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An Apparent Relationship between Molecular Weights and Quantum Yields for the Inactivation of Enzymes and Viruses

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Recently in a review article [1] the quantum yields for the inactivation of a number of enzymes, proteins and viruses have been tabulated. It was stated that quantum yields are, roughly speaking, smaller the larger the molecular weight, but that no exact inverse proportionality was apparent. Since this writing a few revised molecular weights have been published [2]. The question has therefore been reexamined. In Fig. 1 there is plotted the logarithm of the quantum yields (Φ) against the logarithm of the respective molecular weights (M). It will be seen that the data reveals a trend which conforms to an equation of the type

$$\Phi = \frac{Q}{M^n} \tag{1}$$

where Q and n are constants. Now the quantum yield for inactivation may be defined as

$$\Phi = \frac{c_0 f}{ME} \tag{2}$$

where c_0 is initial concentration in grams of native protein per cubic centimeter, and f is the fraction of the material inactivated during the absorption of E einsteins per cubic centimeter (by the native protein). It will be seen that M bears an inverse relationship to Φ but since f is an unknown function of M, E, structure and composition, (nor is ΦM a constant) our photochemical results, expressible by equation (1) have no implicit explanation within the definition of the quantum yield.

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The problem can perhaps be considered geometrically. Our treatments will be quite elementary since further refinements do not seem justified at this time. Let us assume that in the primary process of inactivation a peptide bond adjacent to a chromophore is broken with a quantum efficiency $\o$ (a constant). We further assume that if such a bond breaks on the surface of a protein molecule the quantum efficiency for the bond is the same, whereas if such a bond is ruptured inside a macromolecule the efficiency will be virtually zero because the protein framework holds the ion or free-radical chain ends so close together that recombination is preferred. There is equal probability for absorption by a given type chromophore irrespective of its position in the molecule (reference 1, page 96). Successful splitting of a weak bond will mean inactivation. The fraction of the total aromatic residues with adjacent CONH linkages at the surface will be (assuming spheres as a model of diameter d, as a first approximation)

$$\frac{\text{Volume of surface residues of thicknes } \delta}{\text{Volume of molecule } V} \simeq \frac{6\,\delta}{d} \simeq \frac{k}{M^{1/3}} \tag{3}$$

taking the volumes of protein molecules to be proportional to their respective molecular weights. For the overall quantum yield we can then write

$$\Phi \,\alpha\, \frac{k\,\o}{M^{1/3}} \,\alpha\, \frac{Q}{M^{1/3}} \tag{4}$$

The resulting value of n is $^1/_3$ whereas the experimental value is about twice this. On the other hand, consider that a molecule has some sensitive volume of crossectional area, a. The sensitive volume is taken as the same size in all the proteins. The probability of hitting a in a spherical protein molecule placed in a beam of parallel light will be proportional to a/A where A is the cross sectional area of V, if the molecule is almost opaque to λ 2537 Å. The quantum yields is now

$$\Phi = \frac{k\,a\,\o}{A} \simeq \frac{k'\o\,a}{M^{2/3}} \simeq \frac{Q}{M^{2/3}} \tag{5}$$

which is of the correct form (compare (1)). Since only about 10 % of the residues in a protein are aromatic this is a very poor approximation for low molecular weight substances, however.*

* The possibility that $\Phi = \o\, \varepsilon a\, [a] \,/(\varepsilon a\, [a] + \varepsilon p\, [p]) \simeq \frac{Q}{M}$ (where εa, εp and $[a]$, $[p]$ are the molecular extinction coefficients and moler concentrations of sensitive volume and protein respectively) must also be disposed of since n is not unity. Here $\varepsilon a\, [a] / (\varepsilon a\, [a] + \varepsilon p\, [p])$ gives the fraction of absorbed light absorbed by the sensitive volume. In view of the small spread of M for the low molecular weight proteins and the scattering of points, the applicability of this approximation in the region of low M can not be excluded at present, however. Equation (5) may tend to be most applicable at very high molecular weights.

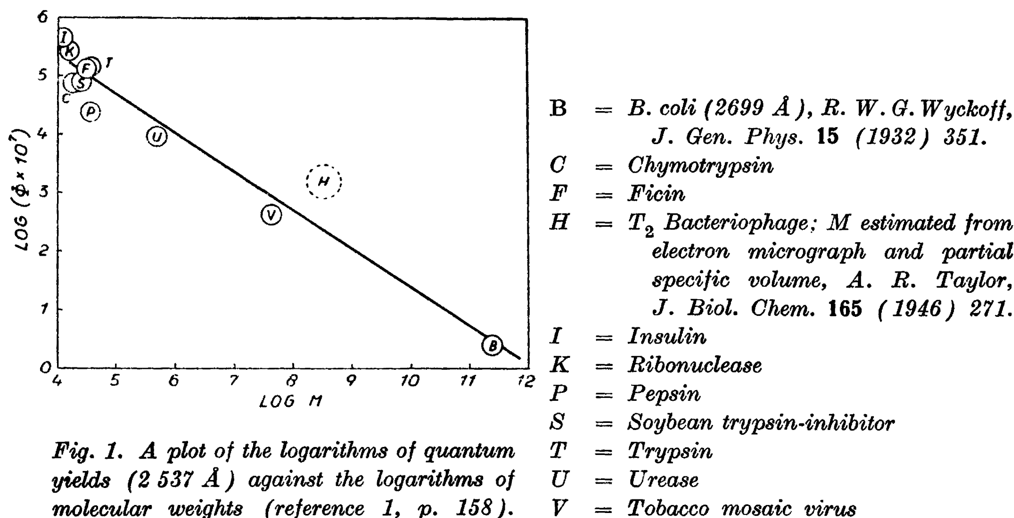

Fig. 1. A plot of the logarithms of quantum yields (2 537 Å) against the logarithms of molecular weights (reference 1, p. 158).

B = *B. coli (2699 Å), R. W. G. Wyckoff, J. Gen. Phys.* **15** *(1932) 351.*
C = *Chymotrypsin*
F = *Ficin*
H = *T_2 Bacteriophage; M estimated from electron micrograph and partial specific volume, A. R. Taylor, J. Biol. Chem.* **165** *(1946) 271.*
I = *Insulin*
K = *Ribonuclease*
P = *Pepsin*
S = *Soybean trypsin-inhibitor*
T = *Trypsin*
U = *Urease*
V = *Tobacco mosaic virus*

In Fig. 1 the best line through the experimental points with a slope of $-{}^2/_3$ has been drawn. It will be noted that a bacterium, a bacteriophage and a virus are also near this line.

The idea that the living cell has a sensitive spot is not new. It has been concluded by several authors that the life of each cell depends on a special molecule of this cell and that any injury to this molecule is spread over the whole cell. Conversely, we can also postulate, in view of our experimental curve, that the cell behaves like a single protein molecule.[3]

It is also of interest to estimate the possible size of the sensitive volume. If we associate the sensitive volume with some particular peptide bond and adjacent chromophore we can calculate from the curve the value of Φ for $M = 100$ (the number 100 is close to the average amino acid residue weight in a protein). The corresponding value of Φ is 0.51. It is surprising indeed to note that Rideal and Mitchell [4] report a value of 0.5 for the quantum efficiency of splitting peptide bonds in gliadin protein monolayers.

Finally, is there evidence that there is only one sensitive volume or area on an enzyme molecule? We tentatively identify this volume element with part of the functional group since it is the loss of enzymatic or hormonal activity which we measure. Trypsin loses its activity when combined with one molecule of inhibitor [5]. Chymotrypsin loses its activity after combining with one molecule of diisopropylfluorophosphate [6]. Both facts support the idea of a single functional group in these at least. Incidentally the tyrosyl residue is one of the strong chromophores in proteins and its presence is

known to be required for the activity of chymotrypsin, pepsin, insulin and tobacco mosaic virus, and doubtless others[7].

Whether or not an enzyme can be inactivated by the breakage of a bond not near the functional group or whether or not an enzyme can be inactivated without undergoing other changes associated with denaturation are related questions for which there is a paucity of data[1]. Work is in progress to help clarify these points.

SUMMARY

The apparent relationship between the quantum yields, Φ, for inactivation of enzymes, viruses and related proteins and *B. coli* and the corresponding molecular weights, M, is $\Phi = Q\ M^{-2/3}$ where Q is a constant. No satisfactory derivation of this relationship has been found although some possibilities have been examined.

The evidence can be interpreted to mean that each molecule has a single sensitive volume, possibly related to the active center in enzymes, which undergoes photolysis during inactivation. The larger the molecule the smaller is the fraction of the absorbed light which is absorbed by the sensitive volume and hence the lower the quantum yield for inactivation.

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