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Relationship between *in vivo* degradative rates and isoelectric points of proteins

(protein degradation/mammalian cells/isoelectric focusing)

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ABSTRACT **Previous studies have shown that in mammalian cells proteins of large molecular weight are degraded more rapidly than small ones. Evidence is presented here that half-lives of proteins are also related to their isoelectric points. A double-isotope method was used to compare degradative rates of soluble proteins separated by isoelectric focusing. In rat liver, skeletal muscle, kidney, and brain, more rapid rates of catabolism were found for acidic protein fractions than for neutral or basic ones. Acidic proteins also tended to be degraded faster in several mouse tissues. A literature survey confirmed this trend. For 22 proteins from rat liver, a highly significant correlation was found between rates of degradation and isoelectric points ($\bar{r} = 0.824$; $P < 0.01$). This relationship between isoelectric point and half-life appears to be distinct from that between protein size and half-life.**

Proteins within animal and bacterial cells vary widely in their rates of degradation (1–4). There is appreciable evidence that the rates of degradation of proteins are determined to a large extent by their conformation (reviewed in ref. 5). For example, proteins with altered conformations resulting from genetic mutations (6–12), incorporation of amino-acid analogs (13, 14), or errors in protein synthesis (14) tend to be degraded much faster than their normal counterparts. Similarly the binding of substrates, coenzymes, or other ligands to polypeptides can both alter a protein's conformation and affect its degradative rate (15–22). The precise structural features of a protein that influence its half-life under normal conditions are not known. In a wide variety of tissues and organisms, proteins of large molecular weight are degraded more rapidly on the average than are small proteins (23–34). However, this relationship between polypeptide size and degradative rate is only a general tendency, and many exceptions to this correlation exist (34).

In an attempt to find other features of protein structure which might be related to rates of catabolism, we have compared degradative rates of different protein fractions after separation by isoelectric focusing. We now report that in several mammalian tissues acidic proteins are degraded faster on the average than neutral or basic ones.

MATERIALS AND METHODS

Isotope Incorporation and Preparation of Proteins. Relative degradative rates of soluble proteins were compared by the double-isotope technique first described by Arias *et al.* (35). In a typical experiment, male rats (Charles River) weighing 125–150 g were injected intraperitoneally with 100 μCi of [*U*-^{14}C]leucine (300 mCi/mmol, Schwarz BioResearch), and 4 days later with 500 μCi of [4,5-^{3}H]leucine (40 Ci/mmol, Schwarz BioResearch). After 4 hr the animals were killed and the liver, kidneys, thigh muscles, and cerebral hemispheres were removed, weighed, and placed on ice. Two volumes (v/w) of ice-cold 25 mM KCl were added, and tissues were homogenized as described previously (25). After centrifugation at 10,000 × *g* for 10 min and 100,000 × *g* for 1 hr, the proteins of the supernatant were dialyzed overnight against 25 mM KCl to remove free amino acids prior to electrofocusing. Radioactive proteins from *Escherichia coli* (14) were prepared as described earlier (26).

Isoelectric Focusing and Counting of Radioactivity. Isoelectric focusing was performed at 2° in a 110 ml capacity LKB 8100 Ampholine column (LKB-Produkter, Bromma, Sweden). Ampholytes were purchased from LKB (Ampholines), and the pH gradient (3.5–10) was established with 1% Ampholines in a 0–1.4 M continuous sucrose gradient. The sample (20–100 mg of protein) was added to the dense sucrose solution. The starting voltage was 300 V but was increased to 500 V as the amperage fell. The experiment was stopped when the current through the column reached a constant minimum value (30–48 hr). Fractions (1–2 ml) were collected and their pH was measured. The proteins were then precipitated by addition of 50% trichloroacetic acid to give a final concentration of 10%, and the precipitate was washed once with 5% trichloroacetic acid and once with ethanol:ether (1:1). The samples were solubilized and prepared for liquid scintillation counting as described previously (36). Correlation coefficients between the isoelectric point and ^{3}H/^{14}C ratio of protein fractions were calculated in the standard way (37).

Literature Survey. Published data on protein half-lives, isoelectric points, and subunit molecular weights were chosen according to criteria listed in an earlier publication (34). Product moment correlation coefficients were calculated (37) in order to evaluate the correlation between isoelectric point and degradative rate.

RESULTS

We have used the double-isotope technique of Arias *et al.* (35) to compare the relative degradative rates of soluble proteins. In this method [^{14}C]- and [^{3}H]leucine are administered at different times to the same animal to establish two time points on the exponential curve describing degradation of the protein. For any protein, the amount of the first isotope remaining (^{14}C) relative to amount of isotope most recently incorporated (^{3}H) reflects the amount of degradation during the time between administrations. Therefore, proteins that are synthesized and degraded rapidly have high ^{3}H/^{14}C ratios. The validity of this approach and its underlying assumptions have been discussed previously (5, 24, 35, 39).

Fig. 1 shows the elution pattern of double-labeled soluble proteins from rat liver after isoelectric focusing. The amount

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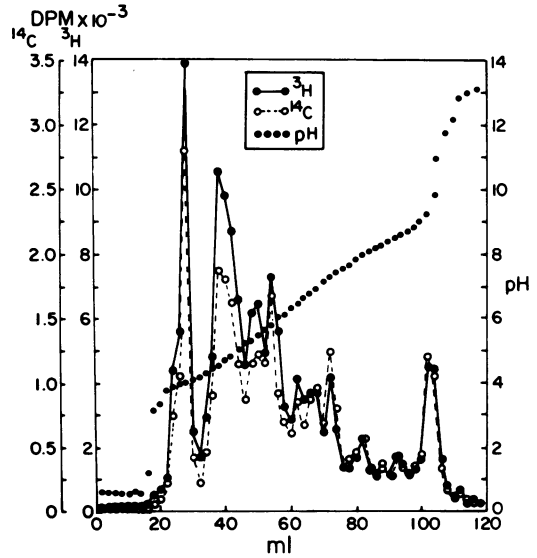

FIG. 1. The pattern of radioactivity of soluble proteins from rat liver after isoelectric focusing. A male rat (125 g) received 100 μCi of [^{14}C]leucine 4 days prior to 500 μCi of [^{3}H]leucine. The proteins (30 mg) were fractionated, precipitated with trichloroacetic acid, washed, and counted as described in the *text*. pH = ...; ^{3}H = ●———●; ^{14}C = O-----O.

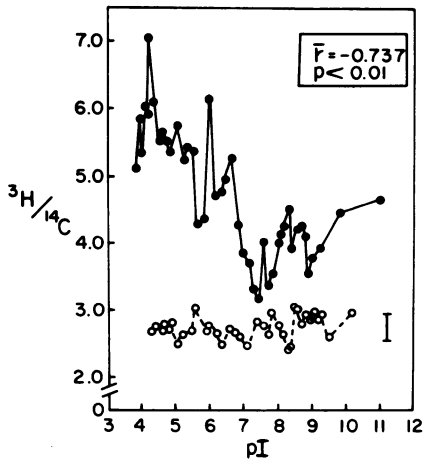

FIG. 2. Relative degradative rates of soluble proteins from rat liver separated by isoelectric focusing. The data represented by solid points (●———●) were calculated from the ^{3}H and ^{14}C radioactivities shown in Fig. 1. The results are representative of five separate experiments. The open points (O-----O) indicate the results of a control experiment using soluble liver proteins from a rat which had been given 50 μCi of [^{14}C]leucine and 500 μCi of [^{3}H]leucine simultaneously 4 hr before being killed. The bracket indicates one standard deviation of the control data. Similar standard deviations were found in two separate experiments.

of protein in the different fractions was measured after dialysis for 72 hr to remove ampholytes. Approximately 55% of the soluble proteins measured by the method of Lowry *et al.* (40) or by absorbance at 280 nm have isoelectric points less than 7. However, 75% of the ^{3}H radioactivity resided in these acidic fractions. One possible explanation for the higher specific activity of the acidic proteins is that they turn over more rapidly and thus are more extensively labeled by the injection of isotope than the basic ones (pI > 7).

The ^{3}H/^{14}C ratios of these protein fractions are plotted against their isoelectric points in Fig. 2. It is clear that acidic proteins have higher ^{3}H/^{14}C ratios, which indicates that they are degraded more rapidly on the average than are the neutral or basic proteins ($P < 0.01$). We have found very similar relationships between protein isoelectric points and half-lives in five separate experiments. The broken line in Fig. 2 indicates the results of a control experiment in which an animal received [^{14}C]leucine and [^{3}H]leucine simultaneously 4 hr before being killed.

Fig. 3 illustrates the results of similar analyses of soluble proteins from rat skeletal muscle, kidney, and brain, and from mouse liver. The elution patterns of proteins from these tissues are not shown. Rat muscle, rat kidney, and mouse liver soluble proteins appeared similar to those from rat liver (Fig. 1). However, soluble proteins from the cerebral hemispheres contained fewer basic proteins than did the other tissues. In each of these tissues there was a significant correlation between isoelectric point and ^{3}H/^{14}C ratio ($P < 0.01$ for muscle, kidney, and mouse liver and $P = 0.01$ for brain). A similar correlation (data not shown) was evident among soluble proteins from the extensor digitorum longus muscle in mice. Thus, in a variety of tissues, the degradative rates and isoelectric points of proteins seem to be related.

Control Experiments. We performed a number of experiments to eliminate possible experimental artifacts which might lead to an apparent correlation between isoelectric points and half-lives. (*a*) Acidic proteins also appeared to be degraded faster when the order of isotope administration was reversed (i.e., when ^{3}H administration preceded ^{14}C by 4 days). (*b*) We found similar results when the electrodes

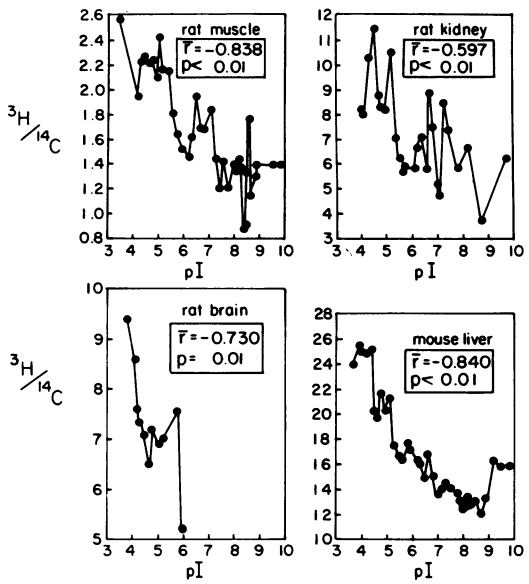

FIG. 3. Relative degradative rates of soluble proteins from rat muscle, kidney, and brain (cerebral hemispheres) and from mouse liver. Soluble proteins from each of these tissues were prepared as described in *Materials and Methods*. Muscle proteins were prepared from the hind limbs of a rat (150 g) which had received 150 μCi of [^{14}C]leucine and 400 μCi of [^{3}H]leucine. Kidney and brain proteins were from a rat (125 g) which had received 100 μCi of [^{14}C]leucine and 4 days later 500 μCi of [^{3}H]leucine. The liver proteins were prepared from four mice weighing 25 g each which had received 20 μCi of [^{14}C]leucine and 4 days later 300 μCi of [^{3}H]leucine. There were very few soluble proteins with basic isoelectric points in brain, so isotope ratios could not be calculated above pI 6.0 in this tissue. The statistical significance of the correlation between pI and ^{3}H/^{14}C ratio is shown for each tissue. These results are representative of two separate experiments for brain and kidney, three for muscle, and four for mouse liver.

Table 1. Half-lives, isoelectric points, and subunit molecular weights for 22 intracellular proteins from rat liver

Protein	EC number	$T_{1/2}$ (hr)	pI	Subunit molecular weight ($\times 10^{-3}$)
1. Ornithine decarboxylase	4.1.1.17	0.2 (34)	4.1 (53)	75 (34)†
2. δ-Aminolevulinate synthetase	4.2.1.24	0.3 (54)	5.9 (55)†	42 (55)
3. RNA polymerase, I	2.7.7.6	1.3 (34)	6.5 (56)*	83 (34)†
4. Tyrosine aminotransferase	2.6.1.5	2.0 (34)	4.1 (57)	32 (34)
5. Tryptophan oxygenase	1.13.1.12	2.5 (34)	5.0 (58)*	43 (34)
6. Phosphoenolpyruvate carboxylase	4.1.1.32	5.0 (34)	5.0 (34)	74 (34)
7. RNA polymerase, II	2.7.7.6	12 (34)	6.0 (56)*	83 (34)†
8. Glucose-6-phosphate dehydrogenase	1.1.1.49	15 (34)	5.3 (59)	63 (34)†
9. Ornithine aminotransferase	2.6.1.13	19 (34)	5.4 (34)	33 (34)
10. Pyruvate kinase	2.7.1.40	30 (34)	6.0 (60)	62 (34)†
11. Catalase	1.11.1.6	30 (34)	6.7 (61)*	62 (34)
12. Fructose-1,6-diphosphatase	3.1.3.22	36 (34)	8.1 (62)*	33 (34)*
13. Ferritin		36 (34)	5.7 (41)*	19 (34)†
14. Cytochrome b_5 (microsomal)		55 (34)	5.8 (34)*	17 (34)*
15. Histidase	4.3.1.3	60 (63)	5.4 (64)	76 (65)
16. Aspartate aminotransferase	2.6.1.1	72 (34)	5.6 (66)*	55 (34)*
17. Arginase	3.5.3.1	96 (34)	9.4 (67)	30 (34)
18. Malate dehydrogenase	1.1.1.40	96 (34)	9.3 (68)	33 (34)
19. Aldolase	4.1.2.31	118 (34)	10.1 (69)	40 (34)
20. Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	130 (34)	8.0 (70)*	36 (70)
21. Lactate dehydrogenase, isozyme 5	1.1.1.27	144 (34)	8.5 (71)†	18 (34)*
22. Cytochrome *c*		150 (34)	9.2 (72)†	14 (34)†

The rationale for selecting these values from the literature is given elsewhere (34). The numbers in parentheses indicate the appropriate references. An asterisk (*) denotes the values determined for the protein from a mammalian liver other than the rat. A dagger (†) signifies that the value was obtained from mammalian tissues other than the liver. Unfortunately, space limitations do not permit us to acknowledge all original papers here. Many specific references are cited in a previous manuscript (34).

were reversed in the electrofocusing run so that proteins of a given isoelectric point focused in different regions of the sucrose gradient. (*c*) The medium in which tissues were homogenized did not affect the elution pattern of soluble proteins from liver. There were similar relationships between isoelectric point and half-life whether distilled water, 25 mM KCl, a phosphate buffer at pH 7.2 (25), phosphate-buffered NaCl (1.25 M), or an isotonic Tris·HCl buffer at pH 7.4 (31) was used. (*d*) We varied the interval between administration of the two isotopes between 3 and 10 days, since Glass and Doyle (24) have shown that altering this time interval results in selective labeling of proteins of different half-lives. Theoretically, changing the time interval might affect the observed relationship between protein charge and half-life. However, in each case acidic proteins had higher $^3H/^{14}C$ ratios. (*e*) Another possible artifact might be selective degradation of neutral and basic proteins during the isoelectric focusing. For example, tissue proteases might have neutral and basic isoelectric points and might selectively degrade proteins of high $^3H/^{14}C$ ratios in these fractions in the course of the experiment. However, when we electrofocused nonradioactive proteins from rat liver and added highly radioactive *E. coli* proteins to each fraction, less than 3% of the protein was degraded to acid-soluble form after even 5 days at 4°. (*f*) Since [^{3}H]leucyl-tRNA might electrofocus in the acidic region (41) and increase the $^3H/^{14}C$ ratio of these fractions, we hydrolyzed RNA from each fraction by hydrolysis at 90° for 15 min or by extensive RNase digestion prior to counting radioactivity. Less than 2% of the radioactivity was made acid-soluble by these treatments, and the $^3H/^{14}C$ ratio of none of the fractions was significantly affected. (*g*) It is possible that these experiments indicate more rapid secretion of acidic proteins rather than more rapid degradation, although secretion of radioactive proteins from the liver should have been completed by 4 hr after administration of the second isotope (35). In some experiments 12 hr elapsed between the final isotope injection and killing the animal to insure that secretion of labeled extracellular protein was complete. Once again, acidic proteins appeared to be degraded most rapidly. (*h*) Finally, these same experiments also rule against the possibility that our results indicate that many intracellular proteins undergo a postsynthetic maturation process which results in their becoming more basic. This explanation appears unlikely since protein maturation appears to be a rapid process (42–46) that should be completed within 4 hr and certainly within 12 hr after administration of the second isotope.

Literature Survey. We have surveyed the literature to determine whether published values for the degradative rates of proteins from rat liver correlate with their isoelectric points and to learn more about the nature of this relationship. We found 22 proteins for which data on rates of degradation and isoelectric points were available (Table 1, Fig. 4). There is a highly significant correlation between isoelectric point and degradative rate for these proteins ($\bar{r} = 0.824$; $P < 0.01$) in confirmation of the double-isotope studies.

The half-lives of the proteins listed in Table 1 are also related to the logarithm of their subunit molecular weight ($\bar{r} = -0.587$; $P < 0.01$), in line with previous studies (34). By calculation of partial correlation coefficients, it was possible to test statistically whether the influence of molecular weight on degradative rates can explain the observed relationship between half-life and isoelectric points or *vice versa* (37). When the contribution of molecular weight was held constant in this way, a highly significant correlation remained between isoelectric point and half-life ($\bar{r} = 0.780$; $P < 0.01$). Conversely, the partial correlation coefficient between the log of subunit size and degradative rate remained significant

($\bar{r} = -0.449$; $P < 0.05$) when the isoelectric points were held constant. Furthermore, protein isoelectric point and subunit molecular weight are not related for these 22 proteins when half-lives are held constant ($r = 0.107$; P = not significant). Therefore, neither correlation can be explained by the other. In addition, recent experiments using the double-label approach also support the conclusion that protein size and isoelectric point are separable factors influencing rates of degradation (Dice and Goldberg, in preparation).

DISCUSSION

The present experiments indicate that in several mammalian tissues proteins with acidic isoelectric points tend to be degraded more rapidly than those with neutral or basic isoelectric points. This result was confirmed by statistical analysis of 22 proteins from rat liver whose degradative rates and isoelectric points had been previously reported. Our recent experiments also indicate more rapid degradation of the acidic proteins in rat serum and in skeletal muscle of both normal mice and mice with genetic muscular dystrophy. It is important to test whether this correlation also holds for proteins in non-mammalian and prokaryotic cells and for proteins in rat liver that are associated with membranes and cell organelles. It is interesting that the basic ribosomal proteins are degraded more slowly than average soluble proteins, and the very basic histones turn over slowly, if at all, in liver (Dice and Goldberg, in preparation).

There are many possible explanations why acidic proteins may turn over more rapidly than neutral or basic ones. On one hand, this correlation may reflect special chemical properties of the acidic or basic polypeptides:

(*1*) The simplest explanation for the diversity of half-lives among intracellular proteins is that they reflect differences in the inherent sensitivity of proteins to the cell's proteolytic enzymes (5, 25, 47–49). In both animal and bacterial cells, the catabolic rates of proteins correlate well with their relative sensitivities to a variety of proteases *in vitro*. For example, the more rapid degradation of large polypeptides may be explained by the finding that the larger proteins are on the average more susceptible to hydrolysis by trypsin or Pronase *in vitro* than smaller ones (25). The present findings could be explained if the acidic proteins also tend to be inherently more sensitive to proteolytic attack than neutral or basic ones. Unfortunately, this possibility has been difficult to examine definitively, because some proteins appear to be denatured by the electrofocusing process (Dice and Goldberg, unpublished).

(*2*) It is also plausible that the native conformations of the acidic polypeptides are inherently less stable. These proteins may be more susceptible to spontaneous denaturation, which may represent the rate-limiting step in protein degradation (5, 50, 51). Further information comparing the chemical properties of the acidic, neutral, and basic proteins may thus help elucidate the basis for this correlation.

On the other hand, the correlation between isoelectric point and degradative rate may reflect properties of the intracellular proteolytic mechanism(s) responsible for protein catabolism.

(*1*) The crucial protease(s) may preferentially hydrolyze polypeptides at acidic residues or may preferentially bind to proteins of a certain charge. Thus far, intracellular proteases specific for acidic residues have not been described. The proteins with acidic isoelectric points will be least charged if protein degradation occurs in the acidic environment of the lysosomes, but will be most negatively charged if it takes place at the neutral pH of the cytoplasm.

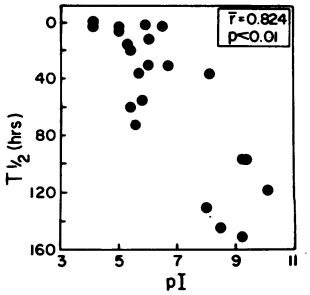

FIG. 4. The relationship between protein isoelectric point and half-lives for the 22 proteins listed in Table 1.

(*2*) It is also possible that acidic proteins preferentially accumulate in regions of the cell where the degradative enzymes are localized. For instance acidic proteins may be taken up by lysosomes more rapidly, or once inside such organelles, they may be released more slowly (52) than neutral or basic proteins. Similarly, if degradation occurs on membranes or other organelles, the acidic proteins may bind more tightly to such sites.

Whatever the basis for the relationship between isoelectric point and protein half-life, it is but one of several factors which may influence a protein's degradative rate. Clearly, there are exceptions to this general relationship (Fig. 4; see Note Added in Proof). For instance, proteins with isoelectric points of 6 vary in half-lives between 0.3 and 60 hr. Other factors which may account for this variability include protein size, ligand binding properties, and the existence of specific proteases for certain of the enzymes (reviewed in ref. 5).

Similarly, many proteins are known that do not appear to fit the correlation between protein size and half-life. Their half-lives may appear less exceptional if the relationship between isoelectric point and degradative rate is also taken into consideration. It may be possible to develop an empirical formula that accurately predicts degradative rates using both the subunit size and isoelectric points.† Hopefully, by defining the relative contributions of subunit size and isoelectric point in determining half-lives, it will be possible to obtain further information about the degradative process and to evaluate the importance of other factors in influencing protein degradation.

Note Added in Proof. Recently we have found published data for the lysosomal enzyme β-glucuronidase (EC 3.2.3.31). Although it is an acidic protein [pI = 5.8; Lin, C.-W., Orcutt, M. L., & Fishman, W. H. (1975) *J. Biol. Chem.* **250**, 4737–4743] and its subunits are relatively large [molecular weight 75,000; Stahl, P. D. & Touster, O. (1971) *J. Biol. Chem.* **246**, 5398–5406], it is degraded extremely slowly [$T_{1/2}$ = 15–30 days; Wang, C.-C. & Touster, O. (1975) *J. Biol. Chem.* **250**, 4896–4902]. If true, this exceptional behavior supports the suggestion that degradation of lysosomal components may show different characteristics than degradation of soluble proteins [Wang and Touster; Dean, R. T. (1975) *Biochem. Soc. Trans.* 554*th Meeting* 3, 250–252].

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† Since this analysis is limited by the available data, the authors would appreciate receiving information from other investigators about recent determinations of the isoelectric points, subunit molecular weights, and half-lives of proteins in rat liver.

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