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16 December 1974; revised 3 February 1975

Rates of Protein Evolution: A Function of Amino Acid Composition

Abstract. *Conservation of secondary and tertiary structure in proteins suggests that rates of sequence variation reflect differences in the total number of amino acid replacements that are compatible with preservation of structure. Consequently, rates of sequence variation depend on whether the constituent amino acids of individual proteins are, overall, more subject or less subject to evolutionary substitution than normal. Such rates correlate well with a mutability term based on amino acid composition.*

The rates of evolution of different proteins vary greatly. At one extreme is histone IV; only two changes in amino acid sequence have been accepted since the divergence of the calf and pea ancestral lines 1.5×10^9 years ago (*1*). Toward the other extreme is pancreatic ribonuclease; although the bovine and pig lines have diverged relatively recently, the sequences of their ribonucleases differ by 22 percent (*2*). Rates of such protein evolution can be expressed quantitatively as the number of accepted mutations per 100 residues per 100 million years. Such rates vary over three orders of magnitude (*2, 3*).

One plausible explanation for such variation is that constraints are placed on the overall structures of proteins and that these structural constraints are less stringent for the more rapidly evolving proteins. This argument works well for some proteins. In other cases, however, the argument may not be quite as applicable. Although at first there would seem to be only one major constraint for ribonuclease (that of maintaining activity), there is at least one other plausible constraint, namely, resistance to ready digestion by the various proteolytic enzymes found in the small intestine. Consequently, one would expect a somewhat slow rate of evolution for ribonuclease, whereas its rate is actually one of the fastest.

Other evidence also suggests that variation in structural constraint may not, in general, be sufficient to explain the variation in rates of protein evolution. X-ray crystallography has shown that there is a great conservation of secondary and tertiary structure in certain sets of proteins despite large variations in primary structure. Two examples will be cited here. One, the overall structures of myoglobin and of the individual chains of hemoglobin are similar despite changes in 80 percent of the amino acids (*2, 4, 5*). Two, the structures of trypsin, chymotrypsin, and elastase are quite similar despite their divergence in the remote past by gene duplication (*2, 6*). These two examples are sets of different, though similar, proteins; conservation of secondary and tertiary structure would be expected to be even greater for a single protein as long as its function is unchanged.

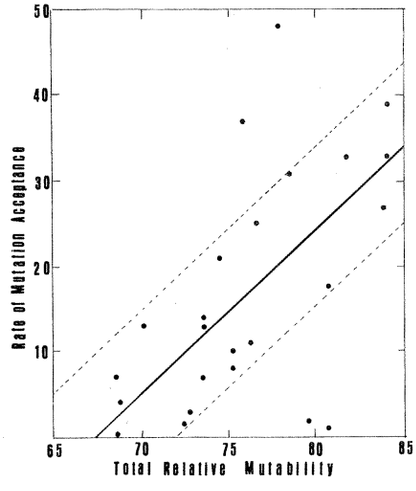

Fig. 1. Rate of mutation acceptance as a function of total relative mutability. Rates of mutation acceptance are in PAM's per 100 million years (one PAM = one accepted point mutation per 100 residues) (*2*). In the case of conflicting rates of evolution (trypsinogen) (*2, 10*), the data used are based on a comparison of mammalian sequences, which is the basis for most of the rates. The total relative mutabilities were calculated from average amino acid compositions of sequenced proteins; for simplicity, insertions and deletions were ignored. When available, the average amino acid compositions given by Dayhoff (*2*) were used; for the other proteins used, average amino acid compositions were calculated with the use of all the complete sequences listed in references (*2, 3, 10*). The immunoglobulin sequences used were those given in the alignments in Dayhoff (*2*). The proteins used were (in order of increasing rate of mutation acceptance): histone IV, glucagon, corticotropin, glyceraldehyde-3-phosphate dehydrogenase, cytochrome c, insulin, melanotropin beta chain, myelin membrane encephalitogenic protein, gastrin, lysozyme, pancreatic secretory trypsin inhibitor, luteinizing hormone beta chain, myoglobin, hemoglobin, trypsinogen, luteinizing hormone alpha chain, lactalbumin, lambda chain C region, gamma chain C regions, kappa chain V region, pancreatic ribonuclease, growth hormone, kappa chain C region, and amyloid protein A.

The above considerations lead to a second plausible explanation for the variation in rates of protein evolution: Stringent constraints on the secondary and tertiary structure of most proteins limit accepted mutations primarily to those in which an amino acid is replaced by another amino acid with similar properties. Consequently, proteins that evolve more rapidly should contain an increased percentage of the amino acids that have the greatest number of acceptable replacements.

In this report, I present a correlation between the rates of protein evolution and a

mutability term dependent upon amino acid composition and the mutabilities of individual amino acids. (Mutability here means the frequency with which evolutionary substitutions occur and is broader than the term in its strict genetic sense.) Such a correlation was suggested by the second explanation for rates of protein evolution. In general, a large number of considerations need to be taken into account for a detailed analysis of the possible replacements for an amino acid: Interior hydrophobic groups may be replaced by other hydrophobic groups; exterior polar groups may be replaced by other polar groups; the size of an interior side chain would restrict the number of amino acid side chains that could replace it; amino acids in a structural element (helix, β sheet, turn) may be replaced by amino acids either favoring the same structure or at least compatible with it.

To perform such an analysis for an individual protein and determine the number of acceptable replacements for each amino acid is certainly a difficult task. There is, however, a set of empirical values that would seem to approximate the results of a detailed analysis. These values are the relative mutabilities of the amino acids, which are proportional to the ratio of the number of accepted mutations of an amino acid over the number expected if all amino acids were equally likely to have acceptable replacements (*2*). Values calculated for individual families of proteins are similar to the average relative mutabilities calculated for all the protein families considered (*2*). It appears, then, that the empirical value, the average relative mutability of an amino acid, will approximate the result of a detailed analysis of possible replacements.

From the relative mutability (*2*) (m_i) of each amino acid and its fractional occurrence (f_i) in a protein, it is possible to calculate a mutability term ($\Sigma m_i f_i$) for that protein. I shall call this term the total relative mutability of a protein and have calculated its value for a number of proteins. The larger this term, the greater the number of amino acid replacements (per unit length of protein) that could occur without disrupting the overall structure of the protein. Consequently, the greater this mutability term, the faster the rate at which mutations are accepted in the evolution of a protein sequence (given that overall structure is maintained and that mutations in general occur randomly and uniformly with time). This expectation is borne out by Fig. 1, which plots the rates of sequence evolution against the total relative mutability. The overall correlation is good; statistical analysis gives $P < .01$ and a Pearson correlation coefficient (r) of .58. Of the 24 proteins tested, 20 follow the correlation quite well ($P < .001$, $r = .85$); they lie in a band about the regression line, $y = 1.92x - 130$.

The four proteins that deviate most markedly are growth hormone and amyloid protein A (above the main trend) and glucagon and glyceraldehyde-3-phosphate dehydrogenase (below the main trend). There may be special reasons why these are exceptional. At least two of them are exceptional in other ways; for example, the rate of evolution of growth hormone is not constant when different sets of mammals are compared (*8*), and the sequences of human amyloid protein A differed by 8 of 76 amino acid positions when determined for two different subjects (*9*).

Whatever the explanation for these exceptional proteins, they are only a fraction of all the proteins examined. For most of the proteins there is a strong relation between the rate of evolution and a mutability term dependent upon amino acid composition. This relationship was predicted by, and is compatible with, one possible explanation for the variability of rates of protein evolution, namely, that fairly stringent structural constraints apply to most proteins and rates of evolution reflect the number of structurally acceptable replacements for the amino acids of a protein.

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17 December 1974; revised 25 February 1975

Immunofluorescent Localization of Cyclic AMP in Toad Urinary Bladder: Possible Intercellular Transfer

Abstract. *By use of an immunofluorescent cytochemical staining technique, adenosine 3′,5′-monophosphate (cyclic AMP) has been localized in toad bladder epithelial cells. Within 2 minutes after addition of vasopressin, staining intensity increases in both mitochondria-rich and granular cells. This finding, taken together with the precise anatomieal relation between these two epithelial cell types and the observation that after separation of the two cell types vasopressin stimulates cyclic AMP accumulation in only mitochondria-rich cells, suggests that cyclic AMP may be transferred from mitochrondria-rich to granular cells as part of the response of the toad urinary bladder to vasopressin.*

The hormone-sensitive epithelial cell layer of the toad urinary bladder consists of two principal cell types: a mitochondria-rich (MR) cell, whose surface is covered by microvilli, and a cell containing numerous electron-opaque cortical granules, whose surface is covered by arborizing ridges (granular cell). These two cell types are arranged in a precise anatomical relationship; each MR cell is surrounded by four or five granular cells, and these anatomical units cover the entire epithelial surface (*1–3*). Addition of arginine vasopressin (AVP), a posterior pituitary antidiuretic hormone, produces several striking changes in the structure and function of the epithelial cell layer; there is an immediate increase in both water permeability and transepithelial sodium transport (*4*), both MR and granular cells swell (*1*), and the surface processes of the granular cells elongate and develop microvilli (*3*). These changes are accompanied by increased tissue adenosine 3′,5′-monophosphate (cyclic AMP) content (*5*). However, when epithelial cells are separated by Ficoll density gradient centrifugation into MR and granular cell fractions, an increase in cyclic AMP can be demonstrated only in the MR cell fraction after addition of AVP to the isolated cells (*6*). This apparent discrepancy in cellular localization between the physiological and biochemical response of the toad bladder epithelium to AVP suggested that cyclic AMP might be transferred from MR to granular cells and prompted our immunofluorescence localization study of cyclic AMP in intact toad bladder.

The immunofluorescent technique has