Ultracentrifugation of Yeast Cells in a Gradient of Acrylamide Gel and Sucrose

The combined techniques of density gradient ultracentrifugation and the photopolymerization of acrylamide can be used to separate and immobilize the molecular components of cells[1-3].

Heterogenous intact cells can also be separated from each other in this way and isolated into homogenous bands. This technique utilizes the physical differences between individual cells in a synchronized or non-synchronized population of living cells.

Materials and methods. The technique consists of layering a suspension of intact cells on a gradient of combined sucrose and acrylamide in a dim light to maintain a liquid phase. After ultracentrifugation, the acrylamide is polymerized by a fluorescent light, thus immobilizing the bands of cells that have been formed in a gel phase. The bands are then scanned in a densitometer and recorded for comparative studies and future reference.

The organism used in this study were yeast cells (*Candida albicans* No. 11651) obtained from the American Type Culture Collection, grown in Sabouraus's dextrose broth, then equally divided into 'starved' and 'non-starved' cultures. The 'starved' cells were starved in sterile distilled water for 16 days at 10 °C and the 'non-starved' cells were kept in nutrient broth for 16 days at 10 °C. Before use, the cells were washed with sterile distilled water and diluted so that 1 ml contained 1.5×10^5 cells.

The gradient of sucrose and acrylamide was formed by diluting the working solutions of 'A', 'C', and 'E', which are used in Canalco[4] disc electrophoresis, with different molar concentrations of sucrose. Solution 'A' consisted of 48.0% 1 *N* HCl, 36.3% *Tris* (2-amino-2-(hydroxmethyl)-1, 3-propanediol), and 0.23% TEMED (tetramethylethylenediamine) in distilled water; final pH 8.8. Solution 'C' consisted of 60% acrylamide and 0.4% *Bis* acrylamide (N, N'-methylenebisacrylamide) and Solution 'E' consisted of 4.0% riboflavin, both in distilled water. The 'IM' solution contained 6.0 ml 1 *M* sucrose, 0.25 ml of solution 'A', 1.0 ml of 'C', and 0.25 ml of 'E'. The '1.5 *M*', '2.0 *M*', and '2.5 *M*' molar solutions contained the same volumes of solutions 'A', 'C', 'E', and sucrose as in the '1 *M*' solution except the molarity of the sucrose was increased to 1.5 *M*, 2.0 *M*, and 2.5 *M* respectively. The combined mixtures of sucrose and acrylamide were layered in 1 ml aliquots in cellulose nitrate tubes (1.25 × 5 cm), thus forming a gradient of 4 layers with the '2.5 *M*' solution at the bottom and the '1.0 *M*' at the top of the centrifuge tube.

A swinging bucket rotor, Model SW50L, in a Spinco (Beckman) Model L2-65B Ultracentrifuge was used at 1000 rpm for 45 mins. Photopolymerization was induced by a fluorescent light in 30 min and the layered polymerized bands of cells immobilized in the gel were scanned in a horizontal position in a Gelscan Automatic Recording and Integrating Scanner, Model 39372. A modified carrier that could accommodate both the tube and the gel in the densitometer was constructed.

Results and discussion. Figures 1, a and b, are photographs of the tubes containing bands formed by the yeast cells; Figure 1a shows two separate and distinct bands formed by the 'starved' cells and Figure 1b shows only one band produced by the 'non-starved' yeast cells. The bands are recorded in Figure 2. The yeast cells were still viable after this treatment.

The intact gel can also be removed from the tube and sliced for further study by staining or culturing. Stains can also be used to identify and intensify the bands before scanning.

This technique can be used to separate cells which differ from each other according to their weight, volume, density, or rate of migration in a sucrose and acrylamide

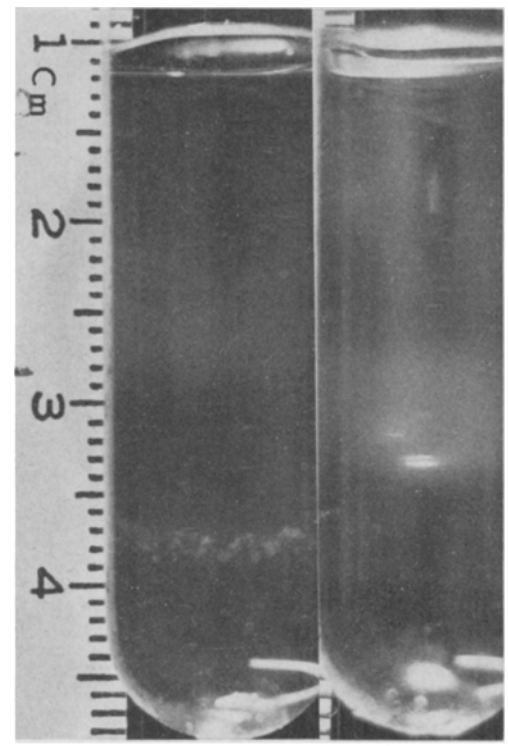

Fig. 1. (a) A cellulose nitrate tube containing photopolymerized acrylamide gel with 2 separate bands formed by the 'starved' yeast cells. (b) Same as (a), but containing one band formed by the 'non-starved' cells.

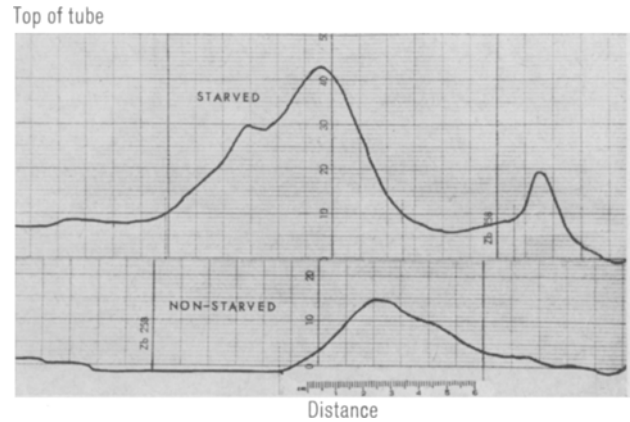

Fig. 2. A recording made of the bands in 1(a) and 1(b).

[1] M. K. BRAKKE, J. Am. chem. Soc. *73*, 1847 (1951).
[2] C. W. BOONE, G. S. HARELL and H. E. BOND, J. Cell Biol. *36*, 369 (1968).
[3] W. B. JOLLY, H. W. ALLEN and O. M. GRITHITH, Analyt. Biochem. *21*, 454 (1967).
[4] Canalco Co., 5635 Fisher Lane, Rockville (MD. 20852, USA).

density gradient. It can also be used as a diagnostic or investigative tool to indicate variation in cell parameters in clinical or research material. Such variations would be meaningful in studying ovulating periods, hormonal inbalances, and/or the beginning of cancerous conditions in clinical material which contain both normal and abnormal cells. It can also be used to physically separate groups of cells or microorganisms for continued study and growth, the monitoring of cell cultures, and the separation of cell generations in synchronous cultures[5].

Zusammenfassung. Dichtegefälle-Ultrazentrifugation, verbunden mit Photopolymerisation von Akrylamid, ist geeignet um lebende, unverletzte Zellen unbeweglich in Bänder zu trennen, insofern sie sich punkto Gewicht, Volumen, Dichte oder Beweglichkeit im Dichtegefälle unterscheiden. Verschieden ernährte Hefezellen-Gruppen (*Candida albicans*) zeigten verschiedene Bandenverteilungen.

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Calcium Ion Binding to Blood Cell Surfaces

This communication reports the uptake of calcium ions by the peripheral region of the human red blood cell (RBC) and polymorphonuclear leucocyte (PMN), before and after neuraminidase treatment, as studied by cell electrophoresis.

Cell electrophoresis was performed as described previously[1]. Solutions containing different molarities of calcium at constant osmolarity, ionic strength and pH were prepared by dilution of a $0.0483\,M$ aqueous calcium chloride solution made $0.145\,M$ with respect to sucrose, with a $0.145\,M$ aqueous sodium chloride solution. PMNs were separated using the method of Carruthers[2]. Separated PMNs were finally washed twice with 50 vol. of standard saline at room temperature ($100 \times g$; 7 min). Neuraminidase treatment of the erythrocytes and PMNs was carried out as described by Seaman and Uhlenbruck[3]. Supernatant fluids from neuraminidase treated cells and controls were analyzed for sialic acid[4,5]. Normal and neuraminidase treated RBCs and PMNs were washed once in, and re-suspended in, each of the aqueous calcium chloride solutions at pH 7.2 ± 0.2 and examined by electrophoresis.

Using Stern's adsorption model[6] in which the adsorption of ions is assumed to be essentially monomolecular on to widely spaced immobile non-interacting sites it can be deduced

$$\text{that} \qquad \Delta\sigma_{Ca} = 2en_{Ca} = \frac{2e\,N_a}{1+exp\left(-\frac{\overline{\Delta G}_{Ca}/kT)}{\chi_{Ca}}\right)}, \qquad \text{(I)}$$

where $\Delta\sigma_{Ca}$ is the decrease in electrokinetic charge density of the cell in a solution of C g-ions per liter of calcium ions, χ_{Ca} mole fraction of calcium ions where $\chi_{Ca} = C/55.6$, n_{Ca} number of calcium ions adsorbed per cm^2, e the electronic charge, N_a the number of sites available for adsorption (number of anionogenic groups per cm^2), $\overline{\Delta G}_{Ca}$ electrochemical free energy of adsorption of calcium ions, k the Boltzmann constant and T the absolute temperature.

$$\text{Now let} \qquad exp\,(\overline{\Delta G}/kT)/55.6 = K$$

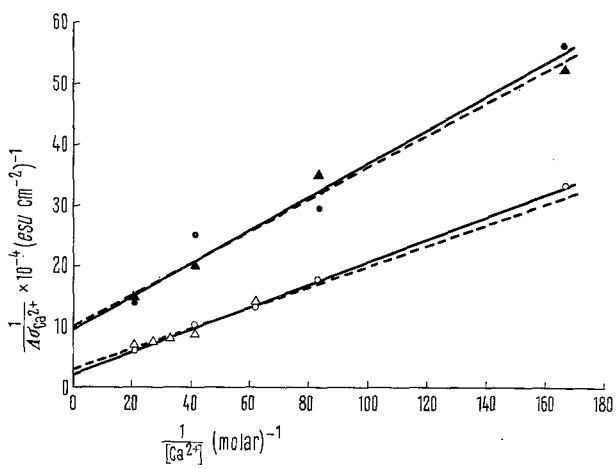
$$\text{then} \qquad \Delta\sigma_{Ca} = \frac{2eN_aCK}{1+CK} \qquad \text{(II)}$$

a standard form for a Langmuir adsorption isotherm, or in linear form,

$$\frac{1}{\Delta\sigma_{Ca}} = \frac{1}{2eN_a} + \frac{1}{C}\,\frac{1}{2eN_aK} \qquad \text{(III)}$$

If calcium ion binding proceeds according to this mass action mechanism, then, the plot of $(1/\Delta\sigma_{Ca})$ versus $(1/C)$ will be a straight line. N_a and $\overline{\Delta G}_{Ca}$ may be evaluated from the intercept with the ordinate and the slope respectively.

The number of anionic sites per square centimeter of cell surface was calculated from the electrophoretic mobilities by use of the Gouy equation for uni-valent electrolytes and converted to a number of sites per cell assuming an area of 1.63×10^{-6} cm^2 for the RBC and 2.84×10^{-6} cm^2 for the PMNs[7]. The Figure shows the plots of $(1/\Delta\sigma_{Ca})$ versus



Calcium ion binding plots for normal (○—○) (Slope 0.185×10^{-4}, intercept $2.1 \pm 0.4 \times 10^{-4}$) and neuraminidase treated (●—●) (Slope 0.276×10^{-4}, intercept $9.5 \pm 2.8 \times 10^{-4}$) erythrocytes and also normal (△---△) (Slope 0.173×10^{-4}, intercept $2.7 \pm 1.2 \times 10^{-4}$) and neuraminidase treated (▲----▲) (Slope 0.261×10^{-4}, intercept $9.9 \pm 2.2 \times 10^{-4}$) polymorphonuclear leucocytes. Symbols represent experimental points.