ΔpH AND MEMBRANE POTENTIAL IN BACTERIAL CHROMATOPHORES

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1. Introduction

The light-induced pH rise in chromatophore suspensions is generally interpreted as an inward electrogenic translocation of protons which, as in chloroplasts, creates a difference in the proton electro-chemical gradient ($\Delta\tilde{\mu}H$) across the chromatophore membrane [1]. Isaev et al. [2] showed energy dependent uptake of some synthetic permeant anions, and Jackson and Crofts observed spectroscopic changes [3] suggesting the existence of a membrane potential ($\Delta\psi$) which is positive inside. Several observations (4–6) indicate the existence of a proton concentration gradient (ΔpH). Moreover, the observation that chromatophores are less sensitive than chloroplasts to amines and nigericin uncoupling [4,5,7–9] and the effect of several ions [10,11] on chromatophore reactions led to the suggestion that $\Delta\psi$ rather than ΔpH provides the major contribution to the high energy state of the chromatophores.

However, quantitative estimation of ΔpH and $\Delta\psi$ of these subcellular structures is still unsatisfactory, due to the uncertainty in the mode of action of several of the monitoring probes used [12–14] and to insufficient data about the particles water volume and their buffer capacity [15].

In the present work the determination of the osmotic volume of the chromatophores enabled the calculation of ΔpH and $\Delta\psi$. The ΔpH was calculated from the distribution of [^{14}C] methylamine similar to such determinations in other subcellular organelles [15–17]. The $\Delta\psi$ was calculated from the distribution of [^{14}C] CNS^- which was shown to be a permeant anion in chromatophores [10,11].

2. Materials and methods

Chromatophores of *Rhodospirillum rubrum* were prepared and stored as previously described [18]. Before the experiment, the chromatophores were washed twice with a solution containing 10 mM sorbitol and 1 mM tricine, pH 8.0.

The tracer distribution between the chromatophore pellet and the medium was determined following the method previously described for chloroplasts (15). The chromatophore suspensions (125 μg Bch/ml) were sequentially preincubated with the proper tracers (10 min at 4°C) and protamine sulfate (0.7 mg/ml unless otherwise stated, 10 min at 4°C followed by 1 min at room temperature). Centrifugation (Beckman Microfuge, Model 152) was conducted in triplicates for 3 min. Illumination (720–1100 nm; 3×10^5 ergs/cm^2/sec) was for 3 min, starting from the one min period at room temperature preceding the centrifugation and continuing during the first 2 min of centrifugation. The supernatant (50 μl) and pellet (slice) samples were mixed with 2.5 ml of a mixture of acetone methanol (7:2 v/v), vigorously shaken and centrifuged (serological centrifuge, 10 min at room temperature). All the chlorophyll of the pellet was thus extracted into the supernatant in which it was determined (772 nM, EmM 96). Then 0.8 ml phtalate buffer (50 mM, pH 5.5) were added to 2 ml of the acetone methanol extracts

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and 0.1 ml of these mixtures were mixed with 10 ml aquasol and counted in a Tri-Carb Scintillation counter (Packard M3320) for both [^{14}C] and [^{3}H] content.

Water content of the pellet, the nonosmotic spaces ([^{14}C] sorbitol, [^{14}C] erythritol, [^{14}C] methanol spaces) and the concentration ratio of [^{14}C] methylamine and [^{14}C] CNS$^-$ between the osmotic compartment and the medium were calculated as previously described [15]. ΔpH was calculated from the distribution of [^{14}C] methylamine (MA): ΔpH=log ([^{14}C] MA in [^{14}C] MA out). Δψ was calculated from the distribution of [^{14}C] CNS$^-$ by the use of the Nernst equation. $\Delta\psi = \frac{RT}{ZF} \ln C_{in}/C_{out}$. The value of $\Delta\tilde{\mu}H$ was calculated from the relation $\Delta\tilde{\mu}H = \Delta\psi + 2.3 \frac{RT}{ZF} \Delta pH$.

Protamine sulfate, from Salmon, grade 1, was from Sigma; Aquasol, from New England Nuclear; Valinomycin was from Calbiochem; Radiochemicals were from the Radiochemical Centre Amersham.

3. Results and discussion

Determination of the distribution of radioactive labelled species between the chromatophore pellet and the supernatant in the light requires a rapid precipitation technique which can be carried out in the light. The usual sedimentation of the chromatophores at 100 000 g for 1 hr in the Spinco is not suitable for this purpose. Nelson et al. [19] have recently reported that in the presence of protamine sulfate subchloroplast particles could be precipitated at low centrifugational force. They have also shown that the presence of protamine sulfate did not lead to any significant loss of photophosphorylation activity. Chromatophores do not sediment at all at low centrifugal force (10 000 g. Beckman microfuge, model 152). We have, however, observed that preincubation of the chromatophores in the presence of protamine sulfate made the suspension turbid and allowed their precipitation in the microfuge.

Using this technique the osmotic response of the chromatophore membrane was determined as summarized in fig. 1. The chromatophores were incubated at various KCl concentrations and the pellet total water content and the sorbitol, erythritol and methanol water spaces were determined. It is seen that the water space which is not penetrated by sorbitol increases linearly with the reciprocal of the osmolarity, while both the non-erythritol water and non-methanol water remain constant (fig. 1B). A fraction of the non-sorbitol space does not react osmotically and is in part not accessible even to methanol or erythritol. The same results were obtained when the chromatophores were precipitated by centrifugation (100 000 g, 1 hr) in the absence of protamine sulfate (fig. 1B). However, in this case, the pellet is tightly packed and the total water content is reduced (fig. 1A). The non-sorbitol water of the chromatophores (which amounts to about 50 µl/mg Bch at the osmolarity of 66 mosmolar used in

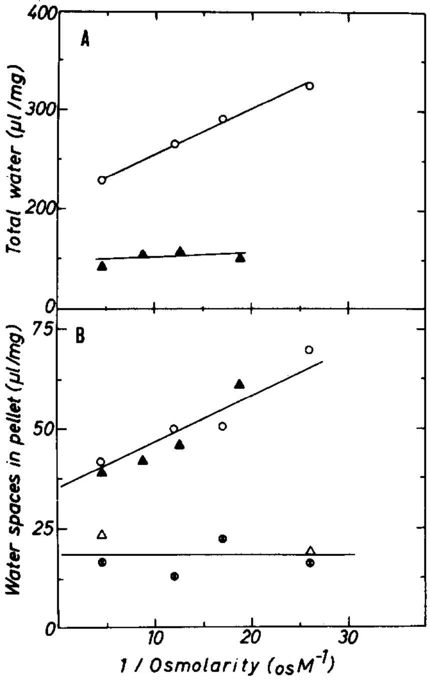

Fig.1. The effect of medium osmolarity on the water compartments of the chromatophores pellet. Reaction was carried out as described under Materials and methods. The reaction mixture contained in a total volume of 1.2 ml: 5 mM Na-tricine (pH 8.0); 12.5 mM sorbitol; 0.84 mg protamine sulfate; KCl to give the indicated osmolarity; [^{3}H]$_2$0, 50 µCi, chromatophores containing 160 µg bacteriochlorophyll and one of the following markers: 3 µCi [^{14}C] sorbitol (○,▲); 0.8 µCi [^{14}C] methanol (△) or, 3.6 µCi [^{14}C] erythritol (●). In one case (▲) the chromatophores were precipitated by centrifugation at 100 000 g for 1 hr in the absence of protamine sulfate. A-total water refers to the [^{3}H]$_2$0 content of the pellet, and B-water spaces refer to the non sorbitol non-erythritol and non-methanol spaces. They are expressed as µl/mg bacteriochlorophyll.

Table 1
Effect of NH_4Cl on the ΔpH formed across the chromatophore membrane in the light.

NH_4Cl (mM)	$\frac{CH_3NH_3^+ \text{ pellet}}{CH_3NH_3^+ \text{ supernatant}}$	$\frac{CH_3NH_3^+ \text{ (in)}}{CH_3NH_3^+ \text{ (out)}}$	ΔpH
0	5.2	23.1	1.4
1	2.8	10.7	1.0
10	1.1	1.6	0.2
50	1.1	1.6	0.2

The reaction was carried out as described under Materials and methods. The reaction mixture contained, in a final volume of 1.2 ml: 20 mM KCl; 330 μM Na-succinate (pH 8.0); 66 μM PMS; 7 mM Na-tricine (pH 8.0); 12.5 mM sorbitol; chromatophores containing 175 μg bacteriochlorophyll; 0.8 mg/ml protamine sulfate and $[^3H]_2O$, 50 μCi. For methylamine distribution determination 3 μCi of $[^{14}C]$ sorbitol or 3 μCi of $[^{14}C]$ methylamine and 40 μM cold methylamine were added. The sorbitol space was 0.81.

Table 2
The behaviour of ΔpH and Δψ under different conditions.

Conditions	ΔpH	Δψ (mv)	$\frac{\Delta\tilde{\mu}H}{\quad}$ (mv)
Dark	1.1	12	76.9
Light	1.8	89	195.2
Light			
+ FCCP 20 μM	0.9	6	59.1
+ KCNS 10 mM	2.0	32	150
+ KCl 100 mM	1.9	76	188.1
+ Val 3 μM	1.9	81	193.1

Conditions as in table 1, except that 50 mM KCl was present in all experiments and only 0.6 mg protamine sulfate was used. ΔpH was determined with methylamine as described in table 1. Δψ was determined by the distribution of CNS^- and for these measurements 3 μCi $[^{14}C]$ CNS and 10 μM cold KCNS were added.

the experiments) was used for the calculation of ΔpH and Δψ. Despite the uncertainty concerning the true osmotic volume these values are relatively insensitive to such inaccuracies in volume.

The determination of ΔpH in chromatophores which were precipitated in the light in the presence of protamine sulfate is shown in table 1. A light-induced ΔpH of 1.4 pH units was measured by the distribution of methylamine. NH_4Cl, which has been shown to inhibit proton uptake and light-induced quenching of atebrin fluorescence without affecting ATP synthesis in chromatophores [5,6,8], did indeed drastically reduce the light-induced ΔpH (table 1). Moreover, this effect was already saturated at 10 mM NH_4Cl, a concentration similar to that reported to inhibit over 90% of the proton uptake [8] and atebrin quenching [6]. These results provide further evidence for the suggestion [5,6] that the quenching of atebrin fluorescence is a reflection of the ΔpH formed across the chromatophore membrane.

When $[^{14}C]$methylamine and $[^{14}C]CNS^-$ are added at low concentrations to suspensions of chromatophores in the dark, they concentrate somewhat in the pellet, but illumination before and during centrifugation greatly increases both these uptakes (table 2). Uncouplers like carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), inhibit the light effect (table 2) and decrease the level of the dark uptake (not shown).

The penetration of CNS^- into the chromatophores is apparent in this work and confirms the conclusions that it is a truly permeable anion [10,11]. Therefore, its equilibrium distribution across the chromatophore membrane must be governed by the membrane potential. High concentrations of CNS^- should thus reduce the membrane potential and increase the concentration gradient of the protons. Indeed 10 mM KCNS were found to decrease the Δψ by 65% while the ΔpH increased so that the overall $\Delta\tilde{\mu}H$ was only slightly reduced (table 2). This concentration of KCNS has also been reported not to affect ATP synthesis [10] while it was shown to increase proton uptake and the quenching of atebrin fluorescence and decrease the light-induced changes in ANS fluorescence [10,11]. An effect similar to that of KCNS, although less pronounced, was obtained either by increasing the potassium concentration or by adding valinomycin to the 50 mM KCl present in the reaction mixture (table 2).

The above results were obtained with chromatophores which were precipitated with protamine sulfate at a ratio of about 5 mg/mg bacteriochlorophyll. This was found to be the minimal amount of protamine sulfate necessary to precipitate most of the chromatophores. A higher ratio should be avoided since protamine sulfate is highly charged and the presence of a large amount of it in the pellet would affect ion distribution between the pellet and the medium in accordance with the Donnan distribution. Cations would thus be less concentrated while anions would be more concentrated in the pellet. This effect was observed in other particle systems (H. Rottenberg, unpublished results) to reach a high magnitude when an excess of protamine sulfate was present in the suspension. The amount of protamine sulfate used in our experiments was as low as possible, so that the error in the calculations of ΔpH and $\Delta\psi$ due to this amount was minimal.

The parallel measurement of both ΔpH and $\Delta\psi$ in the chromatophores shows that during illumination a difference of the proton electrochemical gradient is established across the chromatophore membrane which amounts to about 200 mV (table 2). Thus $\Delta\tilde{\mu}\text{H}$ can serve in chromatophores either as an energy reservoir or energy convertor in photophosphorylation.

The electrical (90 mV) and chemical (1.8 pH units) components of $\Delta\tilde{\mu}\text{H}$ are similar in magnitude. However, the presence of permeant ions (CNS^- or K^+ + valinomycin) decreases $\Delta\psi$ concomitant with an increase of ΔpH while $\Delta\tilde{\mu}\text{H}$ remains constant. These results are compatible with the suggestions that the proton pump is electrogenic and that for uncoupling in chromatophores both $\Delta\psi$ and ΔpH must be collapsed [1, 4,6,8,11].

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