MEASUREMENT OF VISCOSITY IN LIVING CELLS BY A FLUORESCENCE METHOD

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Yeast and Euglena gracilis take up fluorescein diacetate and hydrolyze it to fluorescein in the cells. Fluorescence polarization of fluorescein in cells can be measured accurately in cell suspensions and related to intracellular viscosity. For yeast at 15°C and 27°C, internal viscosities of 14 cp and 10 cp are found and for Euglena values of 6.3 cp and 5.2 cp are found.

The fluorescence polarization of a fluorescent dye solution usually depends on the viscosity of the solution according to a relatively simple relation deduced by Perrin. Weber (1) suggested that this relation could be used to measure microscopic viscosity. Udenfriend (2) described fluorescence measurements of aminonapthylalanine labeled cells in suspension and showed that quantitative measurement of fluorescence is possible in highly scattering environments. Rotman and Papermaster (3) showed that non-fluorescent fatty acid esters of fluorescein enter mammalian cells and are cleaved to give the fluorescent dye fluorescein, which is the only product accumulated in the cells. Fluorescein was not deleterious to the cells. Guilbault and Kramer (4) found that lipase, acylase, or chymotrypsin were the only enzymes capable of hydrolyzing fluorescein diacetate or dibutyrate to give fluorescein.

The present studies show that the fluorescence polarization of fluorescein in yeast and Euglena cells can be measured and related to intracellular viscosity.

Methods

Fluorescence measurements were made with a Perkin-Elmer 203 spectrofluorometer. Polarization filters were Polacoat MB105 and PB105. Polarization

was obtained from the formula $P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}$, where I_{VV} and I_{VH} are the measured fluorescence intensities with the analyzers vertically and horizontally oriented and the correction factor $G = I_{HV}/I_{HH}$ is obtained with the incident beam horizontally polarized (5). For fluorescein measurements the excitation monochromator was set at 475 mμ and the emission monochromator at 520 mμ which are the excitation and emission maxima. When cell suspensions are used, scattered stray light interferes with the measurements unless 475 mμ and 525 mμ monopass interference filters are inserted. Baird-Atomic type B-3 filters were used for this purpose. A thermostatted flow cell was used for the 15°C measurements.

Log phase yeast S. cerevisiae S1237, was grown in G & S synthetic medium (6), harvested, washed and resuspended in G & S at 3×10^7 cell/ ml for all measurements. Euglena gracilis was grown in the dark (to inhibit chlorophyll synthesis) in the medium described by Kempner (7).

Fluorescein diacetate was from the Nutritional Biochemicals Corp.

Results and Discussion

The first set of experiments was designed to relate polarization to viscosity for fluorescein in media of varying viscosity and composition. Fluorescein was dissolved at 5×10^{-8} M in aqueous glycerol or sucrose solutions of known viscosity. Polarization does not depend on concentration for fluorescein concentrations below about 5×10^{-6} M (5). Fig. 1 shows reciprocal polarization versus T/η where T is absolute temperature and η is viscosity in poise. The Perrin relation is $1/P - 1/3 = (1/P_o - 1/3)(1 + RTT/\eta V)$ (8) and shows that reciprocal polarization is linearly dependent on T/η, provided the other molecular constants of the equation do not depend on T/η. Fig. 1 demonstrates that the relation holds for fluorescein for T/η below about 15×10^3 and the constants are the same for either sucrose or glycerol solutions. The value of P_o from extra-polation is 0.44, which is in close agreement with published values (9,10). The variation of polarization with pH for solutions of $T/\eta = 42 \times 10^2$ degrees/poise was also determined.

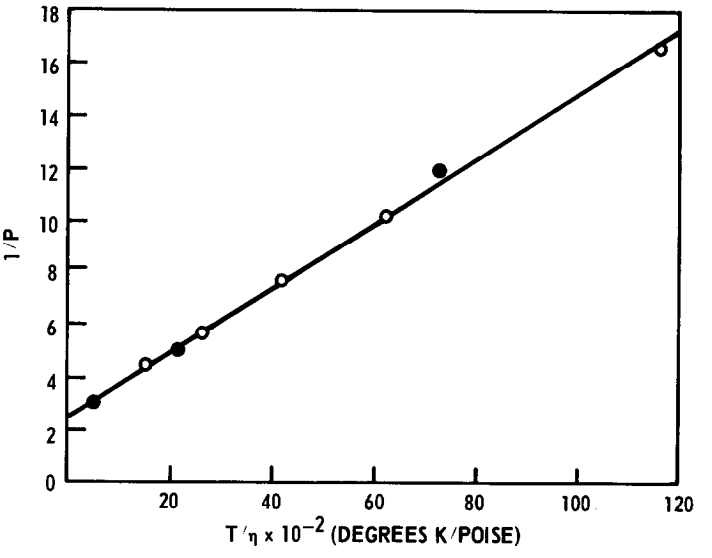

Fig. 1. Reciprocal polarization of fluorescein in sucrose (●) or glycerol (○) solutions versus T/η. T is degrees Kelvin, η is viscosity in poise.

P is constant within ± 5% over the range of pH 4 to 8, although fluorescence intensity increases 18 fold from pH 4 to 8. This is an important consideration for intracellular measurements because exact pH values for protoplasm are not known, although indicator measurements show protoplasmic pH to be near neutrality.

In the second series of experiments, growing yeast cells were exposed to fluorescein diacetate at 1 μg/ml (2.5×10^{-6} M) and fluorescence intensity and polarization measured. Fluorescein diacetate is not fluorescent but upon entering the cells and undergoing hydrolysis, the fluorescein released is fluorescent. The measured fluorescence is from intracellular fluorescein only initially. Fig. 2 shows the increase in fluorescence intensity as the diacetate is taken up and hydrolyzed in the cells and the rapid increase in polarization of fluorescein in the cells. For comparison, fluorescein in water or the G & S medium in which the cells are suspended has a measured polarization of .02. It is noteworthy that fluroescein in the earliest stage of accumulation in the cell does not reach the maximum polarization values

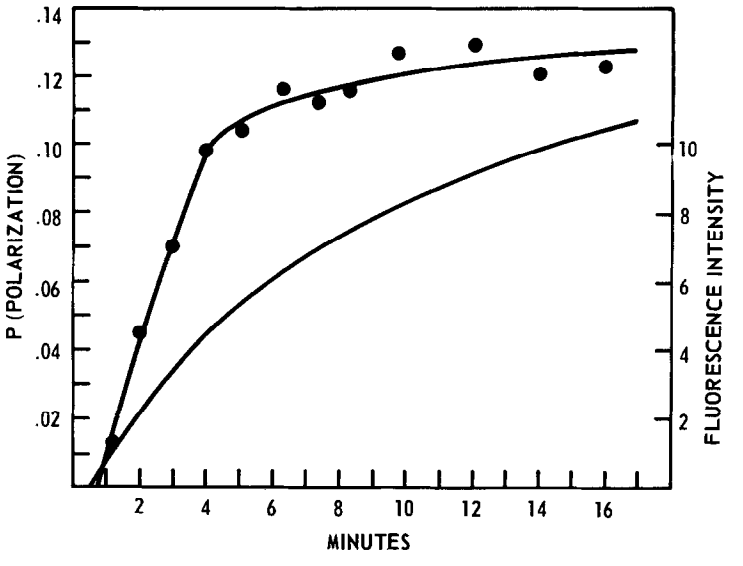

Fig. 2. Polarization (left) and fluorescence intensity (right) of yeast cells given fluorescein diacetate (1 μg/ml) at time zero.

seen later. This suggests that the hydrolysis takes place in cellular regions of relatively low viscosity and fluorescein then moves into regions of higher viscosity. In the determination of P, the fluorescence from fluroescein labeled cells is corrected for the low intensity light using identical unlabeled cells. This correction is small - 10% or less. Tests were also made to determine if the presence of light scattering and absorbing materials in the cells introduces error into the determination of fluorescein polarization. The polarization of fluorescein in 45% sucrose was measured and then mixed with a live cell suspension in 45% sucrose. The polarization of fluorescein in the mixture was unchanged, so light scattering and absorption by the cells did not alter polarization. In another test yeast cells were killed by heating at 90°C for 10' and an aqueous suspension was mixed with fluorescein or with fluorescein diacetate. Hydrolysis of the diacetate was slow but measureable. In both cases polarization was .02, the same as fluorescein in water. Fluorescein is apparently not strongly adsorbed or complexed to the large molecules or cell walls - since if this were the case polarization would be high.

Once fluorescein is released in yeast cells it may also leak out to the medium and increase the fluorescence background. Since P of fluorescein in the medium is .02, leakage will cause decline in the measured polarization of the suspension. To determine this effect, cells labeled with fluorescein were filtered rapidly on a millipore filter and resuspended in medium lacking fluorescein or the diacetate. Polarization measurements began one minute after resuspension. Fig. 3 shows the rapid decline in polarization at 27°C and the much slower decline at 15°C. Fluorescence intensity declines only slightly due to the lower pH of the medium. The rate of decline of polarization is then roughly proportional to the leakage rate and it is apparent that fluorescein leaks much more rapidly at 27°C than at 15°C. Extrapolation of the curves to zero time yields the best values of P for fluorescein in

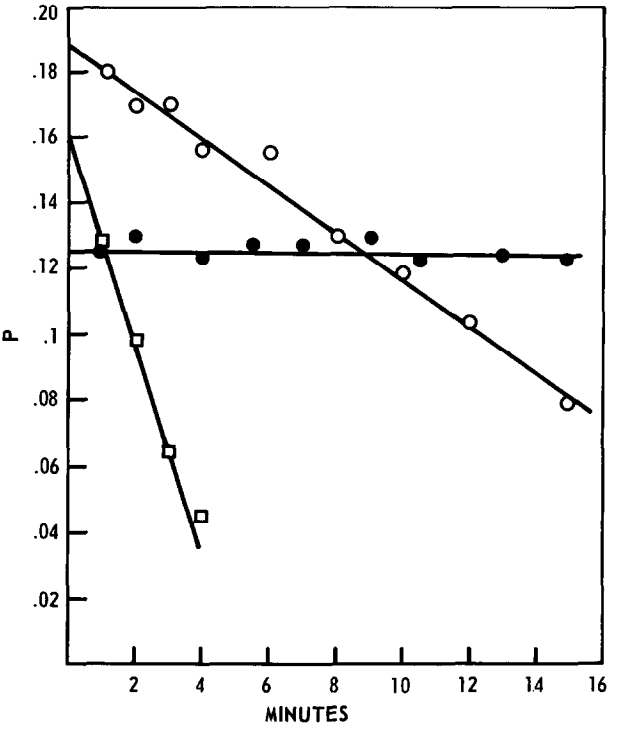

Fig. 3. Polarization versus time for fluorescein labeled cells transferred to fluorescein free medium at time zero. (O) yeast 15° C; (□) yeast 27° C; (●) Euglena 15°C.

yeast cells. These are P = .16 at 27°C and P = .19 at 15°C. Referring these values to fig. 1, the corresponding values of viscosity are 10 cp (centipoise) and 14 cp, respectively.

Measurements similar to those for yeast were also made in Euglena gracilis. The uptake polarization curve was similar to the yeast curve, shown in fig. 2. When labelled cells are filtered and resuspended in unlabeled medium (fig. 3) it was found that fluorescein leakage from Euglena is nil. At 15°C, P remains constant at .125 for at least 13 minutes. Referring this value to fig. 1 the Euglena internal viscosity is 6.3 cp. At 27° C there is again no leakage, and P is constant at .105 corresponding to a viscosity of 5.2 cp. The microviscosity as measured by fluorescein in Euglena is then about half that in yeast.

In relating polarization to viscosity we have assumed that fluorescein is not bound to any structure in the cell. The evidence supporting the assumption for dead cells has been presented but it is also important to note that the values of polarization obtained with live cells would approach P_o = .44 if the dye was bound to a large molecule or structure. The rapid leakage of fluorescein from yeast cells is further evidence that the dye is not bound. Experiments with ethidium bromide (11), which is known to complex with RNA and DNA, show polarization approaches P_o and the dye does not leak out of yeast.

With dark field microscopy it is possible to observe fluorescein fluorescence in individual Euglena cells. The fluorescein is distributed throughout the cytoplasm and does not appear to be concentrated in vacuoles or other organelles. The values of viscosity found by the fluorescein method are within the range found for protoplasmic viscosity by methods such as Brownian movement studies. Heilbrunn (12) in his review discusses values ranging from about 5 to 20 cp. Udenfriend (2) estimates 25 - 30 cp for the intracellular viscosity of Ascites cells.

The method is quick, simple, and can provide continuous measurement

of viscosity as a function of time. Relatively low intracellular viscosities increase the polarization of fluorescein fluorescence by fivefold or more above water values; this large change permits precise measurement. The technique has been employed to measure viscosity changes occuring in cells in response to drug treatment (11).

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