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Assessment of membrane potential changes using the carbocyanine dye, *diS*-C_3-(5): synchronous excitation spectroscopy studies

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Abstract. The fluorescence of the voltage sensitive dye, *diS*-C_3-(5), has been analyzed by means of synchronous excitation spectroscopy. Using this rather rare fluorescence technique we have been able to distinguish between the slightly shifted spectra of *diS*-C_3-(5) fluorescence from cells and from the supernatant. It has been found that *diS*-C_3-(5) fluorescence in the supernatant can be selectively monitored at $\lambda_{exc} = 630$ nm and $\lambda_{em} = 650$ nm, while the cell associated fluorescence can be observed at $\lambda_{exc} = 690$ nm and $\lambda_{em} = 710$ nm. A modified theory for the *diS*-C_3-(5) fluorescence response to the membrane potential is presented, according to which a linear relationship exists between the logarithmic increment of the dye fluorescence intensity in the supernatant, $\ln I/I^\circ$, and the underlying change in the plasma membrane potential, $\Delta\psi_p = \psi_p - \psi_p^\circ$. The theory has been tested on human myeloid leukemia cells (line ML-1) in which membrane potential changes were induced by valinomycin clamping in various K^+ gradients. It has been demonstrated that the membrane potential change, $\Delta\psi_p$, can be measured on an absolute scale.

Key words: Membrane potential – Fluorescence – *diS*-C_3-(5) – Synchronous excitation spectroscopy

Introduction

The carbocyanine dye 3,3′-dipropylthiadicarbocyanine iodide, *diS*-C_3-(5), is a membrane permeating cationic fluorophore (Waggoner 1976; Waggoner 1979), that can be used as a transmembrane-voltage tracer in liposomes and living cells (Hoffman and Laris 1974; Sims et al. 1974). The response of this fluorescent probe to the membrane potential was shown to result from its voltage-dependent partition between the intra- and extracellular phases and between the membrane and aqueous phases (Sims et al. 1974; Waggoner 1976; Waggoner 1979; Hladky and Rink 1976; Bashford and Smith 1979; Bashford 1981). When the membrane potential is negative inside the cell, the positively charged dye molecules accumulate in the cells and the intracellular dye content is increased by hyperpolarization, and vice versa. Observable changes in *diS*-C_3-(5) fluorescence result primarily from the association of a fraction of the dye molecules with different cell structures and/or from the formation of non-fluorescent dimers and higher aggregates.

In dilute aqueous dye solutions ($c \leq 1$ μM), a monomeric form of *diS*-C_3-(5) predominates with maxima in the absorption and fluorescence spectra at 650 and 665 nm, respectively (Sims et al. 1974; Hladky and Rink 1976; Tsien and Hladky 1978). With increasing dye concentration, non-fluorescent *diS*-C_3-(5) dimers, with an absorption maximum at 590 nm, are readily formed (West and Pearce 1965; Krasne 1980; Waggoner et al. 1977; Tsien and Hladky 1978). Together with the dimers higher aggregates may appear, particularly in aqueous media of high ionic strength. Their absorption band is near 760 nm (Tsien and Hladky 1978). They can thus not contribute to the monomer fluorescence at 670 nm. On changing from aqueous media to organic solvents of lower polarity both the absorption and emission spectra of *diS*-C_3-(5) are red shifted by about 17 nm, compared with the values in aqueous solutions (Tsien and Hladky 1978). All the spectroscopically different forms of *diS*-C_3-(5) have also been found in cells and liposomes: i.e. dimers (Sims et al. 1974; Hladky and Rink 1976; Tsien and Hladky 1978), higher aggregates (Sims et al. 1974) and dye molecules bound to nonpolar membrane structures (Sims et al. 1974; Tsien and Hladky 1978; Hladky and Rink 1976; Ivkova et al. 1984).

In our study, the synchronous excitation spectroscopy method (SES) was used to determine *diS*-C_3-(5) fluorescence contributions from cells and the cell medium. This spectroscopic technique, so far little known in the field of biological applications of fluorescence, relies on the simultaneous scanning of fluorescence emission and fluorescence excitation wavelengths, keeping the difference between them constant. Compared with standard fluorescence emission and excitation spectra, SES spectra

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offer a considerably higher chance of discriminating between spectroscopically similar fluorophores and/or in detecting small spectral shifts (Lloyd 1971; Vo Dinh 1981).

After calibration, the fluorescence response of the probe can be used for the quantitative determination of the membrane potential. In many cells, this calibration can be performed against a set of definite K^+ equilibrium potentials which are established after valinomycin clamping of cell membranes in the presence of various gradients of K^+ ions (Hoffman and Laris 1974; Hladky and Rink 1976; Rink et al. 1980), see also Methods.

Several theoretical models for the potential-sensitive response of *diS*-C_3-(5) fluorescence exist (Wilson et al. 1985; Ivkov et al. 1984). In this paper these models have been extended to take into account experimental results on dye aggregation within the cells. The modified theory relates the membrane potential to the free dye concentration in the supernatant. Its predictions have been compared with the results of membrane potential measurements in human myeloid leukemia cells.

Materials and methods

Reagents

The cyanine dye 3,3′-dipropylthiodicarbocyanine [*diS*-C_3-(5)] was obtained from Molecular Probes (USA), valinomycin was from Calbiochem (Switzerland) and other chemicals were from Lachema (Czechoslovakia).

Cell culturing and induction of differentiation

Cells of the human myeloid leukemia ML-1 cell line were kindly supplied by Dr. C. Haskovec, Institute of Haematology and Blood Transfusion, Prague. The cells were cultured in Iscove's medium supplemented with 10% (v/v) heat inactivated foetal calf serum, 50 IU/ml penicillin, 50 μg/ml streptomycin and 540 μg/ml glutamine in a 5% humidified CO_2 atmosphere at 37 °C. The cells were transferred three times a week and kept in the logarithmic growth phase.

Fluorescence measurements

Cell samples were washed three times (120 g × 10 min) in a buffer containing: 137 m*M* NaCl, 5.4 m*M* KCl, 1.43 m*M* $CaCl_2 \cdot 2H_2O$, 0.8 μ*M* $Mg_2SO_4 \cdot 7H_2O$, 1 m*M* $Ha_2HPO_4 \cdot 12H_2O$, 10 m*M* HEPES, 5 m*M* glucose, pH adjusted to 7.35. The cell suspension, concentrated to a density of $2-5 \times 10^6$ cells/ml, was maintained at 4 °C. Before fluorescence measurements, the cells were shaken for 10 min at 37 °C. The cell concentration was then adjusted to $1-5 \times 10^6$ cells/ml and the 1 m*M* stock solution of *diS*-C_3-(5) was added to a final concentration of 0.5–2 μM. The labelled suspension was left to equilibrate for 5 min at 37 °C.

The fluorescence of *diS*-C_3-(5) labelled samples was measured in glass cuvettes at 37 °C. All procedural suggestions recommended by Hladky and Rink (1976) and Tsien and Hladky (1978) were followed. In order to estimate the fraction of dye adsorbed on the cuvette walls, the emptied cuvette was washed for 15 min with stirred ethanol and the absorbance and fluorescence of the extract were measured, (Sims et al. 1974; Krasne 1980).

All fluorescence measurements were performed on an SLM-Aminco 4800 spectrofluorometer. Correction was not carried out for either the spectral distribution of the excitation intensity or the spectral sensitivity of fluorescence detection. The SES spectra were recorded over an excitation wavelength range from 580 to 730 nm. The wavelength difference between λ_{exc} and λ_{em} was $\Delta\lambda =$ 20 nm or $\Delta\lambda = 25$ nm, with slit widths of 8 nm in both emission and excitation.

The wavelength difference was chosen as a compromise between two conflicting requirements: *i)* the $\Delta\lambda$ value should be close to the wavelength difference between the maxima in the excitation and the emission spectra of the examined fluorophore; under such condition the half widths of SES spectral bands are minimal and this is desirable for the easy spectral detection of different fluorescing species, *ii)* at given slit widths the $\Delta\lambda$ value must be high enough to ensure the rejection of scattered excitation light.

The actual contribution of light scattering to the uncorrected SES spectra, and thus the extent of possible scattering artifacts, has been quantitated by measuring the SES spectra in dye-free samples. It was found that the contribution of scattering artifacts to the fluorescence assays presented in this study was negligible.

Calibration of the dye fluorescence response with the K^+ equilibrium potential

The valinomycin stock solution in ethanol (1 m*M*) was added to the cell suspension to a final concentration of 1 μM. Then, the K^+ concentration in the supernatant, $[K^+]_{out}$, was increased in fixed steps: usually from 5.4 m*M* (the K^+ concentration in the standard buffer) to 25.4, 45.4 and 105.4 m*M*. After any manipulation the system was left to equilibrate for 4 min at 37 °C.

The K^+ equilibrium potential was calculated according to the Nernst equation

$$\psi_K = (RT/F) \ln([K^+]_{out}/[K^+]_{in}) \quad (1)$$

with the intracellular potassium concentration approximated by the value $[K^+]_{in} = 145$ m*M*, which was found earlier in a very similar leukemic cell line HL-60 (Gargus et al. 1984). Based on (8), the calibration curves are presented as double-logarithmic plots of $\ln(I/I^\circ)$ vs $\ln[K^+]_{out}$, where I° and I are the *diS*-C_3-(5) fluorescence intensities in the supernatant measured before the addition of valinomycin (in standard buffer) and in the same sample after adding valinomycin and increasing the potassium concentration to the value of $[K^+]_{out}$.

Theory

Let us consider cells containing abundant mitochondria and having plasma and mitochondrial membrane potentials equal to ψ_p and ψ_m, respectively. For dye solutions in

aqueous media, the voltage-dependent partitioning of the probe between the extracellular medium, the aqueous internal space of the cell and its mitochondria can be satisfactorily described by the equations

$$\psi_p = -(\mathrm{RT}/z\,\mathrm{F})\ln(a_c/a_s) \quad (2a)$$

$$\psi_m = -(\mathrm{RT}/z\,\mathrm{F})\ln(a_m/a_c) \quad (2b)$$

which obviously yield

$$\psi_m + \psi_p = -(\mathrm{RT}/z\,\mathrm{F})\ln(a_m/a_s) \quad (2c)$$

where a_s, a_m and a_c are the activities of the monomeric dye dissolved in the extracellular, intramitochondrial and cytoplasmic aqueous media, respectively; z is the effective charge of the cationic dye molecule and R, F and T have the usual meanings, e.g. (Rottenberg 1979). These equations alone are obviously not sufficient to explain the observable changes in the probe fluorescence intensity, as they include neither the effects of dye binding to the cell membranes, organelles and cytosol macromolecules nor the effect of dye aggregation.

As demonstrated earlier, *diS*-C_3-(5) molecules readily aggregate in aqueous solutions containing inorganic anions. Their aggregation threshold is about 3.3 μM in solutions with ionic strength similar to that of the cytosol (the solid dye soon precipitates under such conditions), e.g. (Tsien and Hladky 1978). In our samples the typical rate of dye uptake was about 20–30% of the total dye added to the cell suspension (based on the assessment of both the dye fraction adsorbed on the cuvette walls and the dye fraction remaining free in the supernatant). The total cell volume of 1×10^6 ML-1 cells may be approximated by a value of 0.1–0.2 μl (Wilson et al. 1985). Hence the average dye concentration in the cell volume may exceed 1–3 m*M*, which is about three orders of magnitude above the expected aggregation threshold. This suggests that *diS*-C_3-(5) aggregates may exist in our samples.

From the very beginning of the cell labelling process, the intracellular dye tends to be accumulated in the mitochondria because of their high negative membrane potential (Wilson et al. 1985). Simultaneously, a fraction of the intracellular dye can bind to non-polar cell components, probably cell membranes. If the total amount of accumulated dye is high enough the concentration of the dye solution in the inner mitochondrial space will reach the concentration of the saturated solution, c_m^* (the corresponding dye activity will be denoted a_m^*). Substitution into (2b) and (2c) yields

$$a_s = a_m^* \exp[z\,\mathrm{F}(\psi_m + \psi_p)/\mathrm{RT}] \quad (3)$$

and

$$a_c = a_m^* \exp(z\,\mathrm{F}\,\psi_m/\mathrm{RT})\,. \quad (4)$$

At a certain level of dye uptake the accumulation capacity of the mitochondria will be exceeded. Then the dye activity in the cytosolic solution will not obey the simple relationship given by (4). It will increase above this limit.

On increasing the dye-to-cell concentration, the total dye uptake by cells can also exceed the level at which the free dye concentration in the cytosol become equal to the saturated solution value, c_c^*. For this condition, further variations in the total dye uptake (e.g. variations caused by changes in membrane potential) are expected to be followed either by an increase in the extent of dye aggregation or by partial dissolution of the existing aggregates.

The c_c^* value is a constant dependent on the temperature and the ionic strength of the cytosol. Consequently, the activity of the free dye in the supernatant, a_s, can be simply related to the plasma membrane potential ψ_p. We get

$$a_s = a_c^* \exp(z\,\mathrm{F}\,\psi_p/\mathrm{RT}) \quad (5)$$

where the constant a_c^* is the dye activity corresponding to c_m^*. Assuming that the dye activity is roughly proportional to the dye concentration, this equation can be expressed in terms of dye concentrations c_s and c_c^*:

$$c_s = \alpha\, c_c^* \exp(z\,\mathrm{F}\,\psi_p/\mathrm{RT}) \quad (6)$$

where α is a constant coefficient.

Furthermore, the dye fluorescence intensity in aqueous cell medium solutions was found to be proportional to the dye concentration for $c < 1-2$ μM (data not shown here). As a result, (6) can be rewritten in terms of relative fluorescence intensities as follows:

$$\ln(I/I^\circ) = \frac{z\,\mathrm{F}}{\mathrm{RT}}(\psi_p - \psi_p^\circ) \quad (7)$$

where ψ_p and I are the membrane potential and the dye fluorescence intensity in the supernatant, the indexed values ψ_p° and I° being measured under certain standard experimental conditions (see Methods). In conclusion, the logarithmic increment in the *diS*-C_3-(5) fluorescence intensity in the supernatant, $\ln(I/I^\circ)$, represents a linear measure for the underlying change in the plasma membrane potential, $\Delta\psi_p = \psi_p - \psi_p^\circ$.

In the particular case of the K^+ equilibrium potential calibration the ψ_p values are given by (1). Then we have

$$\ln(I/I^\circ) = z \ln([K^+]_{\mathrm{out}}/[K^+]_{\mathrm{out}}^\circ) \quad (8)$$

where $[K^+]_{\mathrm{out}}^\circ$ is the extracellular potassium concentration under the standard experimental conditions.

The principal hazards of this approach obviously arise from the choice of the approximation. At a low dye-to-cell concentration, i.e. at a low level of cell labelling, *diS*-C_3-(5) fluorescence in the supernatant should reflect the sum of the membrane potentials $\psi_p + \psi_m$ according to (3). Next, a range of intermediate dye-to-cell concentrations must exist at which neither (3) nor (6) can be used. In all probability, the existence of such intermediate states and thus the limits of the applicability of particular approximations can be discovered by investigating the fluorescence response to definite membrane potentials at various dye-to-cell ratios.

Finally, all the cautionary notes concerning both the cyanine dye toxicity, e.g. (Johnstone et al. 1982; Chused et al. 1986 and references therein), and methods of the membrane potential calibration (Hladky and Rink 1976; Rink et al. 1980; Kováč et al. 1982) obviously remain essential for further applications of the *diS*-C_3-(5) fluorescent probe.

Results and discussion

Synchronous excitation spectra of diS-C_3-(5) fluorescence in ML-1 cell suspensions

The SES spectra of *diS*-C_3-(5) fluorescence measured in pure buffer at low concentrations correspond to the emission of dye monomers, cf. (Sims et al. 1974; Hladky and Rink 1976; Tsien and Hladky 1978). These spectra are dominated by a sharp maximum at 664 nm which is accompanied by a pronounced shoulder at approximately 640 nm, Fig. 1. The spectral width at half-maximum of the main band is about 20 nm. It is considerably narrower than the spectral band observed in the standard emission spectrum, FWHM ~35 nm Fig. 2.

On adding ML-1 cells to the *diS*-C_3-(5) solution in buffer the monomeric dye band at 664 nm sharply decreased, Fig. 1. Simultaneously, a red-shifted fluorescence component appeared that could be attributed to a dye bound to non-polar cell structures, cf. (Sims et al. 1974; Hladky and Rink 1976; Tsien and Hladky 1978; Ivkova et al. 1984). Its fraction increased monotonically with increasing cell concentration. At high cell-to-dye ratios it became a dominant component of the SES spectra.

A considerable difference exists between the SES spectra of *diS*-C_3-(5) fluorescence in aqueous solutions and in nonpolar media which is much more pronounced than the difference that exists between the standard emission spectra. Unlike the emission spectra, two main components of the *diS*-C_3-(5) fluorescence in cell suspensions could therefore be resolved in the SES spectra. As shown in Fig. 3, the blue side of the SES spectra ($\lambda_{em} \leq 650$ nm) could be satisfactorily fitted to the normalized SES spectrum in aqueous solutions. The difference spectrum obtained in this way was attributed to the *diS*-C_3-(5) fluorescence in non-polar cell environments, Fig. 3. Its maximum is 17 nm red-shifted relative to the SES spectrum of the monomeric *diS*-C_3-(5) fluorescence in buffer; this equals the known spectral shift between the emission spectra of *diS*-C_3-(5) fluorescence in aqueous and nonpolar organic solutions (Tsien and Hladky 1978).

The spectral separation between the two main components of the *diS*-C_3-(5) fluorescence in cell suspensions, as revealed by the resolved SES spectra, suggests that the relative intensities of these components can be measured selectively. For example, suitable spectral choices for independent monitoring of *diS*-C_3-(5) monomers in an aqueous medium and *diS*-C_3-(5) fluorescence in cell membranes are $\lambda_{em} = 650$ nm at $\lambda_{exc} = 630$ nm and $\lambda_{em} = 710$ nm at $\lambda_{exc} = 690$ nm, respectively.

It should be emphasized here that the intensity of the aqueous phase fluorescence can be taken as a measure of the dye concentration in the supernatant for dye concentration ranging from approximately 0.1 μM to 1 μM. As noted above, the intensity of the *diS*-C_3-(5) fluorescence in buffer is proportional to the dye concentration up to approximately 2 μM. Moreover, a fraction of the dye fluorescence from the intracellular aqueous space can be neglected since *i)* the total volume of cells in suspensions is usually less than 0.1–0.5% of the supernatant volume and *ii)* the upper level of the *diS*-C_3-(5) concentration in the aqueous intracellular space is expected to be less than

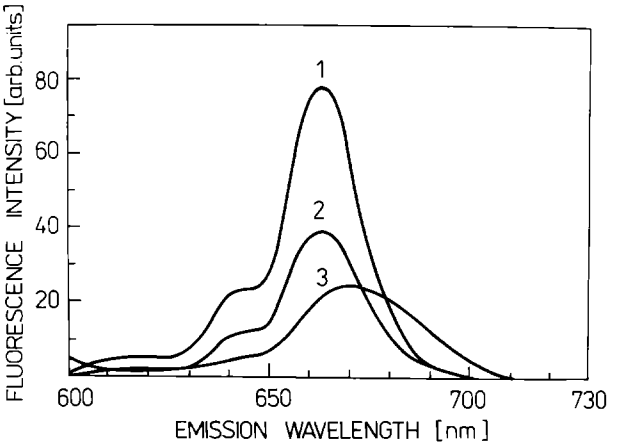

Fig. 1. Synchronous excitation fluorescence of *diS*-C_3-(5); dependence on the ML-1 cell concentration in the suspension: 1 – the solution of *diS*-C_3-(5) in pure buffer, 2 – 0.2×10^6 cells/ml, 3 – 1.7×10^6 cells/ml. The dye concentration was 1 μM in all samples. The wavelength difference $\Delta\lambda = 20$ nm

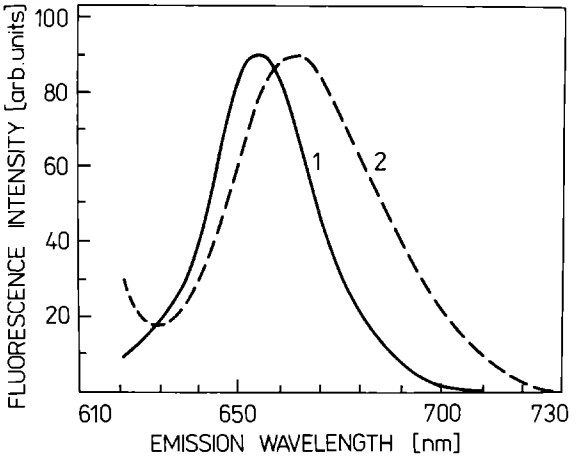

Fig. 2. Emission spectra of *diS*-C_3-(5) fluorescence; 1 – the fluorescence of 1 μM *diS*-C_3-(5) solution in pure buffer, 2 – the fluorescence in the ML-1 cell suspension (1 μM *diS*-C_3-(5), 1×10^6 cells/ml); $\lambda_{exc} = 580$ nm

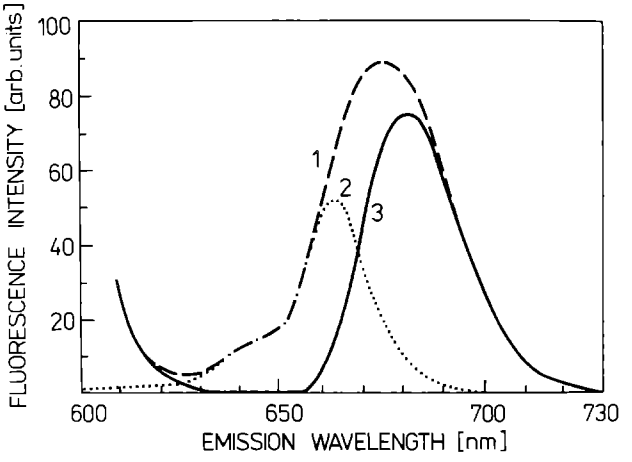

Fig. 3. The difference between the *diS*-C_3-(5) fluorescence in pure buffer and in cell suspensions: 1 – SES spectrum in the cell suspension (1 μM *diS*-C_3-(5), 1×10^6 ML-1 cells/ml), 2 – renormalized SES spectrum of *diS*-C_3-(5) fluorescence in pure buffer, 3 – the difference spectrum. Measured for the wavelength difference $\Delta\lambda = 20$ nm

3 μM, the value typical for the concentrated dye solution at high ionic strength (Tsien and Hladky 1978).

In conclusion, measuring the *diS*-C_3-(5) fluorescence intensity in cell suspensions at $\lambda_{exc}=630$ nm and $\lambda_{em}=650$ nm can be used for easy monitoring of relative changes in the dye concentration in the supernatant instead of the very slow direct method which is based on assaying the supernatant for the dye left after centrifuging out the cells.

DiS-C_3-(5) *fluorescence response to* K^+ *equilibrium potential in ML-1 cells*

The response of the *diS*-C_3-(5) fluorescence to changing K^+ equilibrium potential was studied in resting ML-1 cells at various dye-to-cell concentrations. The fluorescence intensity was measured in the spectral regions of both the supernatant and the cell-associated dye emission.

The fluorescence intensity of the membrane-associated component is almost independent of the K^+ equilibrium potential. In addition, this fraction in the total emission intensity was found to depend significantly on the dye-to-cell concentration, Fig. 1. These facts together offer a plausible interpretation for differences in the magnitude of the voltage-sensitive fluorescence response at varying cell and/or dye concentrations in suspensions which has been noted in most of the previous papers dealing with *diS*-C_3-(5) applications in membrane potential measurement.

A highly voltage-sensitive response of the probe fluorescence is typical of the supernatant component. At dye-to-cell concentrations higher than 1×10^{-9} mol of *diS*-C_3-(5) per 1×10^6 cells in 1 ml linear calibration curves of ln (I/I°) vs ln $[K^+]_{out}$ were observed, Fig. 4. The linearity of the calibration curves supports the theoretical model presented above, i.e. (8).

The calibration curves, however, are strongly concentration dependent. In particular, the apparent null-point value of membrane potential ranges from −87 mV (curve 3) to −38 mV (curve 5), Fig. 4. This means that a depolarization occurs in cells with high dye content; this is a known problem when measuring membrane potential with cyanine dyes, cf. (Sims et al. 1974; Hoffman and Laris 1974; Laris et al. 1976; Hladky and Rink 1976). This effect can be caused either by dye-induced changes in the membrane permeability and/or cell respiration or by a direct effect of the positive charge of the accumulated dye, e.g. (Johnstone et al. 1982). At low dye-to-cell concentrations, the calibration curves deviated from a linear shape which might indicate the expected limit of the applicability of (5) and (7).

Nevertheless, the double-logarithmic calibration curves revealed a new possibility for the use of *diS*-C_3-(5) fluorescence in monitoring the membrane potential. As shown in Fig. 4, the slopes of the linear calibration curves are practically independent of the dye concentration. This suggests that the logarithmic increment in *diS*-C_3-(5) fluorescence intensity is a suitable measure of the underlying membrane potential change, despite the fact that

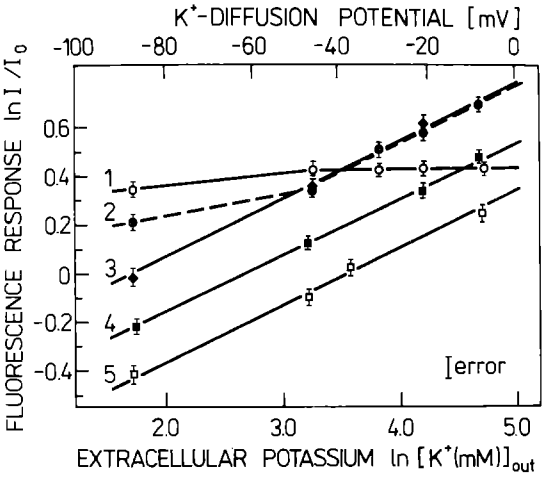

Fig. 4. The calibration of the *diS*-C_3-(5) fluorescence response against the K^+ equilibrium potential in ML-1 cells: curve 1 – 0.5 μM *diS*-C_3-(5), 2×10^6 cells/ml, $\lambda_{exc}=690$ nm, $\lambda_{em}=710$ nm, for other curves $\lambda_{exc}=630$ nm, $\lambda_{em}=650$ nm; 2 – 2 μM *diS*-C_3-(5), 2×10^6 cells/ml; 3 – 1.5 μM *diS*-C_3-(5), 1.2×10^6 cells/ml; 4 – 1.5 μM *diS*-C_3-(5), 0.7×10^6 cells/ml; 5 – 2 μM *diS*-C_3-(5), 1×10^6 cells/ml

the value of the membrane potential itself cannot be reliably assessed. In this sense, cyanine dyes can be further considered as a useful tool for studying the physiological role of membrane potential.

Using (1), the linear calibration curves for ML-1 cells may be converted to the ψ_k scale. Consequently, the logarithmic increment of the probe fluorescence intensity per 1 mV change in the plasma membrane potential of ML-1 cells equals ln $(I/I^\circ)=0.0086$. For quantitative measurement of membrane potential changes in other cell types a new calibration procedure must obviously be carried out.

If no satisfactory calibration procedure is available, measured ln (I/I°) values can be simply taken as a relative scale for measuring changes in the plasma membrane potential. Even in such cases, however, some preliminary studies of the dependence of ln (I/I°) values on the dye-to-cell concentration must also be performed. By comparing results obtained at different levels of cell labelling the limits of applicability of the linear relationship, i.e. (7), can be revealed, at least in principle.

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References

Bashford CL (1981) The measurement of membrane potential using optical indicators. Biosci Rep 1:183–196

Bashford CL, Smith JC (1979) The use of optical probes to monitor membrane potential. Methods Enzymol 55:569–586

Chused CL, Wilson HA, Seligmann BE, Tsien RY (1986) Probes for use in the study of leukocyte physiology by flow cytometry. In: Lansing Taylor D et al. (eds) Application of fluorescence in biomedical sciences. Liss, New York, pp 531–544

Gargus JJ, Adelberg EA, Slayman CW (1984) Rapid changes in bidirectional K^+ fluxes preceding DMSO-induced granulocytic differentiation of HL-60 human leukemic cells. J Cell Physiol 120:83–90

Hladky SB, Rink TJ (1976) Potential difference and the distribution of ions across the human red blood cell membrane: a study of the mechanism by which the fluorescent cation *diS*-C_3-(5) reports membrane potential. J Physiol 263:287–319

Hoffman JF, Laris PC (1974) Determination of membrane potentials in human and Amphiuma red blood cells by means of a fluorescent probe. J Physiol 239:519–552

Ivkov VG, Pechatnikov VA, Ivkova MN (1984) Redistribution of positively charged probes in membrane suspension under the action of transmembrane potential. Gen Physiol Biophys 3:19–30

Ivkova MN, Pechatnikov VA, Ivkov VG (1984) Mechanism of fluorescence response of the probe *diS*-C_3-(5) to transmembrane potential changes. Gen Physiol Biophys 3:97–117

Johnstone RM, Laris PC, Eddy AA (1982) The use of fluorescent dyes to measure membrane potentials: a critique. J Cell Physiol 112:298–301

Kováč L, Bohmerová E, Butko P (1982) Ionophores and intact cells: I. Valinomycin and nigericin act preferentially on mitochondria and not on the plasma membranes of Saccharomyces cerevisiae. Biochim Biophys Acta 721:341–348

Krasne S (1980) Interaction of voltage-sensing dyes with membranes. II Spectrophotometric and electrical correlates of cyanine dye adsorption to membranes. Biophys J 30:441–462

Laris PC, Pershadsingh HA, Johnstone RM (1976) Monitoring membrane potential in Ehrlich ascite tumor cells by meaning of a fluorescent dye. Biochim Biophys Acta 436:475–488

Lloyd JBF (1971) Synchronized excitation of fluorescence emission. Nature 231:64–65

Rink TJ, Montecucco C, Hesketh TR, Tsien RJ (1980) Lymphocyte membrane potential assessed with fluorescent probes. Biochim Biophys Acta 595:15–30

Rottenberg H (1979) The measurements of membrane potential and ΔpH cells, organelles and vesicles. Methods Enzymol 55:547–569

Sims PJ, Waggoner AS, Wang CH, Hoffman JF (1974) Studies on the mechanism by which cyanine dye measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry 13:3315–3330

Tsien RJ Hladky SB (1978) A quantitative resolution of the spectra of a membrane potential indicator *diS*-C_3-(5) bound to cell components and to red blood cells. J Membr Biol 38:73–97

Vo Dinh T (1981) Synchronous excitation spectroscopy. In: Wehry EC (ed) Modern fluorescence spectroscopy, vol 4. Plenum Press, New York London, p 167

Waggoner AS (1976) Optical probes of membrane potential. J Membr Biol 27:17–334

Waggoner AS (1979) Dye indicators of membrane potential. Ann Rev Biophys Bioeng 8:47–68

Waggoner AS, Wang CH, Tolles RL (1977) Mechanism of potential-dependent light absorption changes in lipid bilayer membranes in the presence of cyanine and oxonol dyes. J Membr Biol 27:317–324

West W, Pearce S (1965) The dimeric state of cyanine dyes. J Phys Chem 69:1894–1903

Wilson HA, Seligmann BE, Chused TM (1985) Voltage sensitive cyanine dye fluorescence signals in lymphocytes. Plasma membrane and mitochondrial components. J Cell Physiol 125:61–71