Title

pH GRADIENTS ACROSS THYLAKOID MEMBRANES MEASURED WITH A SPIN-LABELED AMINE

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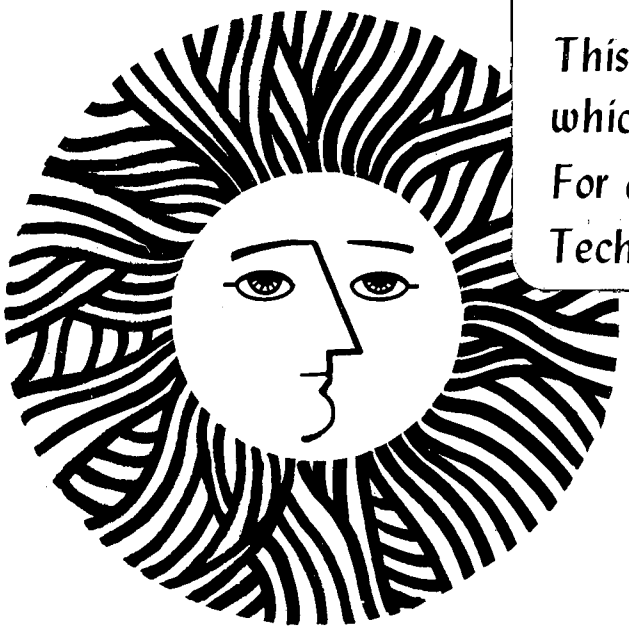

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DISCLAIMER

pH GRADIENTS ACROSS THYLAKOID MEMBRANES MEASURED WITH A SPIN-LABELED AMINE

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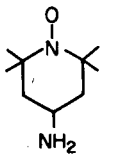
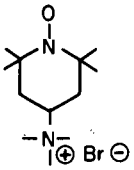
1. Introduction

In their uncharged form amines have been shown to be permeable across membranes and to establish concentration gradients proportional to H^+ gradients [1,2]. In their protonated form they may bind to negative moieties on the surface of proteins or lipid layers. Amines have therefore been used to study pH gradients across membranes [2–9] as well as the surface charge of membranes [10–13]. In particular the quenching of 9-aminoacridine fluorescence has been used to measure the pH gradient across thylakoid membranes [4,8], while that changes in the fluorescence of acridines are claimed [10,12,13] to be closely associated with their strong interaction with the membrane surface and that transport across the thylakoid membrane may not be required.

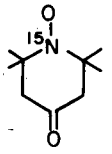
When using amine probes to measure pH gradients across membranes it becomes important to distinguish between the free and membrane bound populations of the probe; we have therefore used the spin-labeled amine 4-amino-2,2,6,6-tetramethyl-piperidine-*N*-oxyl (Tempamine) with pK = 9.5, which shows distinct bound and free EPR signals. An impermeable analogue, 4-trimethyl ammonium-2,2,6,6-tetramethyl-piperidine-*N*-oxyl bromide (CAT_1) and the impermeable spin broadening agent $K_3Fe(CN)_6$ were used to demonstrate that it is the uncharged form of Tempamine that is permeable to the thylakoid membrane. The permeable uncharged spin label 2,2,6,6-tetramethyl-4-piperidone(^{15}N-Tempone) was used to show that at low concentrations of Tempamine (150 μM) virtually no swelling of the thylakoids occurred during illumination.



TEMPAMINE



CAT_1



^{15}N TEMPONE

Our studies have allowed us to determine the internal aqueous concentration of this amine in the thylakoid and to accurately measure the pH gradient across its membrane.

2. Materials and methods

Thylakoid membranes were prepared from spinach leaves in 0.4 M sucrose, 10 mM NaCl buffered with 10 mM tricine, at pH 8, and resuspended in this medium at 6 mg chl/ml [14]. For the present studies thylakoid suspensions (0.6 mg chl/ml) in 90 mM NaCl or KCl, tricine > 1 mM (pH 8), in the presence of spin label and 0.1 mM methylviologen were placed in quartz EPR tubes of 1 mm internal diameter. Where indicated, samples were illuminated by a 400 W tungsten halogen lamp (GE 3476) at heat filtered saturating light intensities (~ 200 W/m^2) inside the microwave cavity and the spectra recorded in an E-109 E spectrometer at microwave power of 10 mW, time constant 0.128 s and scan time 9 min. $K_3Fe(CN)_6$, the spin broadening agent, was usually used at conc. $\leq$ 40 mM. Tempamine was from Aldrich Chemical Co., CAT_1 was synthesized [15] and ^{15}N-Tempone was a gift from A. D. Keith.

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tion and the impermeability of CAT_1 across the thylakoid membrane. We have also been able to demonstrate that at low concentrations of Tempamine ($< 20\ \mu M$) and fairly high concentrations of chlorophyll (0.6 mg chl/ml) virtually no binding of Tempamine to the thylakoid membrane is detectable either in the dark or in the light. Furthermore, this technique allows unambiguous determinations of inside and outside aqueous concentrations of Tempamine in a thylakoid suspension and therefore accurate determinations of the pH gradient across these membranes during illumination. Our results give ΔpH values of the order of 3.2 ± 0.2 in good agreement with the data in [22].

Acknowledgements

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