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Correlation of degradative rates of proteins with a parameter calculated from amino acid composition and subunit size

(protein turnover/protein half-life)

FRANK A. MOMANY, JAMES J. AGUANNO, AND ALLAN R. LARRABEE*

The Department of Chemistry, Memphis State University, Memphis, Tennessee 38152

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ABSTRACT A parameter is developed which relates the amino acid composition and subunit size of a protein to the degradative rate *in vivo*. This parameter was calculated for 11 rat liver proteins and a plot versus the half-lives of these proteins is linear and has a coefficient of correlation of −0.96. Evidence is presented which suggests that the density of excess acidic amino acids on the surface of the protein is the most important factor in determining differential turnover.

The existence of continual intracellular protein renewal or turnover has been studied in many laboratories since the advent of the use of isotopes (1–5). Careful studies have shown that intracellular protein degradation obeys first order kinetics and therefore each protein has a particular half-life or $t_{1/2}$ value. There are many technical difficulties in determining this value for a particular protein, most of which result in an overestimate of the turnover time. As the techniques for the determination of the $t_{1/2}$ value have improved, the published values have tended to decrease (3, 4). Schimke *et al.* (6) showed that in various eukaryote tissues the larger subunits have a tendency to be degraded more rapidly than smaller ones. Dice and Goldberg found a correlation between the logarithm of the subunit molecular weight and the measured or estimated half-life of 33 rat liver proteins (7). A straight line plot of the log of the subunit molecular weight versus the turnover time showed a coefficient of correlation of −0.60. More recently, Dice and Goldberg have obtained a relationship between the isoelectric point and the half-life of 22 rat liver proteins for which both these parameters are known (8). A straight line plot of the isoelectric point for these proteins (sometimes estimated from the isoelectric point of homologous proteins from other animals) versus the $t_{1/2}$ value gives a correlation coefficient of −0.82. These results suggest that the turnover time of a protein must be dependent at least in part upon the composition and structure.

In this work a parameter is developed which is both a function of subunit size and the amino acid composition and is proportional to the turnover time. This parameter and a study of the primary sequence of some of the proteins considered has emphasized that the overall surface polarity of a protein rather than a specific site on the protein surface may be the more important factor regulating its *in vivo* half-life.

RESULTS AND DISCUSSION

In order to calculate a parameter that is based on both subunit size and amino acid composition and that is related to protein turnover, it is necessary to select proteins whose subunit number and amino acid composition are well established or can be reasonably approximated. In addition, the turnover time must have been carefully measured during conditions close to the "steady state." Under these conditions the rate of protein synthesis in moles per minute is balanced by the rate of protein degradation in moles per minute and thus the level of enzyme is unchanged. The half-lives of many adaptive rat liver enzymes have been studied. The steady-state level of these enzymes is very responsive to nutritional perturbations. Deviations from the steady state have in some cases been shown to be effects on the rate of synthesis or the rate of degradation or both (3, 4). Thus, the measured half-life of an enzyme, particularly an adaptive one, may be a result of a basal rate which is accelerated or depressed as a function of the nutritional state.

Dice and Goldberg (7) have listed 33 proteins from rat liver whose half-lives are known or have been estimated. However, the amino acid composition and subunit size are known for only a few of these proteins. Consequently, the following criteria were used in compiling a list of proteins for consideration. If the authors of various papers reported a $t_{1/2}$ value for a particular protein under steady-state or nearly steady-state conditions and the amino acid composition was known, that protein was considered for this work. No attempt was made to estimate the turnover time if it was not so expressed (either as a $t_{1/2}$ value or as the first order rate constant of degradation) by the authors. The values of the half-life of rat liver enzymes determined during starvation and refeeding experiments (e.g., 9, 10) and those after stimulation by hormones (e.g., 11) were not used. In addition certain enzymes, such as serine dehydratase of rat liver, are extremely sensitive to the level of amino acids and glucose in the diet (12) and in those cases the unperturbed or basal rate of turnover is difficult to assess. One exception to the above rule was made in the case of glucose-6-phosphate dehydrogenase. Although the $t_{1/2}$ value for this enzyme was not determined in the steady state, the rate of change of the enzyme was used to estimate the steady state rate of 15 hr as quoted by the author (13). As mentioned above, the measured half-life of any protein has to be interpreted with caution, since it may be a basal rate which can be altered by the effect of adaptive mechanisms in response to dietary or hormonal changes. If more than one $t_{1/2}$ value was reported for an enzyme in rat liver (steady state on any diet) we utilized the smallest value, since many errors in measurements of turnover overestimate the $t_{1/2}$ value because of problems such as recycling of the released amino acids (3, 4). The amino acid composition of rat liver proteins whose $t_{1/2}$ value has been estimated is also not always available (e.g., ornithine decarboxylase). Less commonly, the $t_{1/2}$ value and amino acid composition may be known, but the number and size of subunits of the enzyme is not definitely established (e.g., alanine amidotransferase and acetyl-CoA carboxylase) (14–18). Finally, in the case of the protein ferritin, the $t_{1/2}$ value has been studied but the amino acid composition has recently been shown to be that of two quite different subunits (19). Both the amino acid composition and the steady-state $t_{1/2}$ value have been determined for five rat liver proteins (Table 1). In the six remaining proteins considered, the $t_{1/2}$ value is known and the amino acid composition can be approximated by using compositions of homologous proteins from

* To whom reprint requests should be directed.

Table 1. Half-life, partial amino acid composition, subunit size, and the calculated parameter for 11 proteins

Protein (EC number)	$t_{1/2}$ in rat liver* (hr)	Source for composition*	Monomer molecular weight (× 10^{-3})	Subunits	S†	Glx‡	Asx‡	Lys‡	Arg‡	His‡	$\frac{A-B}{S}$§
Tryptophan oxygenase (1.13.11.11)	2.5 (20)	Rat liver (28)	43	4¶	120	56	39	28	21	10	0.30
Tyrosine aminotransferase (2.6.1.5)	3 (21, 22)	Rat liver (29)	29	4	94	29	24	12	12	5	0.26
Glucose-6-phosphate dehydrogenase (1.1.1.49)	15 (13)	Mouse liver (31)	62	2	157	69	56	39	34	12	0.25
Catalase (1.11.1.6)	25 (24)	Bovine liver (32)	57.5	4	149	46	68	27	31	21	0.23
Ornithine aminotransferase (2.6.1.13)	42 (23)	Rat liver (30)	33	4	103	28	25	17	13	7	0.16
Cytochrome b_5	55 (25)	Rabbit liver (33)	16.7	8	61	15	16	11	4	7	0.15
Aldolase (4.1.2.13)	68 (26)	Rabbit muscle (34)	39.5	4	116	40	29	26	15	11	0.15
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	75 (26)	Pig muscle (35)	37	4	111	17	38	25	10	12	0.072
Lactate dehydrogenase isozyme 5 (1.1.1.27)	84 (26)	Rat liver (36)	35	4	107	29	32	28	10	6	0.15
Arginase (3.5.3.1)	96 (27)	Rat liver (37)	29.5	4	95	22	22	22	8	6	0.084
Cytochrome *c*	146 (25)	Porcine and bovine heart (38)	13.4	1	96	12	8	18	2	3	−0.054

* Numbers in parentheses refer to appropriate references.
† S is the number of amino acids on the surface of the monomer calculated as described in the *text*.
‡ All amino acid residues are per monomer.
§ The sum of the acidic residues (A) minus the sum of the basic residues (B), this result is divided by S.
¶ This enzyme has four equal-sized subunits. There are two pairs of similar but not identical subunits (29).

related species. Clearly, this is an approximation which could obscure the validity of the results. However, such an approximation does allow the input of data from 11 proteins rather than just five.

The turnover of the 11 proteins listed in Table 1 did not appear to correlate with the number of residues of any one amino acid or any combination of amino acids. However, a relation between the number of excess acid residues per unit of surface area of the monomer and the reported $t_{1/2}$ value was found as described below. The computation of the number of residues on the surface of a subunit is made by assuming that the subunits are spherical molecules. Proteins have a density of approximately 1.35 g/cm^3 and dividing the molecular weight by the density and also dividing by Avogadro's number gives the volume of one molecule of protein which is then expressed in Å^3. Formulas for the surface and volume of a sphere were then used to calculate that the surface area of one molecule is approximately equal to $5.5 \times M_r^{2/3}$ Å^2 where M_r is the molecular weight. We have examined molecular models of various proteins, and have found that the area per amino acid residue on the surface is about 50–60 Å^2. The final equation for the number of residues on the surface of a protein molecule is then

Table 2. Comparison of calculated and reported half-lives

Enzyme (EC number)	Composition reference	Source	$\frac{A-B}{S}$	$t_{1/2}$ (hr) (Fig. 1a)	$t_{1/2}$ (hr) (From ref. 7)
Malate dehydrogenase (decarboxylating) (1.1.1.40)	39	Rat liver	0.28	<5	96
Asparate aminotransferase (2.6.1.1)	40	Pig heart	0.23	18	72
Fructose-1,6-diphosphatase (3.1.3.11)	41	Rabbit muscle	0.22	22	36
Phospho*enol*pyruvate carboxylase (4.1.1.32)	42	Pig liver	0.21	27	5
Serine dehydratase (4.2.1.13)	43	Rat liver	0.15	56	4
Pyruvate kinase (2.7.1.40)	44	Rabbit muscle	0.14	61	30
ATP citrate lyase (4.1.3.8)	45	Rat liver	0.44*	7†	24

* Calculated for multimer since subunit size is not known (see Fig. 1b).
† From Fig. 1b.

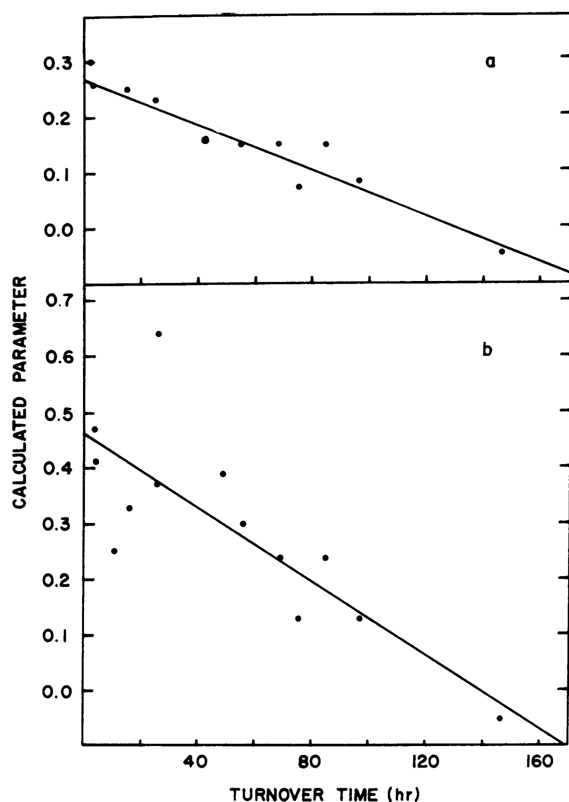

FIG. 1. (a) A plot of the half-life versus the calculated surface polarity parameter for the proteins listed in Table 1. Calculations are based on the monomer of each protein. (b) A plot of the same parameter after recalculation utilizing the oligomeric unit instead of the monomeric unit. This plot also utilizes the data of Table 3.

found to be about $0.1 \times M_r^{2/3}$. Deviations from this value will most certainly be found from protein to protein. However, examination of the sedimentation coefficients for the multimeric proteins whose subunits are considered shows no large deviations from a plot of Svedburg number versus $M_r^{2/3}$. Since the number of subunits in these oligomers is small (usually 4), the subunits may also be approximated as spheres. If the sum of the number of basic residues (His, Lys, Arg) is subtracted from the sum of the number of acidic residues (Asx plus Glx) and the difference is divided by the number of residues on the surface of the monomer, a parameter is obtained (Table 1) which can be considered as the density of excess acidic residues per unit of surface area. This parameter is a function of both amino acid composition and subunit size, since the larger the subunit, the greater the number of amino acids on the surface of the monomer. A straight line least squares fit of this parameter versus the $t_{1/2}$ value is shown in Fig. 1a and gives a coefficient of correlation of −0.96. The validity of this relationship is subject to the effects of the approximations and uncertainties discussed. In addition, experimental turnover times can be too low when the $t_{1/2}$ value is measured during a period of rapid adaptive change. In spite of these obvious difficulties and the relatively few proteins available for comparison, the relationship shown in Fig. 1a is very encouraging. For the relationship of subunit size and pI to turnover time mentioned above, Dice and Goldberg estimated the $t_{1/2}$ value from data from both steady-state as well as nonsteady-state experiments. Table 2 shows a comparison of this compilation of the $t_{1/2}$ value of seven proteins (not included in this work because of insufficient data) with the values predicted from the curve shown in Fig. 1a. In the case of fructose-1,6-diphosphatase there is reasonable agreement between his estimated $t_{1/2}$ value and our calculated value. However, the other pairs of values deviate considerably. These differences may represent two different turnover phenomena. The half-lives from Fig. 1a are suggested to be that of a basal rate, whereas the estimates by Dice and Goldberg may be those of a basal rate superimposed on that of an adaptive mechanism. This concept has already been emphasized by Grisolia *et al.* (46) and by Szepesi and Freedland (47). The latter estimated the half-life of various enzymes by measuring the rate of change from or to the steady state. This method forms the basis for the previously mentioned exception, glucose-6-phosphate dehydrogenase. This concept receives support when we reconsider serine dehydratase. As mentioned, the level of this enzyme is very responsive to dietary glucose and amino acids and the apparent half-life varies between 4.5 and 40.5 hr (12). Perhaps our predicted value of 56 hr is close to a basal unperturbed rate.

Dice and Goldberg have suggested (7) that although the best relation to turnover time is to monomer size, there is a weak correlation to the multimer size. If we repeat the above calculation assuming the multimer to be a sphere and approximate (as before) the number of amino acid residues on the surface of that sphere a new plot may be obtained. The least squares fit to the new points gives a coefficient of correlation of −0.83 (Fig. 1b). This value is lower than before but does include two new data points (Table 3) from proteins whose subunit size is presently not known or is in dispute but whose molecular weight is known. If Dice and Goldberg's plots of the log of the subunit molecular weight and isoelectric point versus $t_{1/2}$ are recalculated considering only those 11 proteins originally considered above, the coefficient of correlation of the plot of the log of the subunit molecular weight versus $t_{1/2}$ value improves from −0.60 to −0.63 and the fit of isoelectric point versus the $t_{1/2}$ value improves from −0.82 to −0.84. This latter method has an advantage in that the pI is known for more proteins than is the amino acid composition, although Dice and Goldberg also used some pI values from homologous proteins. The parameter

Table 3. Half-life, partial amino acid composition, and the calculated parameter for two additional proteins

Protein (EC number)	$t_{1/2}$ in rat liver (hr)	Source for composition	Molecular weight $\times 10^{-3}$	S*	Glx†	Asx†	Lys†	Arg†	His†	$\frac{A-B}{S}$
Alanine aminotransferase (2.6.1.2)	25 (14)	Rat liver (15)	114	235	166	73	22	56	10	0.64
Acetyl-CoA carboxylase (6.4.1.2)	48 (16)	Rat liver (17)	215‡	359	233	179	98	118	57	0.39

* Calculated for the multimer.
† All residues are per multimer.
‡ Rat liver acetyl CoA carboxylase is active only as a linear polymer of this subunit. The assumption made here is that the density of excess acid functions per unit of surface area is the same for the linear polymer as for a single subunit.

developed here appears to be a more fundamental relationship but needs to be tested by the input of more data as such becomes available. An additional source of error may be the paucity of data concerning the number of acidic groups which are actually amidated and occur as glutamine or asparagine. It seems clear that the information for the rate of protein turnover in a cell resides, at least in part, in the makeup of the protein itself (3). The correlation shown in Fig. 1a enhances this conclusion. Moreover, the fact that a correlation can be made between turnover time and the entire amino acid composition argues against a specific site on the protein which is a singularly important recognition signal for the turnover proteases. Rather, the parameter of Fig. 1a stresses the importance of the overall surface polarity of the molecule.

In order to test this hypothesis in a more detailed manner we have searched for possible correlations between the amino acid sequence of a protein and its half-life. Of the proteins treated, only three have been sequenced, namely, cytochrome *c* (38), aldolase (34), and glyceraldehyde-3-phosphate dehydrogenase (35). Lactate dehydrogenase, isozyme 5, has been sequenced from dogfish (48). Another protein, cytochrome b_5, has been partially sequenced and this protein (minus 40 residues on the end) was considered (49). The primary structure of pig heart aspartate aminotransferase is also known (40), but the turnover time in rat liver has not been carefully determined. The data from Fig. 1a were used to estimate the basal turnover time for this enzyme. In order to gain some insight as to probable regions of secondary structure (α-helical regions and β-bends) computational predictive methods were used which require only the primary structure. The helical predictive algorithm utilized was that of Lewis *et al.* (50) and the β-bend algorithm that of Lewis *et al.* (51). The information derived from any predictive algorithms is presently insufficient to allow a determination of the complete three-dimensional structure of these proteins. However, it should be possible to use the information obtained from such algorithms to search for possible correlations between secondary structural properties and the protein turnover or degradation rate. Several criteria were established for this search. First, the assumption was made that there is no large thermal or pH-induced unfolding of cellular proteins *in vivo*; thus, any portions of the polypeptide chain which become, or are naturally, accessible to proteolytic enzymes arise from a structure very close to the native conformation. Second, it has been shown from *in vitro* studies (52) that in general known proteolytic enzymes do not attack residues in the helical regions, or those residues buried inside the protein (53), while they do attack at surface exposed residues (β-bends or extended regions). Further, even if the protein exists in a number of conformational states in the physiological environment, the helix regions most probably persist, relatively intact from one state to the next (54). Third, the kinetics of proteolytic attack of native proteins appears to follow first-order kinetics (55). It has been suggested that the first "hit" or "nick" is the rate-limiting step and that further degradation is rapid, relative to this step (3).

The secondary structural profiles of the six proteins mentioned were calculated and a search was initiated for a correlation between turnover half-life and (*a*) percent helix and (*b*) the percent β-bends or chain reversal sites (four residues per bend, and excluding occurrences of bends which fall in helical regions). No direct correlation between $t_{1/2}$ and amounts of helix or bend in the sequence could be found. This result seems to be in agreement with the empirical correlation, which suggests that an electrostatic effect is dominant in determining turnover. No correlation was found between relative numbers of bends with basic groups and $t_{1/2}$. We further examined relative numbers of bends with aromatic groups, with and

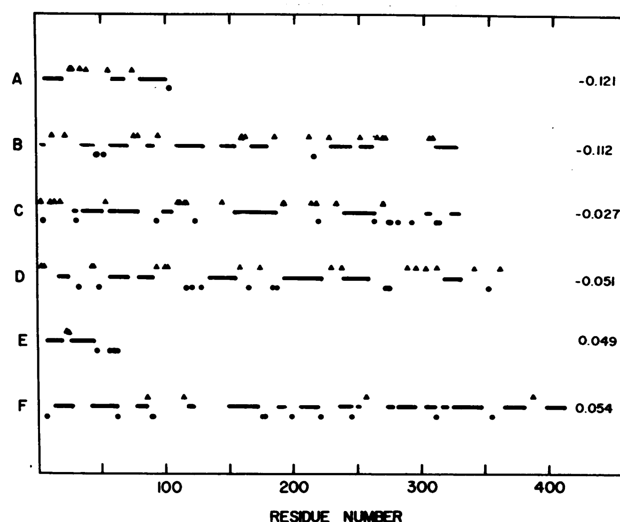

FIG. 2. A representation of the predicted α-helical regions (solid lines) of (A) cytochrome *c*, (B) lactate dehydrogenase isozyme 5, (C) glyceraldehyde-3-phosphate dehydrogenase, (D) aldolase, (E) cytochrome b_5, and (F) aspartate aminotransferase. The half-lives of these proteins are 146, 84, 74, 68, 55, and 18 hr, respectively. Also indicated is the location of isolated acidic (●) and basic (▲) residues. The α-helical regions for lactate dehydrogenase are those shown by x-ray analysis (56) rather than those of a predictive algorithm as described in the *text*. The number to the right of each line is the turnover parameter calculated for the monomer as described in the *text*.

without basic residues, and again found no correlation with $t_{1/2}$. Since the correlation developed above utilized the excess of acidic groups over basic groups, a study was initiated of the occurrence of charged amino acids (basic or acidic) which are not in close proximity to a residue of opposite charge. Any charged residues which occur in helixes and any charged residues which are three residues or less from an oppositely charged residue were excluded from consideration. The neutral Asn and Gln residues are not included in this analysis. The parameter developed above $[(A - B)/S]$ was recalculated utilizing only these isolated acidic and basic residues. The results are shown in Fig. 2 for the four proteins whose sequence can be approximated from homologous sources. The sequence of lactate dehydrogenase from dogfish was included even though this species is not closely related phylogenetically to the rat. Finally, pig heart aspartate aminotransferase was also included. The sequence is known and the $t_{1/2}$ was predicted from the empirical correlation of Fig. 1a to be 18 hr. Here again the proteins with shorter half-lives (compare Table 1) tend to have a higher ratio of acidic residues to basic residues, but this excess is not necessarily localized at one section of the sequence to form an obvious charged site. In spite of the obvious assumptions made in gathering the data shown in Fig. 2 and also the small number of proteins considered, a plot of $t_{1/2}$ versus the calculated parameter, utilizing only the acidic and basic residues indicated in Fig. 2, shows a coefficient of correlation of −0.85. The fact that the new calculated parameters have more negative values is merely a reflection of the fact that α-helixes contain more acidic residues than basic residues. There are at least two possible explanations that these correlations suggest concerning protein turnover. First, if the protease responsible for degradation had an affinity for surface-exposed negatively charged residues, one might expect that the greater the number of accessible sites, the higher the probability of suitable binding and the greater chance of the protein's being nicked. It may well be that in some specific cases cofactor or substrate molecules may inhibit proteolysis when present on the protein by covering

an important acidic group (57). The acid residues then could be effective in binding the attacking protease only when the cofactor or substrate is absent. This effect could explain the relationship of various substrates and cofactors to $t_{1/2}$. The second possible explanation arises from the overall magnitude of net excess charge, but does not require protease directed to a specific polar charge. If cellular proteins, by their intrinsic surface charge, are directed in some manner to regions in the cell that are rich in degradative enzymes, the observed differences in $t_{1/2}$ should be related to their *in vitro* electrophoretic mobility. Thus, both molecular weight and ionic charge may play important roles in rates of transfer. As noted before, cofactors or substrates could be important for such transfer, since charged groups could be masked or uncovered under different conditions. The new element to this second explanation is the role that membranous material would play in allowing for differential transport of proteins through the cell. The highly acidic proteins would move most rapidly on or through membranes to the degradative site. The proteases at this site will then attack the larger proteins (which may even be transported more slowly) at a more rapid rate than smaller proteins. This concept agrees with a previous suggestion that the size relationship of subunits to $t_{1/2}$ is not explained by, and may be a separate phenomena from, the addition relationship to isoelectric point (8). This second membrane-related explanation of turnover may well explain why a cell-free soluble system that shows differential turnover has never been found, whereas soluble systems showing the size relationship to turnover continue to appear (58). The essential elements of this second explanation have been expressed previously (3, 4) but the correlation found in this work offers additional support for such models.

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