View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by idUS. Depósito de Investigación Universidad de Sevilla

Post-print of Photosynthesis Research, March 2011, Volume 107, Issue 3, pp 279-286

Effect of crowding on the electron transfer process from plastocyanin and cytochrome c6 to photosystem I: a comparative study from cyanobacteria to green algae

Manuel Hervás, José A. Navarro

Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Universidad de Sevilla & CSIC, Américo Vespucio 49, 41092 Sevilla, Spain

Abstract

Plastocyanin and cytochrome c 6, the alternate donor proteins to photosystem I, can be acidic, neutral or basic; the role of electrostatics in their interaction with photosystem I vary accordingly for cyanobacteria, algae and plants. The effect of different crowding agents on the kinetics of the reaction between plastocyanin or cytochrome c 6 and photosystem I from three different cyanobacteria, Synechocystis PCC 6803, Nostoc PCC 7119 and Arthrospira maxima, and a green alga, Monoraphidium braunii, has been investigated by laser flash photolysis, in order to elucidate how molecular crowding affects the interaction between the two donor proteins and photosystem I. The negative effect of viscosity on the interaction of the two donors with photosystem I for the three cyanobacterial systems is very similar, as studied by increasing sucrose concentration. Bovine serum albumin seems to alter the different systems in a specific way, probably by means of electrostatic interactions with the donor proteins. Ficoll and dextran behave in a parallel manner, favouring the interaction by an average factor of 2, although this effect is somewhat less pronounced in Nostoc. With regards to the eukaryotic system, a strong negative effect of viscosity is able to overcome the favourable effect of any crowding agent, maybe due to stronger donor/photosystem I electrostatic interactions or the structural nature of the eukaryotic photosystem I-enriched membrane particles.

Keywords

Arthrospira, Cytochrome c6, Crowding, Laser flash photolysis, Monoraphidium, Nostoc, Photosystem I, Plastocyanin, Synechocystis

Abbreviations

BSA

Bovine serum albumin

Cyt c 6

Cytochrome c6

k 2

Second-order rate constant

k 2 0

Second-order rate constant in the absence of any added viscogen

k 2/k 2 0

Relative second-order rate constant in the presence of viscogens

k obs

Observed pseudo first-order rate constant

Pc

Plastocyanin

PSI

Photosystem I

Introduction

In photosynthetic organisms, the transfer of electrons from the cytochrome b 6-f to photosystem I (PSI) complexes—which are both membrane-embedded—is carried out by the two soluble metalloproteins cytochrome c6 (Cyt c6) and plastocyanin (Pc) (Hervás et al. 2003). PSI reduction has been extensively analysed in vitro in a wide variety of organisms, revealing that the kinetic mechanisms for the reaction of either Pc or Cyt c 6 with PSI from the same organism are similar, although they have increased in complexity and efficiency while evolving from prokaryotic cyanobacteria to algal and plant eukaryotic organisms (Hope 2000; De la Rosa et al. 2002; Hervás et al. 1995, 2003).

In eukaryotes, the donor proteins to PSI are strongly acidic, and interact with a well-conserved positively charged docking site in PSI by means of attractive electrostatic interactions (Ben-Shem et al. 2003; Hervás et al. 2003). However, in cyanobacteria, both Pc and Cyt c 6 can be acidic, neutral or basic, and thus the role of electrostatic forces in the interaction with PSI varies accordingly (Hervás et al. 1994, 1996, 2005; Molina-Heredia et al. 1998).

The importance of molecular crowding for reactions occurring in vivo is of outmost interest (Zimmerman and Trach 1991; Zimmerman and Minton 1993; Ellis 2001; Minton 2006). Intracellular environment is crowded due to the high concentration of proteins and other macromolecules (100–400 mg ml−1) (Zimmerman and Trach 1991). Crowding is considered to affect reaction rates in two ways: first, there is a positive effect on protein activities because a reduction of the volume available to large macromolecules increases the effective concentration, and so reaction equilibria and rates of interaction; second, there is a negative effect due to the increase in viscosity at high concentrations of macromolecules, resulting in

limited protein diffusion. Thus, the net result of these two opposite effects varies from one system to another (Zimmerman and Minton 1993; Ellis 2001; Minton 2006).

In the photosynthetic electron transfer chain, in particular, it is considered that crowding affects both the lateral diffusion of membrane proteins and carriers along the thylakoid lumenal membrane, as well as the activity of soluble proteins in the lumen (reviewed in Tremmel 2008). The lumen-confined space is believed to be tightly restricted, and a concentration of at least 20 mg ml−1 has been estimated for soluble proteins (Dekker and Boekema 2005; Schlarb-Ridley et al. 2005).

The effect of molecular crowding on the photosynthetic electron flow has been mainly investigated regarding diffusion of electron carriers within the photosynthetic membrane (Kirchhoff et al. 2008; Tremmel 2008), but little study has been done concerning the influence of crowding on the processes involving soluble electron carrier proteins, such as Pc and Cyt c 6 (Schlarb-Ridley et al. 2005). Thus, there is an open concern regarding to what extent the previous in vitro results concerning PSI reduction reflect the situation in vivo (Durán et al. 2006). Therefore, it is of relevant interest to understand how molecular crowding affects the interaction between the different donor proteins and PSI, as well as to determine whether these effects can be influenced by changes in the electrostatic properties of the donor proteins. With this objective in mind, the effect of different crowding agents on the kinetics of the reaction between Pc or Cyt c 6 and PSI from three different cyanobacteria, Synechocystis PCC 6803, Nostoc PCC 7119, Arthrospira maxima and a green alga, Monoraphidium braunii, has been investigated by laser flash photolysis. The differences in the effect of molecular crowding on the interaction between the alternate Pc/Cyt c 6 couple and PSI in the prokaryotic versus eukaryotic systems are also discussed.

Materials and methods

Purification of Cyt c 6 and Pc from Nostoc sp. PCC 7119, Synechocystis sp. PCC 6803 and Monoraphidium braunii, and Cyt c 6 from Arthrospira maxima was carried out as described elsewhere (Ho et al. 1979; Hervás et al. 1995; Molina-Heredia et al. 1998). Synechocystis, Nostoc, Arthrospira and Monoraphidium PSI were purified as previously described (Hervás et al. 1995).

Kinetics of flash-induced absorbance changes associated to PSI photooxidation and further re-reduction, either by Pc or Cyt c 6, were followed at 830 nm as described by Hervás et al. (2003), with some modifications. Excitation light is provided by a INDI-HG Nd:YAG pulsed laser from Spectra-Physics (wavelength, 532 nm; pulse duration, 4 ns). The laser flash is attenuated with calibrated neutral density filters so as to provide a just saturating excitation energy flash. The analysing light is provided by an 830 nm continuous diode-laser (model LQN830-150C, from Newport). The measuring detector is a silicon photodiode (Melles Griot 13DSI009), protected from actinic light by a narrowband glass filter. The photodiode signal output is amplified through a Melles Griot 13AMP005 amplifier (with wide bandwidth transimpedance) and recorded by a Nicolet 450 digital oscilloscope. Unless otherwise stated, the standard reaction mixture contained in a final volume of 0.25 ml, 20 mM Tricine–KOH, pH 7.5, 10 mM MgCl2, 0.03% β-dodecyl maltoside, an amount of PSI particles equivalent to 0.35 or 0.75 mg of chlorophyll ml−1 for the cyanobacterial or algal PSI, respectively, 0.1 mM methyl viologen, 2

mM sodium ascorbate and Cyt c 6 or Pc 60 μM. Ficoll-70, Dextran-70 and bovine serum albumin (BSA) were used as crowding agents and purchased from Sigma-Aldrich (USA). The concentration of each crowding agent in the reaction mixture was adjusted by adding small amounts of 57% (w/v) stock solutions, and the observed rate constants (k obs) for PSI reduction were corrected taking into account the dilution effect. Similar experiments were carried out with sucrose, as a control of a effect of viscosity. The viscosity of all the solutions after the addition of the different agents was measured with a viscosimeter Visco Star Plus (Fungilab SA, Spain). All the experiments were performed at 22°C in a 1 mm path-length cuvette. Kinetic data collection and analyses were as previously described (Hervás et al. 1995). The estimated error in rate constant determination was ≤15%.

Results

To analyse the influence of molecular crowding in the interaction between the soluble donors with PSI, we have examined the effect on the kinetics of electron transfer from Pc or Cyt c 6 to PSI of increasing concentrations of different agents—sucrose, BSA (MW 66 kDa), Ficoll-70 (MW 70 kDa) and Dextran-70 (average MW 64-76 kDa). PSI reduction has been followed by laser flash photolysis in three different species of cyanobacteria (Synechocystis, Nostoc and Arthrospira) and a green alga (Monoraphidium). Sucrose is commonly used as a control of viscosity effects, whereas ficoll, dextran and BSA are also widely employed as crowding agents (Ellis 2001).

Although different reaction models have been reported for donor/PSI systems from different type of organisms (Hervás et al. 1994, 1995, 2005; Navarro et al. 2001), under our experimental conditions (relatively low donor protein concentration), the PSI reduction by the different cyanobacterial donors shows monophasic kinetics for all systems (not shown), as well as linear dependences of the observed rate constant (k obs) upon donor protein concentration, mainly reflecting the PSI/donor association process. From the kinetic traces, the k obs for the PSI/donor interaction can be calculated, and from this figure and the donor protein concentration, an estimation of the bimolecular rate constant (k 2) can be obtained (Hervás et al. 1994, 1995).

Figure 1 (left) shows raw data of the effect of sucrose and the different crowding agents on the k 2 for the PSI/Cyt c 6 system of Arthrospira. Increasing concentrations of sucrose (and so increasing solvent viscosity) makes the k 2 slow down in a linear manner; however, the addition of BSA at the same concentration has no apparent effect in the interaction. Both ficoll and dextran favour the interaction, although this effect is more pronounced with the later agent (Fig. 1, left). The observed effects are however better seen by plotting the relative k 2 of the interaction versus the relative viscosity of the solution after adding increasing amounts of sucrose or any crowding agent (Fig. 1, right). Sucrose addition produces a small increase in the viscosity of the solution (a factor of ca. 1.2), but the k 2 of the interaction linearly goes down to 50%. However, the addition of BSA to increase the viscosity of the solution by a factor of more than 2 produces no net effects (Fig. 1, right). Addition of both ficoll and dextran produces an exponential increase of the k 2/k 2 0 ratio, reaching values of ca. 1.5 and 2.3, even though the viscosity of the solution increases by a factor of 5 and 7, respectively, at the end of the experiment (Fig. 1, right).

The effect of sucrose, ficoll and dextran on the PSI/donor systems of Synechocystis (Fig. 2) and Nostoc (Fig. 3), for both Pc and Cyt c 6, is qualitatively similar to that observed for Arthrospira (Fig. 1, right). However, the effect of sucrose on the interaction in these two organisms is more pronounced than in Arthrospira (k 2 decreasing to 25–30%), and a smaller positive effect of ficoll and dextran is observed in Nostoc, for both Pc and Cyt c 6, as compared with the other two cyanobacteria (Fig. 3). Moreover, the positive effect of ficoll and dextran is more pronounced with Cyt c 6 in Synechocystis as compared with Pc (Fig. 2), whereas the opposite effect is observed in Nostoc (Fig. 3). In strong contrast with the absence of effect on the Arthrospira system, in Synechocystis the addition of BSA increases the k 2 value by a factor of 2 (Fig. 2), whereas in Nostoc BSA has a negative effect on k 2/k 2 0 values similar to sucrose, although at higher values of relative viscosity (Fig. 3).

With respect to the Monoraphidium system, it has been previously shown that this algal system follows biphasic kinetics for PSI reduction (Hervás et al. 1995), as widely described for other eukaryotic photosynthetic organisms (Hope 2000; Fromme et al. 2003; Hervás et al. 2003). A first initial fast phase is assigned to the electron transfer step in a preformed donor/PSI complex, which has been shown not to be affected by the addition of increasing amounts of viscogens, as glycerol or sucrose (Hervás et al. 1995). A second observed slower phase of PSI reduction is assigned mainly to the initial donor/PSI association process, and this is the phase here studied. Sucrose makes reaction rates slow down drastically to 10%, with a parallel behaviour for both Pc and Cyt c 6 (Fig. 4). The addition of any of the three crowding reagents also promotes a decrease in the electron transfer process, the more drastic negative effect being obtained with BSA, for which similar low rate values than those obtained with sucrose are attained at low viscosity values (Fig. 4). In the case of algal Pc, ficoll decreases k 2 to ca. 35% at a relative viscosity increase of 2, k 2 remaining constant at higher viscosity. A similar effect is observed with Cyt c 6, but in this case k 2 decreases only to 60% (Fig. 4). More remarkable differences between both donor proteins are noted when using dextran as crowding reagent. In the case of Monoraphidium Cyt c 6 the negative effect of dextran is exerted even at low concentration, up to reach a 15% of the initial k 2 (Fig. 4, right). However, algal Pc shows a bell-shaped profile, with a maximum of k 2/k 2 0 at ca. 1.5 of relative viscosity, although higher concentrations of dextran finally lead to similar values than those observed in the case of Cyt c 6 (Fig. 4).

Discussion

Checking the effect of crowding agents on reaction rates is a way to mimic physiological cell conditions (Ellis 2001). Here three different agents (ficoll, dextran and BSA), with similar high molecular mass, were used. As high molecular mass viscogens can affect reaction rates in several ways, out of a pure crowding effect, the use of different crowding reagents is mandatory in order to discriminate possible effects due to specific interactions of the added macromolecules with the system under study (van den Berg et al. 1999; Ellis 2001). In addition, sucrose, a low molecular weight viscogen, is widely used as a control of the effect of increasing solvent viscosity, which usually limits diffusional association of protein partners (Harris et al. 1997); furthermore, sucrose is similar in polarity to ficoll and dextran at the same concentration (w/v) (Jiang and Guo 2007).

The use of donor/PSI systems from different organisms is justified by the fact that Pc/Cyt c 6 couples from different species differ in their surface electrostatic potential distribution and show different reaction mechanisms (Hope 2000; De la Rosa et al. 2002; Hervás et al. 2003). Focusing first in cyanobacteria, PSI and the slightly acidic donors from Synechocystis and the basic ones from Nostoc react according to repulsive and attractive electrostatic interactions, respectively (Hervás et al. 1995; Molina-Heredia et al. 1999), whereas the interaction of PSI with the neutral Cyt c 6 from Arthrospira—there is no Pc in this organism—is independent of ionic strength (Hervás et al. 2005).

Figure 5 shows in a comparative way the effect exerted on the PSI reduction rates by the different reagents at the higher concentrations shown in Figs. 1, 2, 3, 4. The effect of sucrose, ficoll and dextrane on the PSI/donor systems of Arthrospira, Synechocystis and Nostoc, for both Pc and Cyt c 6, is qualitatively similar (Fig. 5). Although only promoting moderate increases in the relative viscosity, the addition of sucrose produces a clear adverse effect in all cases, because the negative effect of viscosity on the diffusion of the reactants is not counterbalanced by a relevant positive crowding effect. However, the effect of sucrose in Synechocystis and Nostoc is more pronounced than in Arthrospira. Although usually considered as a pure viscogen agent, concentrated sucrose solutions have drastically decreased water activity, and this may have some effect on protein–protein interactions (Reiser et al. 1995). Thus, sucrose probably can exert a slight negative effect on the electrostatic interactions involved in the reaction of the charged donors with PSI in Synechocystis and Nostoc with respect to the neutral, hydrophobic system of Arthrospira. However, other explanations (i.e., subtle differences in PSI and/or donor proteins size or shape) cannot be discarded.

In the cyanobacterial systems here studied, ficoll and dextran favour the interaction in a parallel and similar manner (Fig. 5). The excluded volume effect at high concentrations of both crowding agents may increase the effective concentration of both PSI and the donor protein, which would cause the increase in the observed effective k 2, largely overcoming the negative effect of viscosity. This positive effect is, however, more pronounced with Pc in Nostoc as compared with Cyt c 6, whereas the opposite result is obtained in Synechocystis (Fig. 5). Taking into account the similarities of each Pc and Cyt c 6 couple from the same organism concerning the features relevant for the interaction with PSI, there is no a clear explanation for these differences, probably related again with small disparities in the size or shape of the different donor proteins, or to subtle differential electrostatic interactions with the crowding reagents.

An unexpected result is the different effect of BSA on the distinct donor/PSI systems (Fig. 5). In Synechocystis, the addition of BSA has a significant positive effect on the k 2 values, whereas in Nostoc BSA exerts a strong negative effect similar to sucrose. This contrasts with the absence of effect on the Arthrospira system. Thus, these results clearly indicate that additional factors besides size exclusion effects are involved. The most feasible explanation is the occurrence of specific electrostatic interactions between BSA (pI of 4.6) and the electrostatically charged donor proteins from Synechocystis (pI ≈ 5.6) and Nostoc (pI ≈ 9). Whereas acidic BSA may interact by means of electrostatic repulsions with the acidic Synechocystis proteins, it could experiment electrostatic attractions with the basic donor proteins of Nostoc, thus hindering in both cases its interaction with PSI. Specific effects of BSA, beyond a mere effect of increasing

molecular crowding of the solutions, have been previously reported for other systems (van den Berg et al. 1999; Minton 2000).

When analysing the results obtained with the eukaryotic Monoraphidium system, it is important to note that Monoraphidium PSI preparations here used are PSI-enriched membrane particles, and thus the effect of crowding agents is not directly comparable to that in the cyanobacterial preparations, for which PSI is obtained in its pure trimeric form (ca. 1,100 kDa) (Hervás et al. 1995; Fromme and Grotjohann 2006). However, a comparative study of the effects observed when using Pc or Cyt c 6 in this eukaryotic system can be carried out. Sucrose makes decrease reaction rates as previously observed in the cyanobacterial systems, although the effect is now more severe. Accordingly, in the presence of the three crowding reagents, the strong negative effect of viscosity overcomes the favourable crowding effect in all cases, the more drastic behaviour being obtained with BSA (Fig. 5). The negative effect of BSA cannot be now easily explained by an electrostatic interaction with the protein donors, because in this case they are strongly negative (pI ≈ 3.7) and thus a positive effect, similar to that of Synechocystis and opposite to Nostoc, should be expected. However, eukaryotic PSI has acquired an extra loop in the PsaF subunit in the acceptor side, with a strong basic character, to better interact with its negative soluble donors (Ben-Shem et al. 2003). Thus, electrostatic attractions between BSA and the positive PsaF subunit could hinder again the interaction of the donors with PSI.

It is considered that experimentally observed crowding effects are the result of two opposite forces. First, crowding agents reduce diffusion, slowing down association rates. Second, there is the positive effect of reducing the available volume to large molecules, increasing its effective concentration. Thus, the net result of these two opposite effects depends upon the nature of the interaction; however, at very high concentration of crowding agents, any rate will eventually fall due to the effect of viscosity (Ellis 2001). This indeed is the result observed when using ficoll or dextran in the Monoraphidium systems (Fig. 5). Thus, ficoll impedes the PSI/donor interaction even at low concentration, and a similar effect is observed with Monoraphidium Cyt c 6 when using dextran as crowding agent (Fig. 4). Interestingly, algal Pc shows a bell-shaped profile indicating that, at low concentration of dextran, the favourable effect of crowding initially overcomes the negative effect of viscosity, and consequently the rate increases to reach a maximum. Further increasing the concentration of viscogen slows down the interaction, due to the negative effect of viscosity. From the results, it is possible to point to a higher sensitivity of the algal PSI/donor system to viscosity as compared with the cyanobacterial ones or, alternatively, to a reduced sensitivity of the eukaryotic system to crowding. The role of electrostatics in the donor interaction with PSI varies from cyanobacteria to algae and plants (De la Rosa et al. 2002; Hervás et al. 2003). Eukaryotic PSI has extended the PsaF subunit to offer an additional positively charged area to interact with strongly negative patches in the protein donors (Ben-Shem et al. 2003), resulting that in the eukaryotic system the interaction relies more on electrostatic forces and less on entropy-driven binding, more sensitive to crowding effects (Hervás et al. 1996). This is also in agreement with the fact that the positive effects of ficoll and dextran are somewhat less pronounced in Nostoc, which is the cyanobacterial system with stronger electrostatic interactions. Regarding the differences observed between Pc and Cyt c 6 in the eukaryotic system, they have to be attributed to the different structure of the ficoll and dextran reagents and its interaction with the large PSI

vesicles, more than to a direct effect on the two soluble donors (van den Berg et al. 1999; Mukherjee et al. 2009). Dextran-70 is a flexible and linear molecule that behaves as a quasirandom coil, whereas Ficoll-70 is a compact and highly cross-linked reagent that can be more closely approximated to a sphere (Luby-Phelps et al. 1987; Venturoli and Rippe 2005).

To conclude, it is interesting to discuss the results from the point of view of its physiological relevance. As the thylakoidal membrane could promote both packaging and diffusional motions of the soluble proteins in the thylakoidal lumen, it has been proposed that in vivo PSI reduction is limited by the exchange of the donors from the PSI complex (Drepper et al. 1996; Finazzi et al. 2005). Here, it is shown that, in cyanobacteria, donors with very different electrostatic features behave in a similar way, doubling its efficiency under a crowded environment. With respect to the eukaryotic systems, the most relevant conclusion is that, contrary to the cyanobacterial ones, the negative effect of viscosity is able to overcome the favourable effect of crowding agents, as ficoll and dextran, indicating a higher sensitivity of the algal PSI/donor system to viscosity or, alternatively, a less sensitivity to crowding effects, maybe due to an evolution towards electrostatically based stronger donor/PSI interactions.

Acknowledgments

Research work was supported by the Spanish Ministry of Innovation and Science (MICINN, Grant BFU2006-01361) and the Andalusian Government (PAI BIO-022). The authors thank Prof. Miguel A. de la Rosa for helpful discussions, Pilar Alcántara for technical assistance, and Prof. David Krogmann for providing with Arthrospira cells.

References

Ben-Shem A, Frolow F, Nelson N (2003) Crystal structure of plant photosystem I. Nature 426:630–635

De la Rosa MA, Navarro JA, Díaz-Quintana A, De la Cerda B, Molina-Heredia FP, Balme A, Murdoch PS, Díaz-Moreno I, Durán RV, Hervás M (2002) An evolutionary analysis of the reaction mechanisms of photosystem I reduction by cytochrome c 6 and plastocyanin. Bioelectrochemistry 55:41–45

Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta 1706:12–39

Drepper F, Hippler M, Nitschke W, Haehnel W (1996) Binding dynamics and electron transfer between plastocyanin and photosystem I. Biochemistry 35:1282–1295

Durán RV, Hervás M, De la Rosa MA, Navarro JA (2006) A laser flash-induced kinetic analysis of in vivo photosystem I reduction by site-directed mutants of plastocyanin and cytochrome c6 in Synechocystis sp. PCC 6803. Biochemistry 45:1054–1060

Ellis RJ (2001) Macromolecular crowding: obvious but underappreciated. TIBS 26:597–604

Finazzi G, Sommer F, Hippler M (2005) Release of oxidized plastocyanin from photosystem I limits electron transfer between photosystem I and cytochrome b 6 f complex in vivo. Proc Natl Acad Sci USA 102:7031–7036

Fromme P, Grotjohann I (2006) Structural analysis of cyanobacterial photosystem I. In: Golbeck JH (ed) The light-driven plastocyanin: ferredoxin oxidoreductase. Springer-Verlag, Dordrecht, pp 47–69

Fromme P, Melkozernov A, Jordan P, Krauss N (2003) Structure and function of photosystem I: interaction with its soluble electron carriers and external antenna systems. FEBS Lett 555:40–44

Harris MR, Davis DJ, Durham B, Millett F (1997) Temperature and viscosity dependence of the electron-transfer reaction between plastocyanin and cytochrome c labeled with a ruthenium(II) bipyridine complex. Biochim Biophys Acta 1319:147–154

Hervás M, Ortega JM, Navarro JA, De la Rosa MA, Bottin H (1994) Laser flash kinetic analysis of Synechocystis PCC 6803 cytochrome c 6 and plastocyanin oxidation by photosystem I. Biochim Biophys Acta 1184:235–241

Hervás M, Navarro JA, Díaz A, Bottin H, De la Rosa MA (1995) Laser-flash kinetic analysis of the fast electron transfer from plastocyanin and cytochrome c 6 to photosystem I. Experimental evidence on the evolution of the reaction mechanism. Biochemistry 34:11321–11326

Hervás M, Navarro JA, Díaz A, De la Rosa MA (1996) A comparative thermodynamic analysis by laser-flash absorption spectroscopy of photosystem I reduction by plastocyanin and cytochrome c 6 in Anabaena PCC 7119, Synechocystis PCC 6803, and spinach. Biochemistry 35:2693–2698

Hervás M, Navarro JA, De la Rosa MA (2003) Electron transfer between soluble proteins and membrane complexes in photosynthesis. Acc Chem Res 36:798–805

Hervás M, Díaz-Quintana A, Kerfeld C, Krogmann D, De la Rosa MA, Navarro JA (2005) Cyanobacterial photosystem I lacks specificity in its interaction with cytochrome c 6 electron donors. Photosynth Res 83:329–333

Ho KK, Ulrich EL, Krogmann DW, Gomez-Lojero C (1979) Isolation of photosynthetic catalysts from cyanobacteria. Biochim Biophys Acta 545:236–248

Hope AB (2000) Electron transfer amongst cytochrome f, plastocyanin and photosystem I: kinetics and mechanisms. Biochim Biophys Acta 1456:5–26

Jiang M, Guo Z (2007) Effects of macromolecular crowding on the intrinsic catalytic efficiency and structure of enterobactin-specific isochorismate synthase. J Am Chem Soc 129:730–731

Kirchhoff H, Haferkamp S, Allen JF, Epstein DBA, Mullineaux CW (2008) Protein diffusion and macromolecular crowding in thylakoid membranes. Plant Physiol 146:1571–1578

Luby-Phelps K, Castle PE, Taylor DL, Lanni F (1987) Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. Proc Natl Acad Sci USA 84:4910–4913

Minton AP (2000) Implications of macromolecular crowding on protein assembly. Curr Opin Struct Biol 10:34–39

Minton AP (2006) How can biochemical reactions within cells differ from those in test tubes? J Cell Sci 119:2863–2869

Molina-Heredia FP, Hervás M, Navarro JA, De la Rosa MA (1998) Cloning and correct expression in E. coli of the petE and petJ genes respectively encoding plastocyanin and cytochrome c6 from the cyanobacterium Anabaena sp. PCC 7119. Biochem Biophys Res Commun 243:302–306

Molina-Heredia FP, Díaz-Quintana A, Hervás M, Navarro JA, De la Rosa MA (1999) Site-directed mutagenesis of cytochrome c6 from Anabaena species PCC 7119. Identification of surface residues of the hemeprotein involved in photosystem I reduction. J Biol Chem 274:33565–33570

Mukherjee S, Waegele MM, Chowdhury P, Guo L, Gai F (2009) Effect of macromolecular crowding on protein folding dynamics at the secondary structure level. J Mol Biol 393:227–236

Navarro JA, Myshkin E, De la Rosa MA, Bullerjahn GS, Hervás M (2001) The unique proline of the Prochlorothrix hollandica plastocyanin hydrophobic patch impairs electron transfer to photosystem I. J Biol Chem 276:37501–37505

Reiser P, Birch GG, Mathlouthi M (1995) Physical properties. In: Mathlouthi M, Reiser P (eds) Sucrose, properties and applications. Blackie Academic & Professional, Glasgow, pp 186–222

Schlarb-Ridley BG, Mi H, Teale WD, Meyer VS, Howe CJ, Bendall DS (2005) Implications of the effects of viscosity, macromolecular crowding, and temperature for the transient interaction

between cytochrome f and plastocyanin from the cyanobacterium Phormidium laminosum. Biochemistry 44:6232–6238

Tremmel IG (2008) Molecular crowding: a way to deal with crowding in photosynthetic membranes. In: Artmann GM, Chien S (eds) Bioengineering in cell and tissue research. Springer-Verlag, Berlin–Heidelberg, pp 543–579

van den Berg B, Ellis RJ, Dobson CM (1999) Effects of macromolecular crowding on protein folding and aggregation. EMBO J 18(24):6927–6933

Venturoli D, Rippe B (2005) Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. Am J Physiol Renal Physiol 288:F605–F613

Zimmerman SB, Minton AP (1993) Macromolecular crowding: biochemical, biophysical and physiological consequences. Annu Rev Biophys Biomol Struct 22:27–65

Zimmerman SB, Trach SO (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of Escherichia coli. J Mol Biol 222:599–620

Figure captions

Figure 1. Effect of different crowding agents on the bimolecular rate constant (k 2) for the interaction of Cyt c 6 with PSI in Arthrospira (left), and dependence upon relative viscosity of the relative k 2 for the same system (right). The standard reaction mixture contained, in a final volume of 0.25 ml, 20 mM Tricine–KOH, pH 7.5, 10 mM MgCl2, 0.03% β-dodecyl maltoside, an amount of PSI particles equivalent to 0.35 mg of chlorophyll ml−1, 0.1 mM methyl viologen, 2 mM sodium ascorbate, and Cyt c 6 60 μM. The concentration of the crowding agent was adjusted at the indicated values by adding small amounts of a 57% (w/v) stock solution. Relative k 2 (k 2/k 2 0) and viscosity (n/n 0) values were obtained by dividing absolute data by the values obtained in the absence of any added sucrose or crowding reagents

Figure 2. Dependence upon relative viscosity (n/n 0) of the relative bimolecular rate constant (k 2/k 2 0) for Synechocystis PSI reduction by Pc (left) and Cyt c 6 (right) in the presence of sucrose or different crowding agents. Other experimental conditions were as described in Fig. 1

Figure 3. Dependence upon relative viscosity (n/n 0) of the relative bimolecular rate constant (k 2/k 2 0) for Nostoc PSI reduction by Pc (left) and Cyt c 6 (right) in the presence of sucrose or different crowding agents. Other experimental conditions were as described in Fig. 1

Figure 4. Dependence upon relative viscosity (n/n 0) of the relative bimolecular rate constant (k 2/k 2 0) for Monoraphidium PSI reduction by Pc (left) and Cyt c 6 (right) in the presence of sucrose or different crowding agents. Other experimental conditions were as described in Fig. 1, except that the amount of PSI-enriched particles was 0.75 mg of chlorophyll ml−1

Figure 5. Relative bimolecular rate constant (k 2/k 2 0) values for PSI reduction by Pc and Cyt c 6 from different organisms in the presence of sucrose, BSA, ficoll or dextran at the higher experimental concentrations showed in Figs. 1, 2, 3, 4. Art Arthrospira, Syn Synechocystis, Nos Nostoc, Mon Monoraphidium

Figure 1

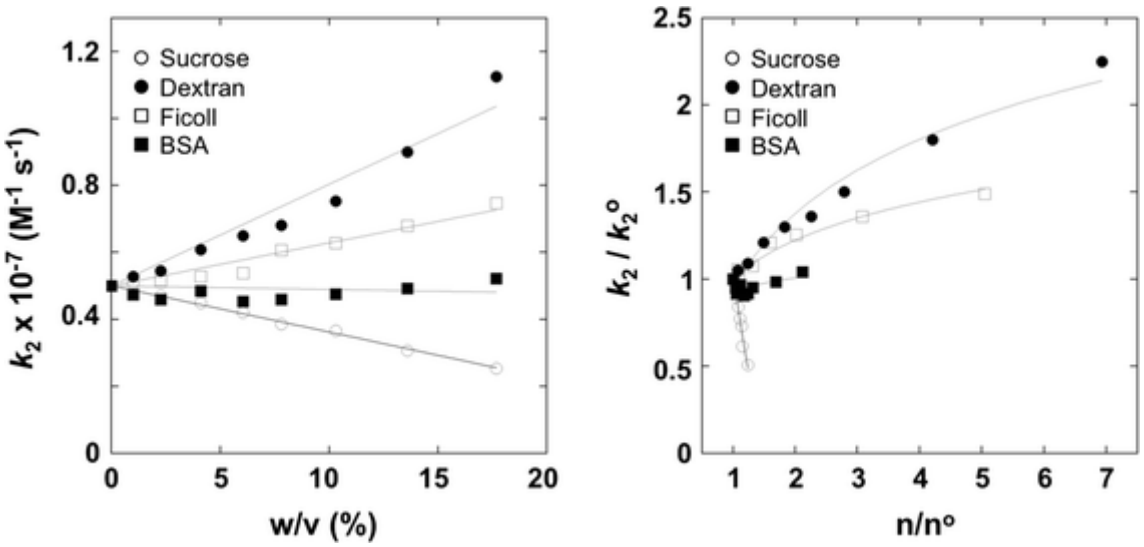


Figure 2

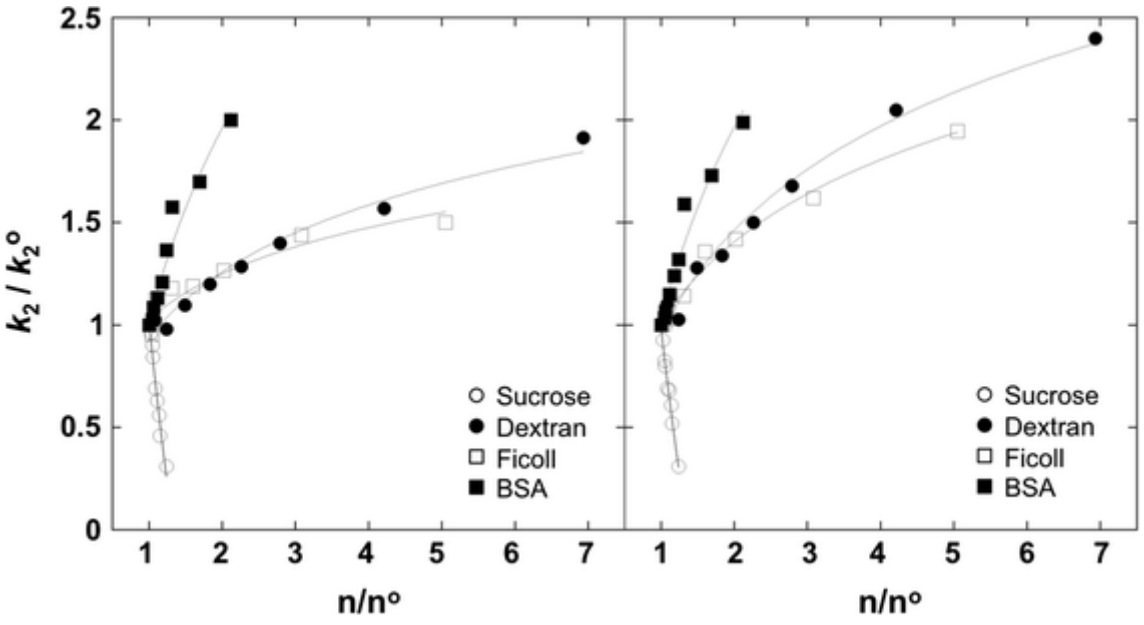

2.5
2
1.5
1
0.5
0
$k_2 / k_2°$
1
2
3
4
5
6
7
n/n°
Sucrose
Dextran
Ficoll
BSA
Sucrose
Dextran
Ficoll
BSA

Figure 3

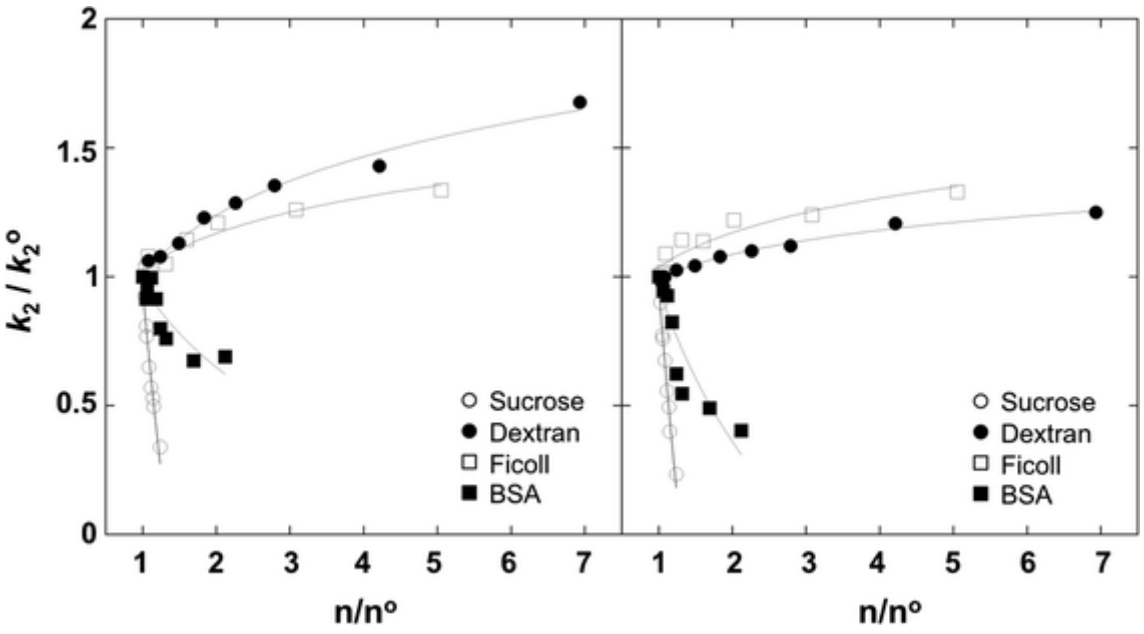

2
1.5
1
0.5
0
$k_2 / k_2°$
1
2
3
4
5
6
7
n/n°
Sucrose
Dextran
Ficoll
BSA
Sucrose
Dextran
Ficoll
BSA

Figure 4

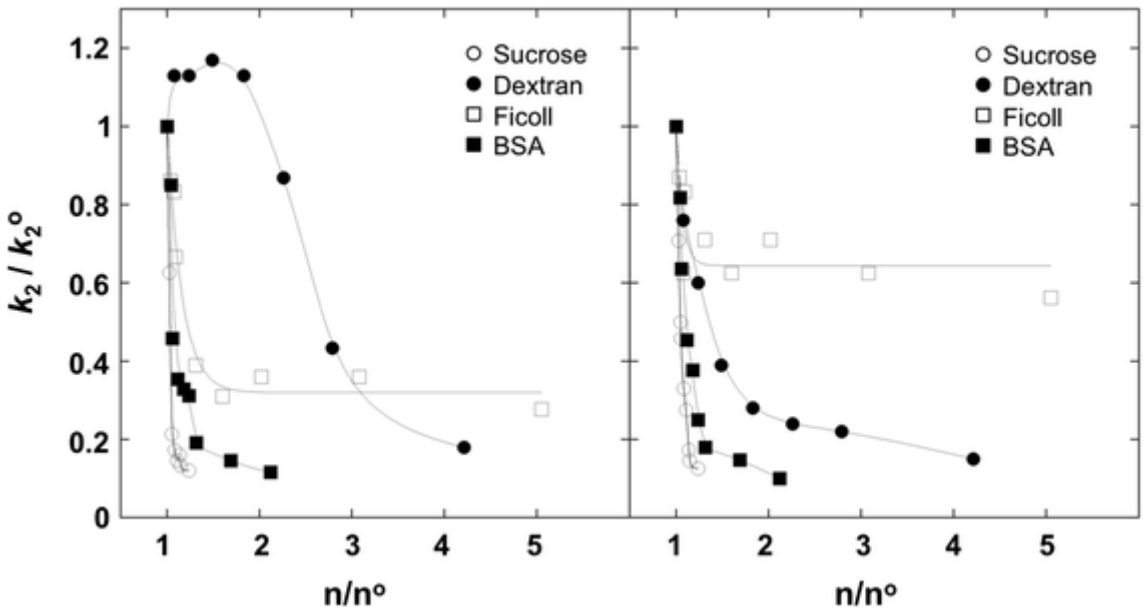
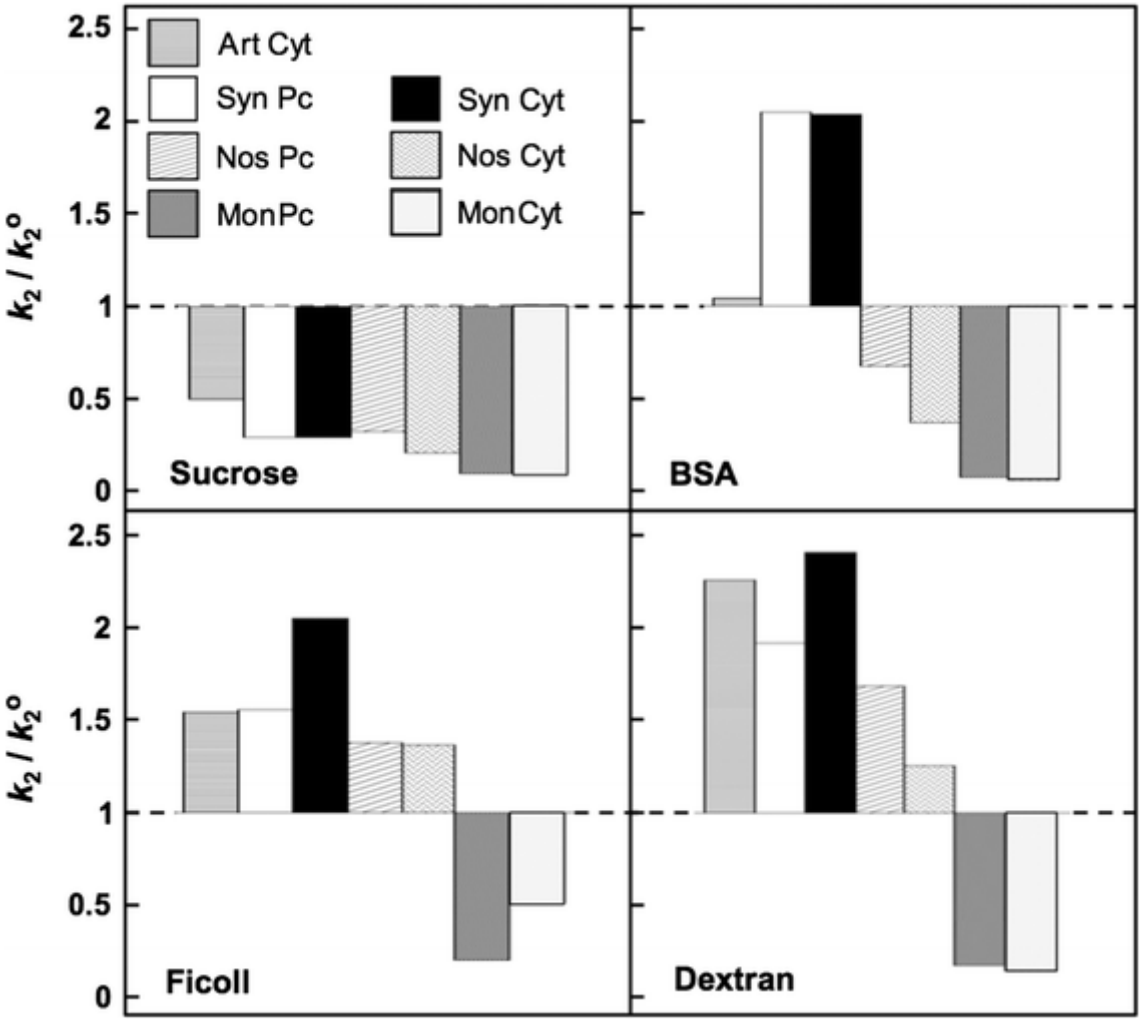

Sucrose
Dextran
Ficoll
BSA
k_2 / k_2^o
n/n°
Sucrose
Dextran
Ficoll
BSA
n/n°

Figure 5


Art Cyt
Syn Pc
Nos Pc
MonPc
Syn Cyt
Nos Cyt
MonCyt
k_2 / k_2^o
2.5
2
1.5
1
0.5
0
Sucrose
BSA
Ficoll
Dextran