of different proteinases of differing specificities and physical properties. These various enzymes were studied in an attempt to find an agent that might duplicate *in vitro* the more rapid hydrolysis of acidic cell proteins observed *in vivo*. Among the proteinases that we tested were some with acidic pH optima, others with basic isoelectric points, and one enzyme that specifically hydrolyses adjacent to acidic amino acid residues. Acidic proteins were found to be no more susceptible to digestion by Pronase (a mixture of proteolytic enzymes with little known substrate specificity), by pepsin and lysosomal cathepsins from rat liver (enzymes with acidic pH optima), by papain and chymopapain (proteinases with basic isoelectric points of 8.75 and 10.0 respectively) or by *S. aureus* V8 proteinase (a proteinase that cleaves preferentially at glutamate and aspartate residues). In these digestions each proteinase was present at 50μg/ml. All digestions were carried out at 22°C in phosphate-buffered saline except the digestions with pepsin and lysosomal cathepsins, which were in 50mM-citrate-buffered saline, pH4.5.

Discussion

We previously reported a correlation between degradative rates *in vivo* and isoelectric points among soluble proteins of rat liver, skeletal muscle, kidney and brain (Dice & Goldberg, 1975) and of mouse liver (Dice & Goldberg, 1975) and skeletal muscle (Goldberg *et al.*, 1977). The present study extends these findings to include rat lung, heart and testes and human fibroblasts and mouse-embryo cells in culture. We conclude that the more rapid degradation of acidic proteins is a general characteristic of degradation of soluble proteins in mammalian tissues.

It is presently unclear whether acidic proteins tend to be degraded more rapidly in prokaryotes. We have attempted unsuccessfully to demonstrate a correlation between isoelectric points and half-lives in *Escherichia coli*, but there are several factors complicating these experiments. Virtually all of the

soluble proteins in *E. coli* are acidic (pI4–7), so the proteins to be compared are only within a narrow range of isoelectric points. Furthermore, *E. coli* during rapid growth has a low rate of protein degradation, and the cultures can be easily maintained only for a few hours. To increase rates of protein degradation so that measurable differences in $^{3}H/^{14}C$ ratios of various proteins could be obtained, we had to deprive our cultures of a required nutrient. However, this approach may have inherent problems, since we have recently found that the relationship between protein charge and half-life normally seen in mammalian tissues is abolished when protein degradation is enhanced due to nutrient deprivation (Dice & Walker, 1978; Dice *et al.*, 1978).

In mammalian tissues, isoelectric point and subunit molecular weight appear to be separable factors influencing rates of degradation. Among the 22 proteins from rat liver for which the rates of degradation, isoelectric points and subunit molecular weights are known, the half-lives of proteins correlated significantly with both isoelectric point and subunit size (Dice & Goldberg, 1975). By calculation of partial correlation coefficients it was possible to show statistically that neither correlation could be caused by the other. Since only 22 proteins were involved in this analysis, it was important to support this conclusion experimentally. We now have several types of such confirming evidence: (*a*) acidic proteins from tissues other than liver are not composed of larger-molecular-weight subunits, and even in liver the size differences are slight, (*b*) proteins of specific isoelectric-point ranges still exhibit the correlation between subunit size and half-life, and (*c*) certain groups of proteins may exhibit the size correlation without the charge correlation.

Our results (Table 1) agree with the report of Duncan & Bond (1977) that acidic proteins from liver cytosol have larger subunits than do basic proteins. These workers separated acidic and basic proteins by DEAE-Sephadex chromatography rather than by isoelectric focusing, however. We find that DEAE-Sephadex chromatography gives a greater difference in average subunit molecular weight of acidic and basic proteins than does isoelectric focusing. These findings suggest that DEAE-Sephadex chromatography fractionates proteins to some extent on the basis of size as well as charge (J. F. Dice & A. L. Goldberg, unpublished work).

Furthermore, the slight correlation between net charge and subunit size for liver soluble proteins does not hold for muscle proteins (Table 1) or for proteins from several other tissues. Two-dimensional electrophoresis with isoelectric focusing in the first dimension and SDS/polyacrylamide-gel electrophoresis in the second has shown that there is little or no correlation between protein isoelectric point and subunit size among proteins from hepatoma cells (O'Farrell & O'Farrell, 1977; Fan *et al.*, 1977), HeLa cells (Milman *et al.*, 1976), skeletal muscle (Whalen *et al.*, 1977), neurons (Wilson *et al.*, 1977), nematodes (O'Farrell, 1975) or *E. coli* (O'Farrell, 1975).

The biochemical explanation of the rapid degradation of acidic proteins is not known. It is not even clear whether the protein net charge itself is the crucial physical parameter in influencing degradative rates, or whether a related property such as charge density may be the important factor (Momany *et al.*, 1976). Many of the known features of protein degradation can be accounted for simply by differential sensitivity of cell proteins to proteolytic attack, including the relationship between protein size and half-life (Dice *et al.*, 1973; Goldberg & Dice, 1974), the effect of many ligands on rates of protein degradation (Schimke *et al.*, 1965*a*,*b*: Goldberg & Dice, 1974), and the very rapid catabolism of various abnormal proteins (Goldberg, 1972*b*; Goldberg & Dice, 1974). The correlation between protein net charge and half-life is the first general characteristic of intracellular protein degradation that apparently cannot be explained by a greater sensitivity of the rapidly turning over (acidic) proteins to known proteolytic enzymes (Fig. 5).

Several possible explanations remain for the more rapid breakdown of acidic proteins *in vivo*.

(1) Acidic proteins may be especially susceptible to proteolytic attack only under specific conditions. We have examined a variety of digestion conditions, including higher temperature, acidic pH, the presence of nutrients found in culture medium and reduction of protein thiol groups, but none of these factors altered the results.

(2) The cellular proteinases actually responsible for intracellular protein degradation may differ from the enzymes tested and may preferentially hydrolyse acidic proteins. On the basis of the present negative results, it is unclear what type of enzyme might show this selectivity. We tested a proteinase that preferentially hydrolyses at glutamate and aspartate residues and proteinases that are themselves highly basic, but they digested proteins of all pI values at equal rates.

(3) Acidic proteins might preferentially accumulate in regions of the cell where degradative enzymes are localized. Thus acidic proteins may be taken up by lysosomes more rapidly or may bind to lysosomal membranes more readily (Dean, 1975) than neutral or basic proteins. Similarly, if degradation occurs after binding to other membranes or organelles (Ballard, 1977), the acidic proteins may preferentially bind to such sites. A group of proteins derived from membranes or organelles do not exhibit a relationship between charge and half-life even though they do exhibit the correlation between subunit size and

half-life (Fig. 4). Most of these proteins are probably membrane components of various cellular organelles, since the 10000*g* particulate fraction had been repeatedly sonicated and washed in water before addition of Triton X-100. One interpretation of these results is that membrane proteins do not exhibit a correlation between isoelectric point and degradative rate, unlike soluble proteins from all tissues tested. Perhaps acidic proteins in cytosol are degraded after an initial rate-limiting step that involves membrane binding (Ballard, 1977). Proteins that are already within cellular membranes might not follow the same correlations.

Recent observations indicate that the tendency of acidic proteins to turn over more rapidly is decreased or abolished in liver and muscle of starved or diabetic animals (Dice & Walker, 1978; Dice *et al.*, 1978). Thus the features of the degradative process that are responsible for the correlation between net charge and half-life must be masked or altered during the enhanced protein degradation seen in diabetes and starvation. Further understanding of the mechanisms underlying the charge and size correlations, and other general features of intracellular protein degradation, should provide insight concerning the basis for heterogeneity in protein degradative rates, the crucial steps in the breakdown process and the ways in which protein degradative rates may be regulated.

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