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Glycolytic Enzymes as a Multi-Enzyme System

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The glycolytic enzymes representing a multi-enzyme system are usually assumed to be a group of soluble and unassociated enzymes which at least in rat liver are found in the cytosol,[1] the mitochondrially bound hexokinase being an exception. Such an arrangement leaves the function of the glycolytic pathway open to interference by the fact that a number of intermediates of this pathway are the same as those of the gluconeogenetic pathway and of the pentose phosphate cycle. Specific heterotropic and homotropic ligands have been identified as modifying the action of key enzymes of either the glycolytic or the gluconeogenetic pathways.[2,3] However, a certain degree of association in the cytosol of the enzymes constituting the glycolytic pathway still seems worth considering for the following reason. In a multi-enzyme system, the diffusion of an intermediate from one enzyme to the next in the sequence (transit-time) is a factor which, in addition to the reaction rates of the component enzymes, is likely to influence the kinetic properties of the whole system.

Webb[4] considered this question by suggesting that at steady-state the transit-time should be less than the molecular activity of the slowest member of a multi-enzyme system. Assuming a molecular activity of 5000, and a diffusion constant of 5×10^{-6} $cm^2\ sec^{-1}$ for a small molecular weight compound (MW 500) in an aqueous medium, he calculated that the diffusion path would be far in excess of the probable distance of enzymes in cells. Webb's calculation may be questioned because it is unlikely that enzymes are reacting in cells at their maximum velocity. This certainly does not apply to hexokinase[5] (EC 2.7.1.1). Furthermore, the inside of a cell is far from being made of water and Crick suggested, therefore, an effective diffusion constant of 8×10^{-7} $cm^2\ sec^{-1}$ for small molecular weight compounds.[6] Finally, Webb's calculations do not consider the probability of collision of a metabolite molecule with an enzyme molecule. The calculation described below takes these considerations into account.

The main assumptions in our calculation are as follows.

(1) The random distribution of the enzyme molecules throughout the cytosol is regarded as resulting in an evenly spaced cuboid lattice.

(2) The substrate molecule is regarded as diffusing away from the centre of a unit cube in the lattice, the corners of which are occupied by the enzyme molecules for which it serves as substrate.

(3) The transit-time is derived from the time it takes for the substrate molecule to

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diffuse the distance between the centre and a corner of the unit cube, corrected for the probability of its collision with an enzyme molecule.

(4) The probability of forming an active enzyme-substrate complex is assumed to be equal to the probability of the substrate colliding with the enzyme. The probability factor used in the calculations represents, therefore, a minimum value.

It is proposed to take phosphofructokinase (PFK, EC 2.7.1.11) as the first example of a glycolytic enzyme. The data on which the calculations are based are as follows. The molecular data of PFK are given in Table I, the DNA content of a single nucleus (D.C.N.) is taken as 0·91 μg of DNA-phosphorous (ref. 12), the DNA content of liver (D.C.L.) as 235 μg of DNA-phosphorous per g (ref. 9) and the diameter of a parenchymal liver cell as 25 μ (ref. 13). By assuming that PFK has a homogeneous distribution throughout the liver the number of enzyme molecules (N) per cell would be as indicated in Eq. (1).

$$N = \frac{\text{S.A.} \times \text{Avogadro's Number} \times \text{D.C.N.}}{\text{M.A.} \times 10^6 \times \text{D.C.L.} \times 10^6} = 4{\cdot}95 \times 10^4 \qquad (1)$$

TABLE I. Molecular data of PFK, ALD and TIM

	Phosphofructokinase (PFK)	Aldolase (ALD)	Triose phosphate isomerase (TIM)
S.A.*	1·0 (ref. 9)	5·3 (ref. 9)	625 (ref. 9)
M.A.†	47,000 (ref. 11)	3,250 (ref. 10)	500,000 (refs. 7, 8)
M.W.‡	360,000 (ref. 11)	159,000 (ref. 10)	43,000 (ref. 8)
Stokes radius§	65 Å	40 Å	25 Å

* S.A., specific activity, μmoles per min per g rat liver.
† M.A., molecular activity, μmoles substrate per μmole enzyme per min.
‡ M.W., molecular weight.
§ Stokes radius, calculated on the assumption that the three enzymes would be globular proteins.

The cell volume of a parenchymal cell would be 8160 μ^3 if the cell were a sphere or 15,625 μ^3 if a cube. Taking the mean of these two estimations the value of 11,900 μ^3 is obtained. However, the volume of the cytosol is considerably less than the cell volume and may be assumed to be only half that volume. Consequently 8·46 molecules of PFK would be present per μ^3 of cytosol. Therefore the mean distance (L) between enzyme molecules in a cuboid lattice is given by Eq. (2).

$$L = \sqrt[3]{\frac{1}{8{\cdot}46}}\,\mu = 0{\cdot}49\,\mu \qquad (2)$$

The diffusion time (t) is given by Eq. (3) where A is a constant and probably 0·5 in

$$t = \frac{A \times r^2}{D} \text{ sec} \qquad (3)$$

biological systems;[6] r, the radius of the sphere circumscribing a unit cube ($\sqrt{3} \times \frac{1}{2}$ mean distance between two enzyme molecules) and D, the diffusion constant is $8{\cdot}0 \times 10^{-7}$

$cm^2\ sec^{-1}$ (ref. 6). Using these values a diffusion time of $1{\cdot}1 \times 10^{-3}$ sec is obtained for PFK.

The probability of collision may be calculated from Eq. (4).

$$\text{Probability factor} = \frac{8 \times \text{Cross-sectional area of enzyme molecule}}{\text{Surface area of sphere circumscribing unit cube}} \quad (4)$$

The value 8 is included as an approximation in regard to the probability of collision of a metabolite with an enzyme molecule in any one of the 8 directions indicated by the corners of the cube. By substituting the appropriate values in Eq. (4) a probability factor of $4{\cdot}67 \times 10^{-4}$ is obtained for PFK and if this is applied to the diffusion time, a value of 2300 msec is estimated for the transit-time.

Analogous calculations carried out for aldolase (ALD; EC 4.1.2.7) and triose phosphate isomerase (TIM; EC 5.3.1.1) give transit-times of 18 msec and 76 msec respectively.

The large difference in transit-times obtained for PFK on the one hand and for ALD and TIM on the other would imply that, at steady-state, the intracellular concentration of fructose 6-phosphate would have to be approximately 30 to 100 times larger than that of either fructose 1,6-diphosphate or of the triose phosphates. However, this is not the case as Wood, Eggleston and Krebs[14] reported the concentrations (μmoles/g wet weight rat liver) of fructose 6-phosphate, fructose 1,6-diphosphate and of glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate to be 0·06, 0·02 and 0·046 respectively.

The transit-time would be of less importance if the glycolytic enzymes were closely associated in multi-enzyme complexes. If this were so one would expect to have approximately equal numbers of molecules of all glycolytic enzymes per cell. The number of enzyme molecules per cell (see Eq. (1)) were calculated to be 5×10^4, 4×10^6 and 3×10^6 for PFK, ALD and TIM respectively. Thus there would be approximately 1 to 2 molecules of PFK only per 100 molecules of ALD or TIM. Even when allowance is made for the participation of ALD and TIM in other pathways occurring in the cytosol, the numerical discrepancy obtained in the above calculation seems too large to justify the assumption that the glycolytic enzymes are arranged in a multi-enzyme complex. It is probably of relevance that de Duve[15] searched for a multi-enzyme complex containing all glycolytic enzymes but was unable to find any evidence for its existence in the cytosol compartment of rat liver cells. It would seem, therefore, that from theoretical as well as experimental considerations, the occurrence of an ideal multi-enzyme complex may be questioned. However, in view of the disagreement between the above calculations and the experimental data of Wood, Eggleston and Krebs,[14] it is equally unlikely that the glycolytic enzymes are evenly distributed throughout the cytosol of the liver cell. Instead, glycolytic enzymes may be concentrated in one or at most a few regions of the cytosol. Such an arrangement would reduce the importance of transit-times without necessitating the occurrence of an ideal multi-enzyme complex. A compartmentalization of the glycolytic enzymes within the cytosol may be brought about by attachment of the enzymes to membranes. In the liver cell, this attachment would have to be rather loose as it is disrupted during conventional homogenization and preparation of subcellular fractions. A firmer attachment of glycolytic enzymes has been reported for the erythrocyte membrane.[16]

The calculations described above for the number of enzyme molecules per cell seem

to be of the right order of magnitude when related to the rate of net glycolysis in rat liver. This rate was recently reported to be less than 0·1 μmoles per g per min in the post-absorptive state.[17] When the DNA values described for Eq. (1) are taken into account this rate may be expressed as 4×10^6 molecules of glucose utilized per second per cell. The number of PFK molecules per cell was calculated (see above) as being 5×10^4 and thus 80 molecules of substrate would be dealt with by PFK per second indicating that the intracellular reaction velocity of this enzyme would be approximately 10% of the theoretical maximal velocity.

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