Biochimica et Biophysica Acta, 732 (1983) 83–91
Elsevier

BBA 71717

ISOELECTRIC POINTS OF MEMBRANE SURFACES OF THREE SPINACH CHLOROPLAST CLASSES DETERMINED BY CROSS-PARTITION

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(Received March 28th, 1983)

Key words: Isoelectric point; Cross-partition; Plasma membrane; Thylakoid membrane (Spinach chloroplast)

The isoelectric points of the membranes surrounding three classes of spinach chloroplasts have been determined by partition at different pH values in aqueous two-phase systems where the electrical potential differences at the interface are opposite (cross-partition). Class I chloroplasts, intact chloroplasts, have an isoelectric point at pH 3.8–4.1 and class II chloroplasts, broken chloroplasts or intact thylakoid membranes, have an isoelectric point at pH 4.7–4.9. The third class of particles, class III 'chloroplasts', that contain one or more chloroplasts, mitochondria, peroxisomes and some cytoplasm all surrounded by a membrane, probably the plasma membrane, have an isoelectric point at pH 3.4–4.0. The partition technique used presumably yields the isoelectric point of the surface of the membranes exposed to the phase system by the three classes of chloroplasts, i.e., the outer envelope membrane, the thylakoid membrane and the plasma membrane, respectively. The isoelectric points obtained with this technique are suggested to reflect protein to charged-lipid differences in the composition of the membranes.

Introduction

When aqueous solutions of dextran and poly(ethylene glycol) are mixed above certain concentrations, the mixture will separate into two water-rich phases [1–3]. Such two-phase systems can be buffered and made isotonic and have been useful in separating cells, cell particles and macromolecules [1–3].

The distribution of charged material between the two phases and the interface depends on the composition of the phase system, especially on the electrolytes added. Introduction of salts into the system usually results in an electrical potential across the interface and the sign and magnitude of it depend on the ions introduced [4,5]. Introduction of covalently bound ionisable groups at the ends of some of the poly(ethylene glycol) molecules also gives rise to an interfacial potential which is higher than that obtained with a salt [6].

The partition coefficient, K, (K = concentration in upper phase/concentration in lower phase) for a substance can be divided into two parts [1,3,7]

$$\log K = \log K_0 + \frac{Z \cdot F \cdot \psi}{\ln 10 \cdot R \cdot T}$$

where ψ is the interfacial potential in the phase system, Z the net charge of the substance, F the Faraday constant, R the gas constant and T the absolute temperature. When the net charge, Z, is zero, e.g., at the isoelectric point for a protein, the partition is independent of the interfacial potential between the phases and depends exclusively on surface properties of the substance other than charge, e.g., hydrophobic-hydrophilic properties, that thus determine K_0.

When an amphoteric substance is partitioned at different values of pH in a two-phase system with

an interfacial potential, the partition of the substance will be pH-dependent. Thus, if a protein is partitioned at a series of pH values in two-phase systems differing only in salt composition, and lines representing the pH-dependent distribution of the protein in the presence of each of the different salts are drawn, the lines would intersect at a pH close to or equal to the isoelectric point of the protein [8]. In phase systems containing positively and negatively charged poly(ethylene glycol) instead of different salts, the lines representing the pH-dependent partition of the protein obtained with each of the charged polymers will have steeper slopes due to the higher interfacial potentials, and the cross-point will become more distinct. The cross-partition method has earlier been used for the determination of isoelectric points of a variety of proteins [8,9], of mitochondrial membranes [10,11] and of peroxisomes [12] using different salts to obtain systems with different interfacial potentials. Cross-partition with positively and negatively charged polymers has been used for determining isoelectric points of soluble proteins [13,14], a membrane protein [15] and of disintegrated chloroplast lamellar membranes [16].

Information on isoelectric points of chloroplast membranes is very limited. Methods used have been measurements of electrophoretic mobility [17,18] and volumetric measurements, such as light scattering [19]. These studies have been performed on intact chloroplasts of the green algae *Nitella* [17] (isoelectric point 4.2), on broken chloroplasts of spinach (isoelectric point 4.7) [19] and on intact and broken chloroplasts of pea (isoelectric point 4.3 for both) [18]. Cells and cell organelles are susceptible to breakage and therefore it is desirable to use a gentle method for the determination of the isoelectric point of such membrane structures. Aqueous two-phase systems have been found to be mild towards membrane structures, and cross-partition is therefore suitable for the determination of their isoelectric points.

In the present work, the three classes of spinach chloroplasts described by Larsson et al. [20,21] have been subjected to cross-partition using either salts or charged polymers to induce the positive and negative potential, respectively, across the interface. The characteristics of the three chloroplast types are briefly [20–22]: Class I chloroplasts: morphologically intact chloroplasts rich in stroma material and surrounded by the envelope membranes. Class II chloroplasts: stripped or broken chloroplasts containing the inner lamellae (thylakoid membranes) and small amounts of stroma material. Class III chloroplasts: membrane-surrounded particles containing one to four chloroplasts and cytoplasm with some mitochondria and peroxisomes. Both class I and class III chloroplasts have an intact appearance in phase-contrast microscopy, i.e., shiny features without any internal structures visible, while class II chloroplasts are dark with visible grana stacks [23]. Class I and class III are, however, easily distinguishable in the transmission electron microscope [21].

Materials and Methods

Dextran T 500, batch No. 5996, M_r 500 000, was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Poly(ethylene glycol), grade Carbowax 4000, M_r 3000–3700, was supplied by Union Carbide Chemicals, New York, U.S.A. The charged polymers, sodium poly(ethylene glycol) sulphonate and trimethylamino-poly(ethylene glycol) bromide, synthesized from Carbowax 4000 [6], were gifts from Dr. G. Johansson, Department of Biochemistry, Lund, Sweden.

Preparation of chloroplasts

Plant material. Spinach, *Spinacea oleracea L.*, was cultured on vermiculite in sunlight or artificial light. Before harvesting, the spinach was protected against light for 24–72 h to reduce the amount of starch in the chloroplasts.

Chloroplasts preparations using batch procedures. Class I, II and III chloroplasts were prepared from 20-g batches of leaves, either full-grown or cotyledons, essentially according to Larsson et al. [21]. The method utilizes a conventional centrifugation technique followed by further purification by separation in aqueous two-phase systems.

The class I chloroplast preparations, obtained as a pellet, consisting of 80–95% intact (class I) chloroplasts, as estimated from phase-contrast micrographs, were suspended in an appropriate volume of 0.3 M sucrose/50 mM potassium phosphate buffer (pH 7.8) to obtain a chloroplast concentration of 10–40 absorbance units at 680 nm

(A_{680}), which corresponds approximately to 125–500 μg chlorophyll per ml.

The class II pellets obtained, containing less than 20% of class I chloroplasts, were suspended in 0.3 M sucrose/50 mM potassium phosphate buffer (pH 7.8) to the same chlorophyll concentration as for class I. The class III preparations, always derived from cotyledons, contained 90–95% chloroplasts with an intact appearance in the phase-contrast microscope. The residual part of the chloroplasts in these preparations was broken, class II, chloroplasts. The pellets were suspended in 0.3 M sucrose/50 mM potassium phosphate buffer (pH 7.8) for the cross-partition experiments.

Separation of the three classes of chloroplasts by counter-current distribution. The three classes of chloroplasts were also prepared from cotyledons by thin-layer counter-current distribution in aqueous two-phase systems [1,2]. The separation was performed according to the procedure of Larsson et al. [20]. Material from the three distribution peaks obtained were pooled separately and diluted with 0.3 M sucrose/50 mM potassium phosphate buffer (pH 7.8) and pelleted by centrifugation. Each of the pellets was suspended in 0.3 M sucrose/50 mM potassium phosphate buffer (pH 7.8) and used in cross-partition experiments.

Cross-partition

The cross-partition experiments were carried out by partitioning the chloroplast material in series of polymeric two-phase systems, where the pH was varied over a range of about 1.5 pH units in steps of about 0.1–0.2 pH unit. The phase systems contained either different salts or charged poly(ethylene glycol)s to produce the interfacial electrical potentials (systems A and B, respectively, Table I).

The phase systems, for example for test series A1 (Table I), were prepared by weighing out two batch systems:

A1-1: 18.9 g 20% (w/w) Dextran T 500, 9.45 g 40% (w/w) poly(ethylene glycol) 4000, 5.85 g sucrose, 6.0 ml 0.5 M Na_2SO_4 and water added to 57.00 g; and A1-2: 18.9 g 20% (w/w) Dextran T 500, 9.45 g 40% (w/w) poly(ethylene glycol) 4000, 5.85 g sucrose, 6.0 ml 0.5 M Na_2SO_4, 6.0 ml 0.05 M citric acid and water added to 57.00 g.

These batch systems were kept thoroughly mixed and 1.90 g in total, containing different proportions of the two, were weighed out in 8-ml test tubes. The proportion of A1-1 to A1-2 required to achieve the final desired pH was determined empirically. The test systems were temperature-equilibrated in a cold room at 2–4°C and 0.1 ml of the chloroplast suspension in 0.3 M sucrose/50 mM

TABLE I

COMPOSITION AND PHASE VOLUMES OF THE TWO-PHASE SYSTEMS USED IN THE CROSS-PARTITION EXPERIMENTS

	Phase system			
	A1	A2	B1	B2
Dextran 500 (%(w/w))	6.3	6.3	6.8	6.8
Poly(ethylene glycol) 4000 (%(w/w))	6.3	6.3	4.1	4.1
Trimethylamino-poly(ethylene glycol) 4000 (%(w/w))	0	0	2.7	0
Poly(ethylene glycol) sulphonate 4000 (%(w/w))	0	0	0	2.7
Na_2SO_4 (mmol/kg)	50	0	0	0
NaCl (mmol/kg)	0	100	0	0
Potassium phosphate buffer (pH 7.8) (mmol/kg)	2.5	2.5	2.5	2.5
Citric acid [a] (mmol/kg)	0–5	0–5	0–5	0–5
Sucrose (mmol/kg)	300	300	220	220
Phase volumes of 2.00 g systems				
Top phase (ml)	1.00	0.99	1.00	0.85
Bottom phase (ml)	0.87	0.88	0.87	1.02

* The concentration of citric acid is varied to give the desired pH in the phase systems after addition of chloroplast suspension.

potassium phosphate buffer (pH 7.8) was added to the tubes. The phase systems were mixed by inverting the tubes 50 times to achieve distribution equilibrium and the phases were allowed to separate for 30–60 min in the cold. A blank system for each test series was prepared in the same way except that the suspension buffer was exchanged for the chloroplast suspension.

750 μl of the top phase and 500 μl of the bottom phase were carefully withdrawn with constriction pipettes and the samples were transferred to 1.5 ml of 0.3 M sucrose. The remaining phase system in the test tube was broken by addition of 1.5 ml 0.3 M sucrose.

The absorbance at 680 nm of the diluted samples from top and bottom phases and the residual, diluted, phase system was measured against corresponding blanks. The absorbance was measured with a Zeiss PMQ II Spectrophotometer.

The pH values of the diluted residues were measured at room temperature with a pH meter 26 (Radiometer, Copenhagen) calibrated against 50 mM potassium hydrogen phthalate, pH 4.00 ± 0.01.

The total amount of chloroplasts in the system was obtained by summing up the amount of chloroplasts in the top phase and bottom phase samples and the residual system calculated from the absorbance values at 680 nm. The amount of chloroplasts distributed to top and bottom phases in each tube, expressed as percentage of total amount added, was calculated as: (A_{680} reading of the diluted sample × dilution of the withdrawn sample × phase volume × 100)/total amount of chloroplasts in the system (in A_{680} units).

Owing to the small amount of class III chloroplasts obtained, only very limited amounts could be included in the phase systems. Consequently, the determination of the distribution of this type of chloroplast based on absorbance measurements at 680 nm would be highly inaccurate. Therefore the number of chloroplasts (particles) was instead counted in an electronic cell counter (Celloscope 302, AB Lars Ljungberg & Co., Stockholm). This cell counter is a slightly modified version of the Coulter counter described by Shibata [24]. The countings were performed in 0.9% NaCl solution as electrolyte, twice filtered through Millipore filter type GS with a pore diameter of 0.22 μm. Spherical particles with volumes less than 5.1. μm^3 (diameter 2.14 μm) were excluded when counting the chloroplasts and the amount of particles were corrected for coincidence loss.

The total amount of chloroplasts added to the phase systems was in the range of 1–4 absorbance units at 680 nm, corresponding to approx. 10–50 μg chlorophyll, for classes I and II. For the cross-partition experiments with class III chloroplasts a total number of $(1–9) \cdot 10^6$ particles were added to each phase system, corresponding to a total absorbance at 680 nm of approx. 0.1–0.9.

Results and Discussion

When particles are partitioned in aqueous two-phase systems, there is generally an adsorption of particles to the interface when the affinity of the partitioned particle is the same for both phases [1]. In the phase systems used, the accumulation of chloroplasts at the interface was in some cases as much as 70% of the total amount of chloroplasts added to the system. Therefore the partition data have been presented as the amount of chloroplasts in the top phase and the bottom phase expressed as a percentage of the total amount of chloroplasts in the system. The same data were used to calculate the partition coefficient, K, i.e., the ratio of the amount of chloroplasts in the top and the bottom phase, respectively, with no consideration being taken of eventual material collected at the interface.

The chloroplasts, especially class I chloroplasts, aggregated at low pH, and caused changes in light-scattering effects. Chloroplasts are known to shrink in acidic solutions and in high salt solutions and this also changes the light scattering. The observed absorbance will therefore differ from that measured in isotonic media at neutral pH [25]. The total amount of chloroplasts added to each phase system was therefore calculated as described under Materials and Methods. The amount calculated in this way was in some instances only 50% of that calculated for the same chloroplast suspension at pH 7.8. The intactness of class I and class III chloroplasts after the cross-partition experiments was occasionally examined in the phase-contrast microscope and, even though mostly aggregated, they were found to retain an intact appearance.

TABLE II

MEAN VALUE OF CROSS-POINTS AND K_0 FOR THREE TYPES OF CHLOROPLAST MEMBRANES

Standard error of mean is given as ±.

Chloroplast class	Phase system (Table I)	pH of cross points calculated from separate experiments. Partition curves representing:			K_0 at cross-point	Number of experiments
		Top phase	Bottom phase	log K		
I	A	> 4.0	3.8 ±0.3	3.8 ±0.2	0.06±0.02	9
I	B	4.6±0.1	4.05±0.2	4.3 ±0.2	0.6 ±0.2	6
II	A	4.9±0.1	4.5 ±0.1	4.75±0.1	1.5 ±1.1	6
II	B	4.7±0.1	4.4 ±0.1	4.6 ±0.1	2.6 ±0.7	7
III	A	4.0±0.2	3.9 ±0.2	4.0 ±0.04	4.2 ±1.8	6
III	B	3.4	3.4	3.4	5.3	2

No systematic difference could be detected in partition behaviour and cross-points obtained for chloroplasts prepared by the batch procedures described under Materials and Methods or by counter current distribution [20]. Neither could any large difference in partition be observed for chloroplasts prepared from leaves grown under different conditions (sun or artificial light) or from young (cotyledons) or full-grown leaves.

Results presented in the figures are a mean of several cross-partition experiments with freshly prepared chloroplast samples. The mean values of cross-points and K_0 together with the corresponding values of S.E. are given in Table II.

Fig. 1a shows the partition behaviour of class I chloroplast preparations in systems A, the salt-containing phase systems (Table I). In these systems there was always a marked aggregation of the chloroplasts in the bottom phase, especially close to the isoelectric point of class I chloroplasts, and more so in the sodium sulphate- than in the sodium chloride-containing systems. The withdrawn samples were therefore heterogeneous and this is reflected in the rather large standard deviation in the cross-point and K_0 in these systems (Table II). Fig. 1b shows the cross-partition experiments of class I chloroplast preparations in phase systems B, with charged poly(ethylene glycol)s, Table I. The slope of the line with trimethylaminopoly(ethylene glycol) is much steeper than the corresponding one with sodium sulphate in Fig. 1a. This is due to the higher interfacial potential obtained with the charged poly(ethylene glycol) compared to that generated by the salt in system A1.

In Fig. 1b the pH for the cross-point in top phase is higher, which indicates that class II chloroplasts present as contamination in the class I preparation also contribute to the cross-point observed in the top phase. This has been verified by phase-contrast microscopy and from the knowledge of the distribution behaviour or class I and class II chloroplasts in comparable two-phase systems [1,20], where class I chloroplasts distribute mainly to the bottom phase and interface and class II chloroplasts mainly to the top phase and interface. The cross-point for class I chloroplasts in bottom phase is, however, approximately the same (within standard deviation limits) as in Fig. 1a (phase systems A, Table I).

Fig. 2a and b shows the results of cross-partition of class II chloroplast preparations in systems A and B, respectively. In both systems, the pH for the cross-point in the bottom phase is 0.3–0.4 pH unit lower than that in the top phase. This might be due to the contamination by up to 20% class I chloroplasts in the class II preparations (see Materials and Methods). The lines in bottom phase therefore derive from the partition of class I, with lower isoelectric point and partition coefficient, and class II with higher ones. The cross-point in bottom phase is therefore an average of the isoelectric points of both these chloroplast types, while that in top phase belongs almost exclusively to class II chloroplasts. The cross-point in the top phase is higher in phase systems A (Fig. 2a) than in systems B (Fig. 2b). A plausible reason is that a

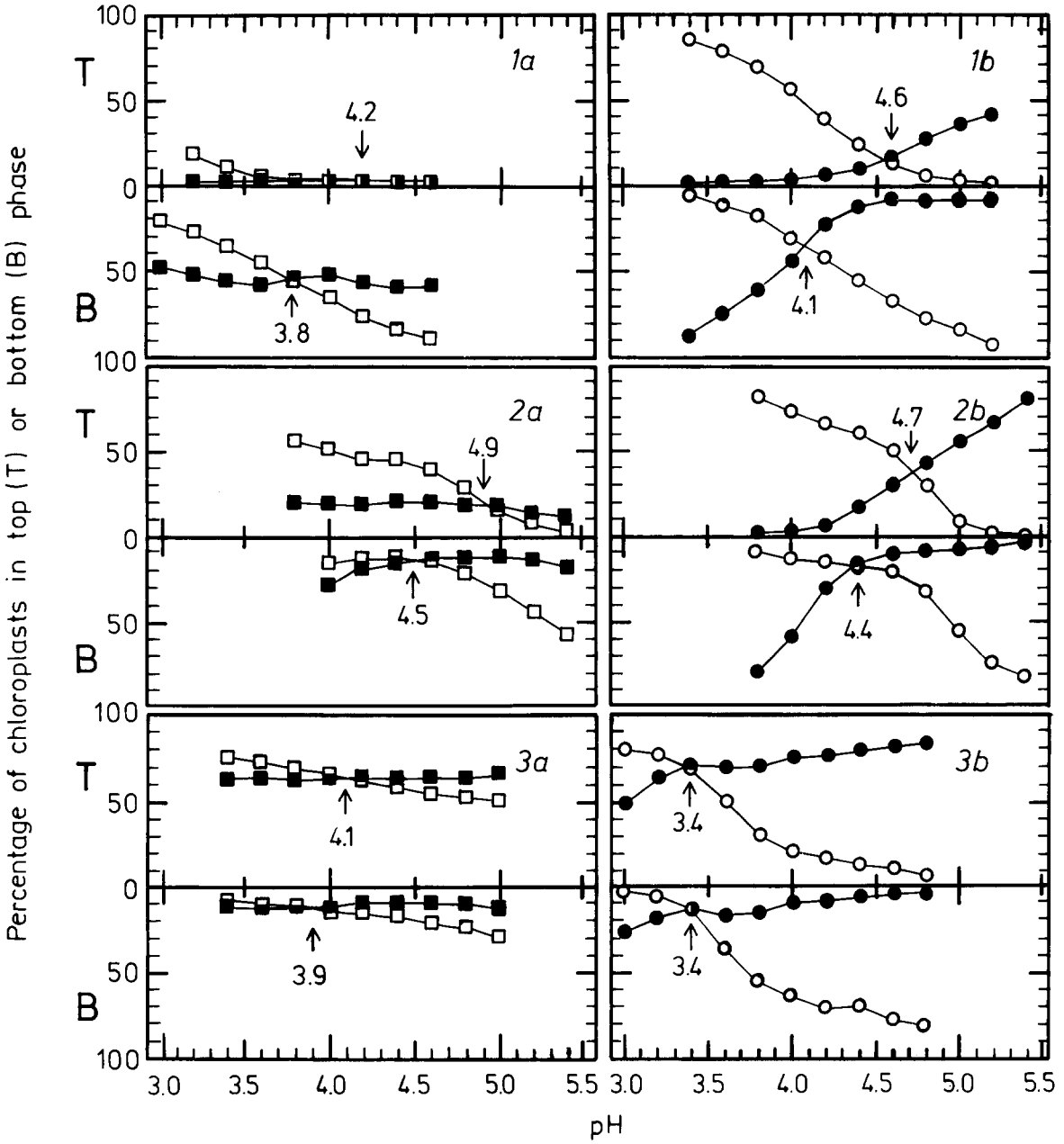

Fig. 1–3. Cross-partition of three classes of chloroplasts. Amount of chloroplasts in the top (T) and bottom (B) phase, respectively, expressed as percentage of total amount in the system, is plotted versus pH. Each data point is a mean of several individual experiments (number of experiments and S.E. are given in Table II). Fig. 1. Class I, intact chloroplasts. Fig. 2. Class II, broken chloroplasts (thylakoid membrane lamellar system). Fig. 3. Class III chloroplasts. Particles surrounded by a membrane different from chloroplastic membranes, probably the plasma membrane, and containing one or a few chloroplasts, some cytoplasm and sometimes mitochondria and peroxisomes as well. Figures a (squares) represent partition in salt-containing phase systems (systems A, Table I) and figures b (circles) show partition in phase systems containing charged poly(ethylene glycol) (systems B, Table I). Open symbols: Phase systems with positive interfacial potential (upper phase negative relative to lower phase, systems A2 and B2, Table I). Filled symbols: Phase systems with negative interfacial potential or interfacial potential close to zero (systems B1 and A1 respectively, Table I).

difference in ionic concentrations and ionic compositions leads to a difference in ionic interactions with the membranes. These interactions may cause conformational changes in the membrane proteins leading to changes in the surface properties of the membranes including the distribution of charges on the surface. This effect of ionic composition seems to hold for all the three types of chloroplast examined (Figs. 1–3 and Table II). Differences in ionic composition in the phase systems are known to affect the cross-point obtained for proteins [9] and for mitochondria [10].

Fig. 3a and b presents the cross-partition data for class III chloroplasts. This particle consists of one or more chloroplasts, mitochondria, peroxisomes and some cytoplasm, all surrounded by a membrane different from the chloroplast membranes, probably the plasma membrane. The difference in the membrane composition compared to the chloroplast membranes can be judged from the extreme distribution of these particles to the top phase and their low isoelectric point. The cross-points obtained in the top and bottom phase are identical in Fig. 3b (systems B, Table I) and the two values in Fig. 3a (systems A, Table I) are closer than that obtained for the other two classes of chloroplast preparation. This is probably because the degree of contamination of the class III chloroplast preparations is very small (see Materials and Methods). The results, figures and Table II, in short, show that class I chloroplasts (with intact envelope membranes) are partitioned to the bottom phase and have a low isoelectric point (pH 3.8–4.1) while class III chloroplasts, which also have a low isoelectric point (pH 3.4–4.1), distribute mainly to the top phase. Class II chloroplasts (the lamellar system) distribute mainly to the interface and top phase at the isoelectric point, which is high (pH 4.7–4.9). Our result for class II chloroplasts in top phase in Fig. 2b is in very good agreement with the result presented by Åkerlund et al. [16] for the intact chloroplast lamellae systems prepared in a slightly different manner.

The relative order of the values of partition coefficient at the cross-point, K_0, is the same as the G values for the different chloroplast classes, obtained from counter-current distribution experiments [1,20]. Thus, in counter-current distribution experiments with chloroplasts, class III chloroplasts show the highest partition, class I the lowest, and class II chloroplasts are inbetween. The absolute numerical values for the partition ratio, G, and partition coefficient, K_0, are, however, not identical as the phase systems used in counter-current distribution and cross-partition experiments differ in composition and also pH, which is above 7 in the former experiments and below 5 in the later. In the counter-current distribution experiments at a pH above 7, all chloroplast membranes carry a net negative charge and the distribution is thus dependent both on net charge and the ratio of hydrophobic and hydrophilic groups on the membrane surface, but at the isoelectric point of the membrane the distribution is dependent only on the latter properties which thereby determine K_0.

It is probably quite valid to suppose that the partition of a substance or a particle in aqueous two-phase systems is determined by the surface properties. Therefore, the partition of a membrane-surrounded particle like the intact chloroplast is determined by the membrane surface exposed to the phase system. For the class I chloroplasts, then, the partition is due to surface properties of the envelope and probably of the outer envelope membrane only, at least in systems with charged poly(ethylene glycol), to which the membrane can be expected to be impermeable. The partition of our class II chloroplasts in turn yield the properties for intact thylakoid membranes, i.e., the thylakoid membrane surface exposed to the chloroplast stroma in vivo.

As mentioned earlier, the slope of the partition line for a given substance depends on the interfacial potential arising from the salt or charged polymer included in the system. The sodium sulphate-containing phase systems (figures a and system A1, Table I) always yield more-or-less straight lines independent of pH, which indicates that this phase system has a zero interfacial potential. Sodium chloride, on the other hand, gives an interfacial potential with the same sign as poly(ethylene glycol) sulphonate-containing systems, but the absolute value of the potential is less and hence the slope of the partition line is less with sodium chloride. It is apparent from the figures that the angle of intersection of the cross-partition lines is larger and the cross-point sharper

in systems containing the charged polymers. Hence those systems are more suitable for determination of isoelectric points.

The slope of the curve log K vs. pH representing the partition of a protein at different pH values in a phase system containing a particular electrolyte yielding an interfacial potential has been shown to be proportional to the change in the net charge of the protein [5,7,14]. For membrane-surrounded particles with a number of different proteins and charged lipids on the surface it is, however, impossible to determine the net charge of each separate particle. The slope of a cross-partition curve for membrane particles is thus a mean of the distribution of a heterogeneous population of particles differing both in size and protein-to-lipid composition and hence probably net charge and charge density as well. From the cross-partition data, log K was also plotted versus pH (not shown). For class I and class II chloroplasts there was no difference in the angle of intersection of the partition lines representing the partition in the charged poly(ethylene glycol) phase systems (systems B, Table I). In the salt-containing systems (systems A), however, a slightly higher angle was obtained for the partition for class II chloroplasts in comparison to that for intact, class I, chloroplasts. The partition lines for class III chloroplasts clearly intersect at a lower angle than the lines for the other chloroplast classes in systems B and still more pronounced in systems A. The conclusion drawn from these observations is that, within a pH range ± 0.4 pH unit from the isoelectric point, the membrane surrounding the class III chloroplast particle bears the lowest net charge, while the thylakoid membranes bear an equal or slightly higher net charge than the outer envelope membrane. The present experiments were designed specifically to determine the isoelectric points of the membranes and nothing can therefore be said about the net charge, for example, at neutral pH. We have, however, previously shown that class II chloroplasts (the thylakoid membranes) bear a higher net negative charge compared to class I chloroplasts (surrounded by the envelope membranes) at pH 7.8 [26]. The conclusion was based on a study of counter-current distribution of the two classes of chloroplasts in charged and uncharged phase systems containing a hydrophobic top phase.

In the literature, a large store of data concerning the lipid and protein composition of the envelope and thylakoid membranes is available. Characteristic for chloroplast membranes is their high content of galactolipids (mono- and digalactosyldiacylglycerols) and the lack of phosphatidylethanolamine, except for the unique content of chlorophylls in the thylakoid membranes. The small, but distinct, differences in the charged lipid (phospho- and sulpholipids) composition reported [27,28] between envelope and lamellar membranes, probably do not alone cause the observed difference in their isoelectric point. The protein pattern in sodium dodecyl sulphate polyacrylamide gel electrophoresis has been shown to differ markedly for envelope and lamellar membranes [29,30] and freeze-fracture electron microscopy has displayed differences in particle size and density for the two envelope membranes as well as different surfaces of the thylakoid membranes [31,32]. There are, however, no large differences in the total amino acid composition between the envelope and thylakoid membrane proteins of broad bean (*Vicia faba* L.) [33]. One would therefore expect that the contribution from the constituent proteins to the net charge of the two membranes would be approximately the same. The observed difference in isoelectric point therefore probably reflects a difference in the relative content of charged membrane lipids and proteins exposed to the surrounding medium.

The membrane surrounding the class III chloroplast particles is most probably the plasma membrane. These particles are formed during homogenization of the leaves [20]. Upon cell disruption, part of the plasma membrane is fused and encloses a part of the cytoplasm containing one or more chloroplasts together with other organelles. This interpretation is supported by electron microscopy, which shows a similar structure of the membrane surrounding class III particles as that of the plasma membrane of the intact cell [20]. From studies of the carbon metabolism and the leakage of metabolites it can also be concluded that it is a plasma membrane which surrounds the class III particles [22]. Also, their partition behaviour is similar to that of protoplasts and isolated plasma membranes (Larsson, C., personal communication). The low isoelectric point of this

particle indicates, however, that the membrane surrounding it has a high relative content of carged lipids to proteins. The low slope of the partition curves especially in salt-containing systems (Fig. 3a) indicate that the membrane contains only a few titratable groups (most probably glutamic and aspartic acid residues of proteins [18]) in the pH interval between 3 and 5, which indicates a low protein content. The extreme distribution to the top phase at all the pH values used (including pH 7.8 [20]) might be due to a low content or complete lack of galactolipids. In contrast, both envelope and lamellar membranes have a high content of galactolipids and the headgroups of these lipids probably contribute to the affinity for the lower, dextran-rich phase shown by these membranes.

This report has shown that the membrane surfaces exposed to the phase systems used have different isoelectric points and net charge at pH values close to the isoelectric point. The proportion of hydrophilic and hydrophobic groups exposed are also different, as judged from the distribution at the isoelectric point. These differences could reflect differences in the lipid-to-protein ratio and different lipid composition of the envelope, thylakoid and plasma membranes, respectively.

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