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Does a cell perform isoelectric focusing?

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A model of intracellular electrical sorting of enzymes and organelles in the cytosol, based on isoelectric focusing, is proposed. The focusing is suggested to take place over a centrally symmetric pH gradient which in the cytosol of the yeast *Saccharomyces cerevisiae* is known to be 7.2–6.4. From published data on the energetic capacity and from the computed electric resistance of the *S. cerevisiae* cell, the maximum value of the electric field that can be maintained in the cytosol was estimated. The results showed that the strength of a centrally symmetric intracytosolic electric field could be as high as 90 mV/cm, which is sufficient to account for sorting of cytosolic proteins according to their isoelectric points. Although direct experimental evidence for intracellular isoelectric focusing is still missing, several phenomena of physiological importance can be understood on the assumption of its real existence.

Keywords: Hypothesis; pH gradient; Electrophoretic sorting; Structure of cytosol.

1. Introduction

The physiological importance of electrophoretic transport of molecules and organelles in a manner analogous to zone electrophoresis has been stressed by many authors; for reviews see DeLoof (1985, 1986), Robinson (1985), Harold et al. (1985, 1986) and Nuccitelli (1988). The existence of a pH gradient in the cytosol (Slavik, 1983; Slavik and Kotyk, 1984; Tsien and Poenie, 1986; Ross and Slavik, 1987; Paradiso et al., 1987) suggests that also some analogy with isolectric focusing could be functioning in a living cell. It is obvious that if both an electric field and a pH gradient exist in the interior of the cell, then cytosolic ampholytes (e.g. proteins) are sorted according to their isoelectric points (pI) to occupy stable positions in distinct regions within the cell. The rates of many, if not all, biochemical reactions in the cytosol are supposed to be limited by the rates of diffusion of the reactants (Snol, 1979). The importance of a process which could concentrate the reactants into small and distinct regions of the cell interior and thus overcome the limitation is evident. In this paper, the possibility of intracellular isoelectric focusing operating in the *S. cerevisiae* cell is considered from an energetic point of view. Experimental data supporting the existance of intracellular isoelectric focusing are reviewed and its possible impacts on different physiological processes in the cell are discussed.

2. The expenditure on intracellular isoelectric focusing in the energetic budget of the yeast cell

Slavik and Kotyk (1984) have demonstrated the presence of a continuous pH gradient ranging from pH 7.2 (cell centre) to pH 6.4 (cell periphery) in the cytosol of a living *Saccharomyces cerevisiae* cell. If such a gradient were to be utilized for isoelectric focusing of cell components, a centrally symmetric electric field with a negative potential in the cell centre would have to exist. One of a number of alternative models of electric field generation is shown in Fig. 1. It assumes that posi-

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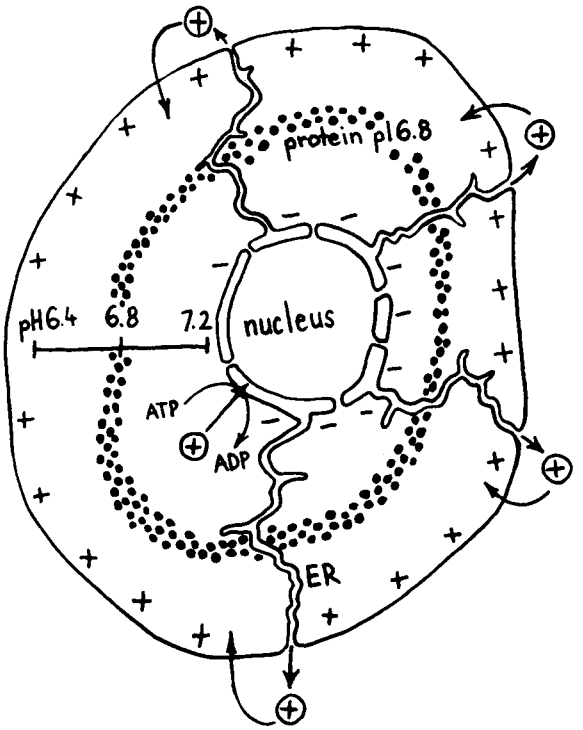

Fig. 1. Model of generation of an electric field in the cell interior and of an isoelectric focusing of a protein, pH 6.8.

tive ions are pumped into cisterns of the endoplasmic reticulum, from which the nuclear envelope is formed and whose lumen is known to communicate through transport vesicles or through channels with the extracellular space (Franke, 1974).

The model does not take into consideration the influence of cytoskeleton and organelles such as lysosomes, mitochondria and microbodies. An interior of these organelles has often different pH from the rest of the cell, suggesting that they are electrically isolated from the cytosol. Such structures, of course, could highly influence on the geometry of the field in the real living cell.

Also the cytoskeleton could play a big role in the real cell. Many cells, especially animal cells, show movements due to the activity of the cytoskeleton. It could be possible that the cytoskeleton keeps structures containing ion pumps in place in the cell interior so that the movements would not disturb the gradients too much.

The maximal strength of the field could be estimated using the published experimental data on *S. cerevisiae* metabolism. To maintain a steady state, as required for efficient isoelectric focusing, a constant power would have to be invested to compensate for the electric current (I) between electrodes. This power (W) can be expressed as

$$W = UI$$

where U is the difference of potentials between electrodes. From Ohm's law we obtain

$$W = U^2/R \tag{1}$$

where R is the resistance of the electrophoretic chamber. The resistance of a thin spherical layer of electrolyte in a spherical electrophoretic chamber with two concentric spherical electrodes (a highly simplified and idealized model of the *S. cerevisiae* cell) can be expressed as

$$dR = (r\,dX)/(4\pi X^2) \tag{2}$$

where r is the resistivity of the electrolyte in a steady state of isoelectric focusing and X is the radius of the infinitesimally thin spherical layer. The total resistance of the electrolyte between electrodes (R) can be calculated as an integral

$$\int_A^B r/(4\,\pi\,X^2)\,dX$$

hence

$$R = \frac{r}{4\pi}\left(\frac{1}{B} - \frac{1}{A}\right) \tag{3}$$

where A and B are the radii of the outer and inner electrode, respectively. During isoelectric focusing, all electrically charged particles migrate toward the electrodes, which results in a continual increase of resistivity of the

electrolyte. Finally, when only H^+ and OH^- are left to transfer the electric current, the resistivity increases up to the value of pure water; for an electrolyte of pH near 7 this is about 1.8×10^7 Ω/cm (Svensson, 1962). for the *S. cerevisiae* cell, we can estimate the radius of the outer and inner electrode as 3.1×10^{-4} cm and 0.62×10^{-4} cm, respectively (Slavik and Kotyk, 1984). Substituting these values into Eqn. (3), we find that the resistance of the interior of the *S. cerevisiae* cell is about 114 Ω. The maximum power which *S. cerevisiae* can invest into isoelectric focusing is 4.4×10^{-12} J/s, the power which the yeast spends on unidentified functions different from the known metabolic processes (Lagunas, 1976). From Eqn. (1) we obtain that this power is sufficient to maintain an intracellular difference of electric potentials equal to about 2.2×10^{-5} V, which represents an electric field of about 90 mV/cm.

This electric field strength seems to be rather low in comparison with the strength of electric fields used for separation of proteins in laboratory devices for isoelectric focusing. The question arises, how much and how quickly could the cellular proteins be focussed in this field. The efficiency of isoelectric focusing in directly proportional to two paramenters, namely electric field strength and the value of the pH gradient. The former is more than three orders of magnitude higher in man-made apparatus for isoelectric focusing; the latter (3200 pH/cm), however, is nearly four orders of magnitude higher in the living *S. cerevisiae* cell. Also the distance which the proteins are expected to migrate ($< 2.5 \times 10^{-4}$ cm) is about five orders of magnitude less in the cell. Therefore, an electric field strength of 90 mV/cm would be more than sufficient for effective isoelectric focusing of intracellular components and their redistribution would be accomplished within seconds.

3. Evidence for existence of intracellular isoelectric focusing

Although the existence of intracellular isoelectric focusing has not been proved experimentally, the actual existence of this phenomenon can be inferred from the following facts:

(1) An intracytosolic pH gradient has been shown to exist in different cells (Slavik, 1983; Slavik and Kotyk, 1984; Tsien and Poenie, 1986; Ross and Slavik, 1987; Paradiso et al., 1987). The simplest mechanism whereby a cell could set up such a gradient is to generate an electric field between the centre and the periphery by some sort of electrogenic transport. The electric field than gives rise to a natural pH gradient (Svennson, 1961) and this, in turn, should lead to isoelectric focusing of ampholytes.

(2) Proteins labeled with fluorescent dyes injected into a cell often form distinct spherical zones in the cytosol (Wehland and Weber, 1980; Wodsworth and Sloboda, 1983; Glacy, 1983). Some results even suggest that the shapes of these zones are spherical layers (Wehland and Weber, 1980), which agrees with predictions based on the model of intracellular isoelectric focusing.

(3) The hypothesis of intracellular isoelectric focusing is strongly supported by the results of ^{31}P nuclear magnetic resonance studies (NMR). When cytosolic pH was estimated by NMR using inorganic phosphate and intermediates of glycolysis, values of pH 7.2 and 7.0 were obtained, respectively (Navon et al., 1979). This difference could be explained by different molecules being focused into different regions of the cytosol; the region of maximal accumulation of glycolytic intermediates probably coincides with the region of maximal occurrence of glycolytic enzymes.

A pH gradient has been demonstrated in *S. cerevisiae* cytosol using a pH-specific fluorescent probe (Slavik and Kotyk, 1984). In contrast, the sharp resonance peaks of most intracellular pH-sensitive molecules obtained with NMR studies would suggest that one distinct pH value rather than a pH continuum exists in the cytosol of the *S. cerevisiae* cell (Salhany et al., 1975; Navon et al., 1979; Barton et al., 1980). This discrepancy can be

explained if the presence of intracellular isoelectric focusing is taken into consideration. The molecule which is used for monitoring pH is not homogeneously distributed throughout the cytosolic compartment but is concentrated, according to its pI or its electric charge, in a distinct region of the cytosol, displaying the pH of that particular region rather than the average pH of the cytosol. It is worth mentioning that the resonance peak of highly diffusible (and, consequently, poorly electrofocusable) ions of inorganic phosphate is far broader than expected from theory and than observed for other ^{31}P-containing molecules (Busby et al., 1970; Busby et al., 1978; Roberts and Jardetzky, 1981). This phenomenon, suggesting the existence of an intracytosolic pH gradient, can be detected in the intact cell only, not in a cell homogenate.

(4) On the basis of the structure of the genetic code, the properties of amino acids, and the character of the process of mutagenesis, a model describing the evolution of proteins has been constructed (Graur, 1986). The model shows that in the absence of strong selection constraints the pIs of proteins should converge toward mildly basic values. The large diversity in pIs among real proteins suggests that this parameter has its biological meaning and that it is controlled by natural selection. Experimental evidence supports the same conclusion. Studies with proteins injected into living cells have shown that a parameter controlling the entry of a protein into a nucleus is its pI (Kreis and Birchmeier, 1982). A similar study has shown that a strong correlation also exists between the pI of a protein and the rate of its degradation in vivo (Dice and Goldberg, 1975). The process of intracellular isoelectric focusing could easily be the missing link between the physical constant, i.e. pI, and the biological properties of the protein.

(5) There is a growing body of evidence suggesting that the cytosol of a living cell exists in a highly organized state. Different cytosolic molecules are separated into distinct regions of the cell rather than homogeneously distributed throughout the cytosol. The rates of diffusion of proteins, but not of polysaccharides of the same molecular weight, are much lower in the cytosol of living cells than in a water solution of the same viscosity. Enzymatic-reaction rates are often different in cell homogenates than in living cells. For a review of this topic see Bhargava (1985) and Kaprelyants (1988).

4. Possible significance of intracellular isoelectric focusing for cell physiology

Data suggest that a cell could generate an electric field of the strength of 90 mV/cm, which appears sufficient for efficient isoelectric focusing of intracellular ampholytes. This section shows that existence of this process would be advantageous from the point of view of cell physiology. In a biochemist's test tube, the molar concentrations of enzymes are usually much lower than the concentrations of substrates, so the reactions follow a pseudo-first-order reaction kinetics. By contrast, the concentrations of enzymes and substrates in a living cell are often nearly identical (Lehninger, 1978). Under such conditions the reaction follows the second-order reaction kinetics and the rate of diffusion of the reactants, rather than turnover number of the enzyme, would be the factor limiting the reaction rate. A process which could overcome this limitation would highly improve the efficiency of intracellular enzymatic reactions. Intracellular isoelectric focusing could be such a process. Of course, this would require a spatial coincidence of enzymes and corresponding substrates. While electrofocusing of enzymes is very likely, this is not the case with the low molecular weight, highly diffusible substrates, mostly lacking ampholytic character (Svensson, 1962). The results of Bernhard (1988), however, show that once trapped by the first enzyme of a particular biochemical pathway, the substrate seldom leaves the molecule of the enzyme to diffuse freely to the second enzyme of the enzymatic cascade.

Substrates are rather transported between enzymes, being bound on their surfaces, via enzyme-enzyme collisions (Srivastava and Bernhard, 1985; Weber and Bernhard, 1982). In such a way, the focusing of enzymes would be sufficient for an enhancement of the rate of biochemical processes.

Electrofocusing of enzymes could not only speed up biochemical processes: it also offers a powerful tool for the regulation of cell metabolism. A cell could avoid futile cycles simply by focusing enzymes of different biochemical pathways into different parts of its interior. Furthermore, phosphorylation, dephosphorylation, glycosylation, or other modifications that could change an enzyme's pI would automatically result in its translocation into a new cell region. This could switch particular biochemical processes on or off. In this case both reactants, i.e. the regulating enzyme and the regulated enzyme, are ampholytes and so they could be electrofocused into the same part of the cell. Theoretically, the substrate specificity of the regulating enzyme could be based on the very fact that both enzymes share the same pI value.

Isoelectric focusing could also participate in processes of intracellular molecular transport. It has been estimated (Whetley, 1985) that the time required for an average protein molecule to move across the interior of a HeLa cell by a simple diffusion would be about 26 or 27 min. Proteins introduced into a cell by microinjection are, however, seen to spread through the cell within seconds (Stacey and Allfrey, 1977). Similar velocities (0.1 – 2.0 μm/s) could be expected for proteins electromigrating in an electric field of 90 mV/cm. Electrophoretically driven transport has been demonstrated in many biological systems (Jaffe, 1966; Jaffe et al., 1974; Woodruff and Telfer, 1974; Jaffe and Woodruff, 1979; Woodruff and Telfer, 1980; Cooper et al., 1989). Intracellular isoelectric focusing appears to offer a more versatile tool for assembling sophisticated material-transporting systems than zone electrophoresis. While the latter is just capable of separating intracellular particles into two regions and the values of the electric charges of the particles merely influence the migration rates, isoelectric focusing could sort the particles into a number of distinct cell regions, thus establishing a finely tune-up distribution of proteins and organelles. For the transport of a molecule from one cell region to another by isoelectric focusing, only one substrate-specific step, i.e. a modification leading to a change of pI of the transported molecule, is necessary. The following step, translocation of the molecule, could be performed by the substrate-non-specific mechanism of intracellular isoelectric focusing.

In the preceding paragraphs, intracellular isoelecric focusing of macromolecules, namely proteins, has been considered. There is no reason, however, to exclude higher structures, e.g. subcellular particles and organelles, from these considerations. Because of their low diffusion coefficients, the transport or stabilization of these structures in particular cell regions could be very efficiently controlled by isoelectric focusing (Vesterberg and Svensson, 1966). Considering that proteins of different pI values could be inserted into different parts of organelle membranes, one might propose that the shape, orientation, and spatial distribution of intracellular membrane structures could all be controlled by the process of intracellular isoelectric focusing.

5. Concluding remarks

Processes analogous to isoelectric focusing have been proposed to participate in the spatial arrangement of intracellular components. The presumed processes have been shown to fall within the range of the energetic capacity of the cell. Some possible physiological functions of intracellular isoelectric focusing have been discussed and some experimental data suggesting its existence have been reviewed.

Intracellular isoelectric focusing could play important roles in many different intracellular

processes connected with compartmentalization of the cell interior, transport of material, or transmission of physiological signals. Such processes could be especially important in a large cell. In nature two distinct types of cell architecture exist — the small prokaryotic cell of bacteria and generally about three orders of magnitude larger eukaryotic cell of other organisms. The presence of endosymbiotically originated organelles, mitochondria and plastids, is often considered a principal difference between the two types of cells. The discrepancy between monophyletical origin of the eukaryotic cell and probably polyphyletical origin of its endosymbionts, however, suggests a possible existence of a more fundamental difference. Such a difference could theoretically be the presence of intracellular isoelectric focusing in the pre-eukaryotic cell and its absence in the prokaryotic one. This mechanism could enable the functioning of large cells; the large size is a necessary precondition for endosymbiosis. For this reason, it could be speculated that the origin of the eukaryotic cell was associated with the evolutionary emergence on the life scene of the mechanism of intracellular isoelectric focusing.

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Corrigendum

Corrigendum to "Does a cell perform isoelectric focusing?" [BioSystems 24 (1990) 127]

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The Author regrets that there was a serious numerical error in the above paper. The correct value for the resistance of *S. cerevisiae* interior is 1.85 10^{10} Ω, i.e. approximately eight orders of magnitude higher than originally claimed. If the correct value is substituted into Eq. 1, we find that *S. cerevisiae* cell can maintain an intracellular difference of electric potential equal to about 0.29 V which represents an electric field of about 1.5 kV/cm. The correct value suggests that the cell can achieve highly effective isoelectric focusing of intracellular proteins and organelles by using a negligible fraction of its energy reserves.

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