A CORRELATION BETWEEN TURNOVER RATES AND LIPOPHILIC AFFINITIES OF SOLUBLE RAT LIVER PROTEINS

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SUMMARY: With the use of alkyl agarose columns which separate proteins according to their lipophilic affinities, a marked correlation has been uncovered between this parameter of proteins and their degradation rates in vivo. The possible relationship between these observations and analogous ones with other properties of proteins and their implications in regard to the mechanism of the turnover process are discussed.

INTRODUCTION

The mechanism of intracellular protein turnover and its regulation remain unresolved (1). Among other suggestions, it has been proposed that gradations among proteins in their affinities for membranes may be a factor in determining their turnover rates (2). Consistent with this hypothesis is a finding of Dean, who has reported a greater adsorption onto lysosomes of rapidly turning over proteins compared to the whole pool of soluble rat liver proteins (3). However, the discrimination was modest and based upon the measurement of material representing only a very small fraction of the total protein pool. Moreover, Huisman et al. in similar experiments could find no such selectivity (4).

We have separated the pool of soluble rat liver proteins according to their lipophilic affinities on agarose columns to which were attached hydrocarbon arms of increasing length, according to the method of Shaltiel (5), and have found a marked and continuous relationship between turnover rates in vivo and lipophilic affinity in this population of proteins.

MATERIALS AND METHODS

In order to distinguish turnover rates in the protein pool, the double label technique was used (6). Animals were male Holtzman rats in the 300 g range and were fasted for 18 hr before isotope injections, which were by the intraperitoneal route. (^{3}H) leucine (250 μc) was injected first, followed 92 hr later by an injection of (^{14}C) leucine (100 μc). Labeled compounds were obtained from Schwarz/Mann. Four hr after the second injection, the rats were sacrificed and the livers perfused thoroughly with 0.9% NaCl, then homogenized in 3 volumes of 0.3 M sucrose in 1 mM EDTA, pH 7.2. The homogenate was centrifuged for 15 min at 3,000 x g and the supernatant fluid therefrom recentrifuged at 112,000 x g for 90 min. The high speed supernatant fluid was dialyzed several times against 0.06 M Na phosphate buffer, pH 7.4, containing 1 mM EDTA. In the first dialysis, 0.1% leucine was also present.

Radioactivity determinations were performed in a Beckman LS-250 liquid scintillation counter. The efficiencies of counting ^{14}C and ^{3}H were 52% and 22%, respectively, and the spillover of ^{14}C counts into the ^{3}H channel was 14%.

The chromatography media were obtained from Miles Laboratories, Elkhart, Ind. and consisted of agarose to which were attached linear hydrocarbon chains of lengths C_2, C_4, C_6, C_8, and C_{10} (5). Column volumes were 10 ml and were equilibrated with the dialysis buffer. Four ml of the liver high speed supernatant fraction, containing 53.4 mg of protein, 438,000 dpm of ^{3}H, and 284,000 dpm of ^{14}C were added to the C_2 column, which was then developed with the dialysis buffer. Approximately 4 ml fractions were collected. After the non-retarded protein had flowed through the column, as monitored by a return of the 280 nm absorbance to the baseline, the adsorbed protein was eluted with 0.1 N NaOH. The non-adsorbed and adsorbed fractions were pooled separately, concentrated on an Amicon ultrafilter (cut off, >10,000 daltons), to 4.0 ml and 2.5 ml, respectively, and 0.1 ml aliquots taken for counting in Merit (Isolab, Akron, Ohio). The remainder of the fraction which passed through the C_2 column was added to the C_4 column and the process repeated sequentially with each column. Yields from each chromatography step were 85% to 94%.

Protein determinations were by the method of Lowry et al. (7).

RESULTS

From the results in Fig. 1, it can be seen that there was a substantial correlation in the soluble protein pool of rat liver between turnover rates, as monitored by $^{14}C/^{3}H$ ratios, and lipophilic affinity, as indicated by the hydrocarbon chain length of the column to which each fraction was adsorbed. Moreover, with each column there was a marked selectivity for the adsorption of proteins of higher turnover rates than the average of the pool added to the

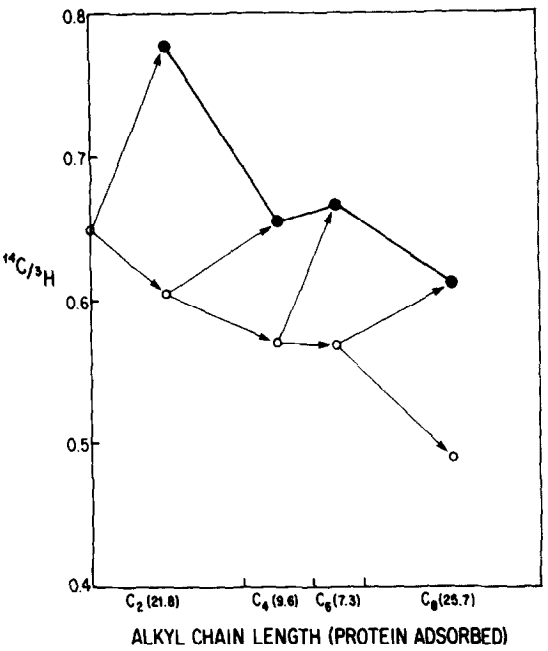

Fig. 1. $^{14}C/^{3}H$ ratios of proteins adsorbed to alkyl agarose columns of increasing chain length. The ordinate indicates the ratios of dpm of ^{14}C to ^{3}H of the several fractions. The abscissa indicates the alkyl chain lengths of the columns employed in sequence. The width associated with each column is in proportion to the protein adsorbed to that column (numbers in parentheses are amounts of protein adsorbed to the column in % of the initial protein, corrected for losses at each step). Filled circles refer to adsorbed protein, open circles refer to non-adsorbed protein or, in the case of the first open circle, to the protein applied to the first column. Arrows lead from the original protein or the non-adsorbed protein to the fractions obtained from it at the next step.

column. The basis for the conclusion that the interaction between proteins and the alkyl agarose columns is a lipophilic one and that the shorter the hydrocarbon chain length of the alkyl agarose to which a protein is adsorbed the greater is its lipophilicity has been discussed by Shaltiel (5). In support of this premise is the finding in the present work that no significant amount of protein was adsorbed by a C_0 column (unsubstituted agarose).

An attempt was made to continue the experiment through the C_{10} column; however, most of the protein which was applied became adsorbed, and very

little of the adsorbed material was eluted with 0.1 N NaOH. Further elutions with 0.25 N NaOH, 50% ethylene glycol, and 1M NaCl in 50% ethylene glycol yielded only small additional increments.

DISCUSSION

Correlations have now been found between half-lives of proteins *in vivo* and their molecular size (8,9), isoelectric point (10), susceptibility to proteases (11), and lipophilicity (this paper). (A correlation with an additional function has been proposed, viz., the surface charge density (12); however, this is a derived term, involving simply a summation of the size and isoelectric point relationships.) What is not known is whether these correlations reflect separate causal relationships, or whether the half-life and the physical parameter with which it is correlated are independently related to some other underlying properties of the proteins or their interactions within the cell, and if so which ones.

Some possibilities that may be considered are the following. The correlation between turnover rate and both molecular size and isoelectric point may result from variations in proteolytic susceptibility which depend upon these parameters. For example, larger peptides may present more favorable targets for proteases simply as a result of increased target size and therefore of a larger number of susceptible bonds per molecule. Similarly a reduced isoelectric point may lead to a greater susceptibility to acid proteases as a result of a reduced net charge at acid pH. These parameters appear to relate to turnover rates independently and not as a result of a primary correlation between themselves (10). In both of these cases the proposals are consistent with the directly demonstrated relationship between turnover rates and proteolytic susceptibility. The implication in the above arguments is that there is a direct

causal relationship between turnover rates and susceptibility to acid (lysosomal) proteases, and that this reaction is a rate-limiting one in protein degradation in vivo (1).

In regard to the present findings, several questions arise. Is the correlation observed based upon an underlying relationship between lipophilicity and one of the other parameters of proteins previously implicated, i. e., molecular size or isoelectric point, or does increased lipophilicity confer greater proteolytic susceptibility on still a different basis? Or is the correlation a result of an effect on the rate of another step entirely in the overall process of protein degradation, e. g., uptake into the lysosomal digestive system? The last possibility is consistent with the proposal that rates of turnover of proteins are dependent upon their differential affinities for the membranes of the vacuolar digestive apparatus and that the uptake step is a specific, rate-limiting one in the overall degradative pathway. Selection among these possibilities cannot confidently be made in the present state of our knowledge of these processes.

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