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ARTICLE

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Alignment of biological microparticles by a polarized laser beam

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Abstract The optical alignment of biological samples is of great relevance to microspectrometry and to the micromanipulation of single particles. Recently, Bayoudh et al. (*J. Mod. Opt.* 50:1581–1590, 2003) have shown that isolated, disk-shaped chloroplasts can be aligned in a controlled manner using an in-plane-polarized Gaussian beam trap, and suggested that this is due to their nonspherical shape. Here we demonstrate that the orientation of various micrometer-sized isolated biological particles, trapped by optical tweezers, can be altered in a controlled way by changing the plane of linear polarization of the tweezers. In addition to chloroplasts, we show that subchloroplast particles of small size and irregular overall shape, aggregated photosynthetic light-harvesting protein complexes as well as chromosomes can be oriented with the linearly polarized beam of the tweezers. By using a laser scanning confocal microscope equipped with a differential polarization attachment, we also measured the birefringence of magnetically oriented granal chloroplasts, and found that they exhibit strong birefringence with large local variations, which appears to originate from stacked membranes. The size and sign of the birefringence are such that the resulting anisotropic interaction with the linearly polarized laser beam significantly contributes to the torque orienting the chloroplasts.

Keywords Birefringence · Chloroplast · Chromosome · Laser tweezers · Optical alignment

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Introduction

In the late 1980s, it became possible to trap micrometer-sized particles at the focal point of a laser beam strongly focused through the microscope objective and to move them around in a controlled way by moving the focal point of the beam (Ashkin et al. 1986). Since then, these optical tweezers have become a powerful tool in physics, chemistry and biology (for reviews, see Ashkin 1997). With independently controllable laser beams, it is possible to trap and displace several particles at the same time (Visscher et al. 1993). Alternatively, two independent laser beams can be used to impose a given orientation to a particle by grabbing it at different positions with each beam and then moving their focal points with respect to each other. This method was used to vary the orientation of a chloroplast in a controlled way (Bayoudh et al. 2001) in order to investigate its shape, its consistency and its adhesion to the cytoskeleton.

The possibility of controlling the orientation of specially prepared, birefringent calcite particles using the polarized light of only one laser beam was discovered some years ago (Friese et al. 1998). This makes it possible in principle to manufacture micromotors for micromechanical systems.

Whereas linearly polarized light can be used to fix the orientation of birefringent particles, circularly polarized light can make them rotate in a continuous fashion, provided that the birefringence is large enough (Friese et al. 1998; Higurashi et al. 1999). Elliptically polarized light, which can be regarded as a combination of linear and circular polarization, may cause a particle either to rotate continuously or to adopt a preferred orientation, depending on the degree of ellipticity of the beam. Optical reorientation by linearly polarized light also occurs in nematic liquid crystals (Jánossy 1999), in which $n_e > n_o$, and thus the optical field tends to orient the director parallel to its electric vector (n_e and n_o denote the extraordinary and ordinary refractive indices, respectively).

It was recently found that helical rotors, manufactured from nonbirefringent materials, may also be rotated by optical tweezers, in consequence of the torque generated by the change in momentum of the light deflected by the helical structure (Galajda and Ormos 2001). In the same work, the first light-driven micromachine was demonstrated showing that two engaged cogwheels could be driven by such a light-driven rotor. These experiments demonstrated the possibility of rotating carefully designed microscopic particles, granted that they reflect or refract light asymmetrically. Further, micrometer-sized isotropic objects with flat shapes can also be oriented with a linearly polarized beam of laser tweezers (Galajda and Ormos 2003). In this case, the torque is produced by the anisotropic scattering of linearly polarized light by the trapped object; as described by the Fresnel formulas. By calculating the scattering fields, based on a theory for full electromagnetic wave scattering, it has also been shown that disk-shaped objects, such as isolated chloroplasts, can give rise to scattering that aligns them with their semimajor axis parallel with the polarization plane (Bayoudh et al. 2003).

Taken together, the data just described suggest that, owing to their asymmetric shape and/or their birefringence, biological entities may align in accordance with a preferential axis at the focus of optical tweezers. There are numerous potential applications for this phenomenon, including (1) the determination of the torsional elasticity of single particles (cell organelles, DNA condensates, macromolecular assemblies, etc.), (2) the measurement of microscopic viscosity, (3) the characterization of the orientation-dependent interaction between particles, which would be invaluable for the study of surface biochemistry, etc., and (4) the spectroscopic study of single particles with well-defined orientations in order to obtain information about their anisotropic internal organization.

We show here that it is possible to orient isolated biological particles of different shapes and sizes. We provide four examples: (1) isolated granal chloroplasts, (2) small fragments of irregular overall shapes prepared from granal thylakoid membranes, (3) isolated lamellar aggregates of the main chlorophyll a/b light harvesting complex of photosystem II (LHCII), and (4) isolated hamster chromosomes. In order to investigate the origin of the torque, i.e., to better understand the orienting mechanism, we have measured the degree and spatial distribution of the birefringence of single, magnetically oriented chloroplasts. This was performed in a laser scanning microscope in which the polarization of the beam was rapidly modulated with a photoelastic modulator. We show that chloroplasts exhibit strong birefringence, with high local values, most likely originating from grana, the stacked regions of the thylakoid membranes. Our analysis shows that although the asymmetrical shape might be involved in the alignment of chloroplasts, the birefringence, in the presence of linearly polarized laser tweezers, is large enough to lead to an efficient alignment. We also point out that the direction of alignment is the same as that expected for nonbirefringent disks, i.e., the two mechanisms of orientation facilitate each other in a linearly polarized beam. However, large inhomogeneities and density variations, due to the presence of the stacked and unstacked membrane regions, and anomalous differential polarization scattering features, arising from the long-range order of the protein complexes, might hamper the expected rotation of this complex multilamellar membrane system in a circularly polarized beam.

Materials and methods

Chloroplasts were isolated from pea (*Pisum sativum*) leaves grown in a greenhouse by a method described earlier (Garab et al. 1988a), and were suspended in a reaction medium containing 30 mM Tricine (pH 7.6), 5 mM $MgCl_2$ and 300 mM sorbitol. Subchloroplast particles, fragments containing a few grana, were obtained by sonication and differential centrifugation (Jacobi 1971); they were resuspended in the same reaction medium. Lamellar aggregates of LHCII were obtained as described earlier (Simidjiev et al. 1997) and suspended in 10 mM Tricine buffer (pH 7.6). Intact thylakoid membranes were oriented in a homogenous magnetic field of 1.5 T, and trapped in the oriented state by inclusion in a polyacrylamide gel (Finzi et al. 1989).

Mammalian metaphase chromosomes were isolated from cultured Chinese hamster-mouse hybrid cell line H1D3 (Kereso et al. 1996) by a standard method (Hadlaczky et al. 1982). Cells were synchronized for mitosis with 0.1% colchicine and were burst in a glycine–hexylene glycol buffer supplemented with 0.1% Triton X-100 (GHT buffer). Chromosomes were purified by differential centrifugation and were resuspended in GHT buffer.

Transmission and confocal fluorescence images of magnetically aligned and gel-trapped chloroplasts were recorded with a Zeiss LSM 410 laser scanning microscope with an excitation at 488 nm. For birefringence imaging, a home-built device on the Zeiss LSM 410, similar to that described by Gupta et al. (1994), was

used. It contained a high-frequency polarization module (Hinds International, PEM) in the polarization state generator unit and a digital phase-sensitive detector (KFKI, Budapest) in the polarization state analyzer unit. The magnitude of the birefringence (phase shift) of chloroplasts was determined taking advantage of the fact that they could be gel-trapped with their long axis oriented along the polarization of the laser beam. Birefringence images were obtained from two consecutive scans with the beam of the 543-nm HeNe laser of the Zeiss LSM 410. The differential polarization unit was calibrated with the aid of calibrated, passive phase retarder plates.

A Zeiss Axiovert 135 microscope was interfaced with a Cell Robotics laser tweezer 980–1,000 unit. In this unit, tweezers are formed by an IR diode laser (SDL 5762 A6 of 994-nm wavelength) focused by an objective lens (Zeiss Plan-Apochromat oil immersion 100×1.4). The maximal trapping intensity is about 20 mW. The beam diameter was approximately 0.5 μm.

Theory

In this section, we consider the role of birefringence. The role of asymmetrical shape has been treated extensively by Galajda and Ormos (2003) and Bayoudh et al. (2003).

The orienting effect of linearly polarized light on birefringent particles is easily understood. An incident beam of linear polarization becomes elliptically polarized and gains angular momentum after impinging on a birefringent particle whose optical axis is not parallel to that of the polarization of the light. The resulting change in angular momentum generates a reaction torque τ on a particle with thickness d (Higurashi et al. 1999):

$$\tau_{\text{lin}} = -\frac{P}{\omega}\sin\left(2\pi\frac{R}{\lambda}\right)\sin(2\theta), \tag{1}$$

where P is the power of the laser beam at the sample, ω and λ are the frequency and wavelength, respectively, of the light, θ is the angle between the polarization direction and the slow birefringence axis of the particle (the axis along which the index of refraction is largest), and R is the retardation of the particle, defined as $R = \Delta n \times d$, where Δn is its birefringence, i.e., the difference in refractive index for light polarized along two perpendicular axes of the particle, and d is the thickness of the particle along the optical path of the laser beam. Particles with relatively small birefringence, i.e., $R < \lambda$, tend to align with their fast axis (lowest value for the refractive index) along the polarization direction. When θ equals zero, the particles are aligned and no torque remains. The torque should be large enough to overcome Brownian motion in order to obtain substantial orientation; in practice, the orientation fluctuates around an equilibrium value (Higurashi et al. 1999). The more the orientation deviates from the equilibrium, the larger the ''restoring torque'' becomes.

By a similar mechanism (Higurashi et al. 1999), circularly polarized light exerts a torque on a birefringent particle, its size being given by

$$\tau_{\text{circ}} = \frac{2P}{\omega}\sin^2\left(\pi\frac{R}{\lambda}\right). \tag{2}$$

As there is no angular dependence, the particle rotates continuously, provided that the birefringence and/or the laser power are sufficiently high relative to the forces of Brownian motion.

Results and discussion

When trapped by the laser beam of the optical tweezers, the disk-shaped chloroplasts with the approximate dimensions of an idealized rotational ellipsoid of 8 μm×8 μm×4 μm, become oriented with their short axis parallel to the focal plane (edge-aligned), and could be realigned with the linearly polarized laser beam (Fig. 1).

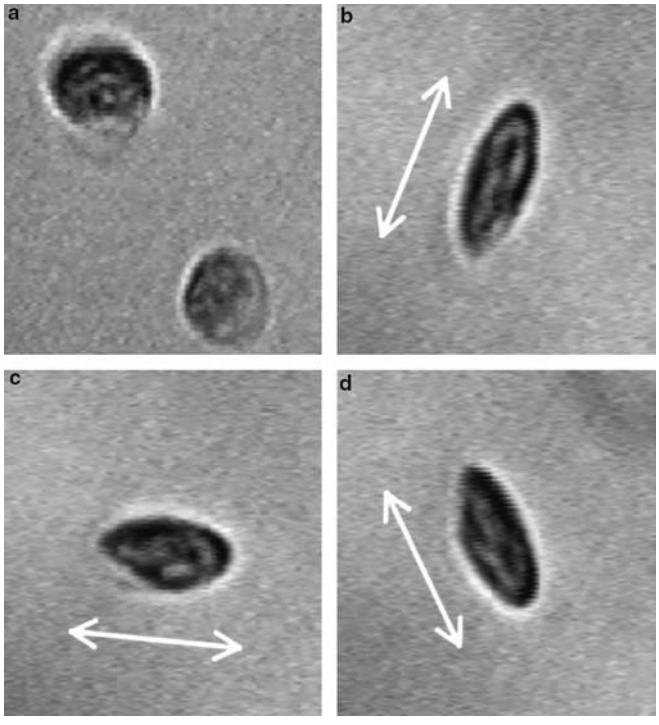

Fig. 1 Microscopic images of isolated chloroplasts before their trapping by the laser tweezers (**a**) and their alignment (**b**–**d**) with linearly polarized light of different polarization planes (indicated by *arrows*). The chloroplasts were continuously realigned parallel to the plane of polarization of the trapping laser beam, with an incident power of 10 mW. A $\lambda/2$ plate was rotated slowly (about 1 rps), which forced the chloroplast to follow this rotation without noticeable slip. (In a nonpolarized laser beam, chloroplasts are trapped in a way that the laser beam aligns them with one of their long axes parallel to the direction of propagation of the light (edge-aligned position), but the second long axis remains free. This latter axis can also be oriented with an external alternating current electric field of about 10–20 V cm^{-1})

The long axis of the thylakoids aligns along the polarization direction. In this particular case, the laser power at the focal point was approximately 10 mW. Similar effects were observed (not shown) for lamellar aggregates of LHCII with dimensions similar to those of chloroplasts.

Figure 2 depicts hamster chromosomes (approximate dimensions 10-μm long and 1–2-μm wide) in the absence and presence of optical tweezers. When a chromosome is trapped, it flips in such a way that the long arms of the two sister chromatids are no longer visible, they are oriented parallel to the direction of propagation of the light. Similar observations have been made for nonbiological elongated micro-objects (Higurashi et al. 1999). When the polarization direction is changed, the chromosomes rotate accordingly (Fig. 2b–d).

In order to investigate whether the orientation effect contains a significant contribution from the birefringent properties of the particles, we measured the birefringence of individual chloroplasts whose orientations were fixed in the presence of an external magnetic field of 1.5 T (see later). Evidently, the results can be applied for subchloroplast particles of 1–2-μm in size. The birefringence in granal chloroplasts and their fragments originates from the same ultrastructure: the granum–stroma assembly. Further, it can be assumed that isolated lamellar aggregates of LHCII exhibit similar birefringence as whole thylakoid membranes. LHCII is a pigment–protein complex that binds approximately 50% of the pigments embedded in the thylakoid membranes. Its spectroscopic properties in relation to its highly organized molecular architecture (Kühlbrandt 1994) have recently been reviewed (van Amerongen and van Grondelle 2001). The anisotropic optical properties, the diamagnetic anisotropy and the electric orientability, i.e., the polarizability in quasistatic alternating current fields, of these liquid-crystalline-like lamellar aggregates are very similar to those of the isolated thylakoid membranes (Garab 1987). Concerning the role of birefringence in the orientation of chromosomes in the linearly polarized beam of laser tweezers, no relevant birefringence measurement could be done. It was not possible to orient the chromosomes in a magnetic field of 1–2 T, and we could not measure their birefringence in the directions defined by the optical trapping. Chromosomes are known to exhibit strong birefringence (Stockert et al. 1990). However, the currently available data do not permit us to evaluate the contribution of chromosomal birefringence in the orientation effect observed in the linearly polarized laser tweezers. The overall elliptical shape of chromosomes in the optical trap may also play a role in their orientation in a way hard to estimate.

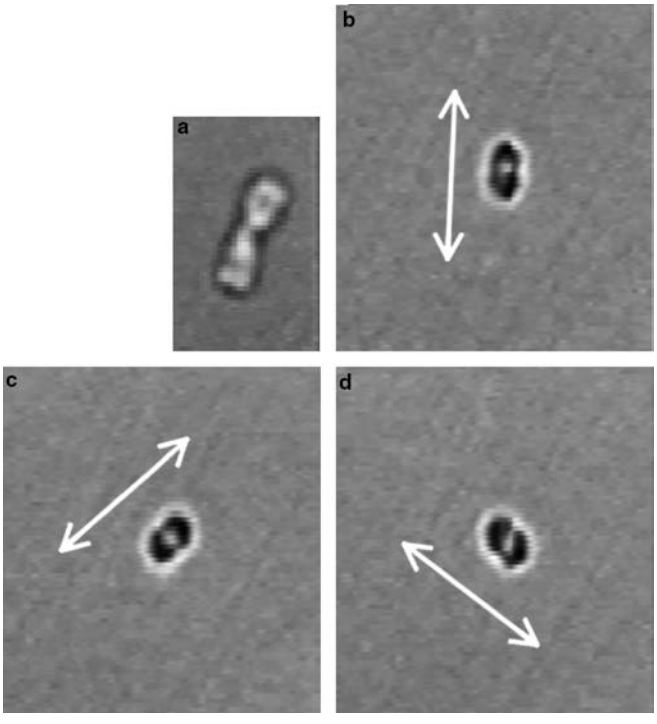

Fig. 2 Isolated hamster chromosomes in the absence (**a**) and presence (**b–d**) of a trapping polarized laser beam. A comparison of **a** and **b** reveals that the laser beam aligns the chromosomes with their "legs" (long axis) parallel to the direction of propagation of the light, while successive frames in **b–d**, as in Fig. 1 for chloroplasts, show their alignment in the polarized beam upon changing the plane of polarization

Figure 3a–c presents transmission, fluorescence and birefringence images of an edge-aligned granal chloroplast. The light gray regions indicate regions in the chloroplasts where the refractive index for light polarized along the long axis of the chloroplast is smaller than that along the perpendicular direction. The observed values of the phase difference $\Delta\,\phi$ ($\Delta\varphi = 2\pi R/\lambda$) varied between -0.1 and 0.4 and the average retardation was 0.12. This average value varies slightly from chloroplast to chloroplast (typically between 0.10 and 0.20).

It is useful to compare these values with those measured in a conventional microscope on agranal and granal chloroplasts. In *Mougeotia* chloroplasts a phase retardation of about 50 nm (i.e., $\Delta\phi{\sim}0.6$) was measured between 500 and 550 nm (Goedheer 1955). The green alga *Mougeotia* contains big (40–120-μm-long, 28-μm-wide and 4–6-μm-thick), homogenously organized, plate-shaped (ribbonlike), lamellate chloroplasts. These chloroplasts exhibited strong birefringence, which could conveniently be measured in a microscope equipped with a Berek compensator (Goedheer 1955). In contrast, granal chloroplasts of *Funaria* displayed much weaker birefringence and linear dichroism. Clearly, this difference is due to the more inhomogenous organization of the thylakoid membranes in granal chloroplasts. Indeed, as revealed by our measurements, the local values in granal chloroplasts alternate between a strong positive and a somewhat weaker negative value (Fig. 3d). This nearly periodic pattern evidently arises from the internal, laterally heterogenous structure of the thylakoid membrane system. Granal chloroplasts contain a more or less regular pattern of the granum–stroma thylakoid

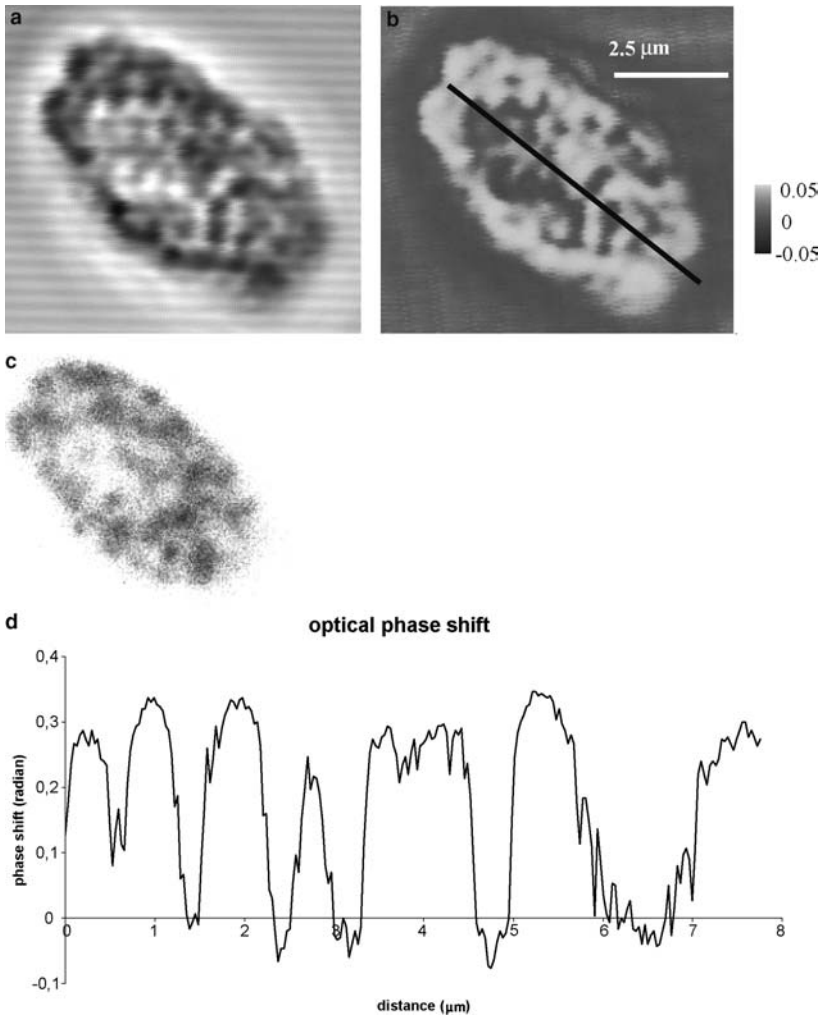

Fig. 3 Transmission (**a**), linear birefringence (**b**) and fluorescence intensity (**c**) images of magnetically oriented (edge-aligned), gel-trapped isolated thylakoid membranes. In **b**, the light regions are those parts of the chloroplasts for which the refractive index for light polarized along the long chloroplast axis is smaller than that for the perpendicular direction. **d** Variation of the phase shift along the line shown in **c**

assemblies (Mustárdy and Garab 2003), stacked and unstacked membrane regions.

Earlier Bayoudh et al. (2003) showed that it is possible to controllably align disk-shaped, spinach chloroplasts in a plane-polarized Gaussian beam trap. This was interpreted as a result of an optical torque originating predominantly from the nonspherical shape of these cell organelles. These authors excluded the possibility of a contribution from birefringence for two reasons: (1) they were unable to observe any birefringence by viewing chloroplasts between crossed polarizers, and (2) it was argued that "if the chloroplasts were sufficiently birefringent so as to align with the plane polarized beam, the circularly polarized beam should have caused them to rotate at a constant rate"; no such rotation was observed. It is clear, however, that edge-aligned chloroplasts exhibit strong birefringence (Fig. 3b), and thus its contribution to the orienting torque, or possibly acting against the torque arising from the shape, might be significant. In the following paragraphs, first we point out that birefringence might not be detectable with chloroplasts that are not properly aligned, then discuss the role of birefringence in the generation of torque in the linearly polarized beam of the tweezers, and make considerations on the lack of rotation in circularly polarized beam. Finally, we argue that the complex, laterally heterogenous membrane organization of granal thylakoids must be taken into account in order to understand better the behavior of chloroplasts in polarized beams of optical traps.

Regarding the absence of birefringence between crossed polarizers (Bayoudh et al. 2003) we point out that birefringence in face-aligned chloroplasts is very weak (data not shown). With this alignment linear dichroism is zero (Breton and Vermeglio 1982). This is in sharp contrast with the strong birefringence and linear dichroism in edge-aligned chloroplasts. This observation might explain the lack of birefringence noticed when the chloroplasts are not previously oriented (Bayoudh et al. 2003). In this case, chloroplasts tend to sediment on their face (Breton and Vermeglio 1982) (see also Fig. 1a), not on their edge. It is to be noted that upon the optical trapping chloroplasts assume an edge-aligned position (Fig. 1b).

The contribution of birefringence to the torque in a linearly polarized beam can be estimated using Eq. 1

and the values of the birefringence measured on spinach chloroplasts at 543 nm. This wavelength is outside the principal absorbance bands, on the long-wavelength side of the Soret region. The birefringence at this wavelength is taken as an approximation for the birefringence at 994 nm, i.e., outside the red absorbance bands of chlorophyll a . In the green and far-red regions, the birefringence is not very strongly dependent on wavelength and is relatively constant in magnitude: in *Mougeotia*, the phase retardation, $\Delta\phi$, between 500 and 550 nm is nearly constant, about 50 nm, and the measured and calculated values decay slowly in the far red, between 700 and 750 nm from around 110 nm to about 80–90 nm (Goedheer 1955).

The torque exerted by a laser beam of 10 mW on a particle with $\Delta\phi = 0.12$ is calculated to be $6.3\times10^{-19}\sin2\theta$ N m. Although chloroplasts could be trapped using a laser power of 1–2 mW, they could not be oriented with powers lower than about 3–4 mW, giving rise to torques of $1.9–2.5\times10^{-19}$ N m. It is interesting to note that these values are comparable with the torque value (2.2×10^{-19} N m) estimated by Bayoudh et al. (2003) at a laser power of 30 mW. There are only minor differences between the two preparations: our medium contains a higher concentration of Mg^{2+}, which plays a key role in membrane stacking (Barber 1982). These are unlikely to explain the difference in the magnitude of the torque. It is more likely that the difference is due to the optics. In our optical system, the torque imparted by a linearly polarized beam of 4 mW to a flat particle with a diameter similar to that of a chloroplast was determined to be about 2×10^{-20} N m (Galajda and Ormos 2003). We cannot rule out that this value of the orienting torque due to the shape is underestimated. Nonetheless, our data clearly show that the torque due to birefringence gives a significant contribution to the orienting torque acting on chloroplasts.

It has been demonstrated (Higurashi et al. 1999) that a torque as low as 1.1×10^{-19} N m, exerted by a circularly polarized beam, still leads to continuous rotation of a strongly birefringent particle with similar dimensions. In other words, the Brownian motion can be overcome by such a torque and it is possible to orient the chloroplast to within 5° of $\theta = 0°$ (Even if the torque is an order of magnitude lower, orientation can still be obtained for somewhat smaller ''smooth'' particles (Galajda and Ormos, unpublished results). It may therefore be concluded that—although the alignment due to the shape also contributes significantly—the average size of the birefringence is sufficient to explain the alignment of the chloroplasts. It is also important to point out that the sign of the birefringence ''predicts'' that the chloroplast will be aligned with its long axis and not its short axis along the direction of polarization. Further, the same orientation is induced by a low-frequency, 10 kHz, external electric field (not shown). This implies that the polarizability of the membranes does not vanish at the optical frequencies (Fredericq and Houssier 1973). We also observed that, similarly to what happens in liquid crystals (Csillag et al. 1982), the optical and quasistatic electric fields can be superimposed on each other. Thus the orientation of chloroplasts can also be varied by varying the amplitude of an orthogonally applied external electric field (data not shown). In many samples the torque of orientation by linearly polarized light might be limited by the laser power because the orienting torque is usually substantially smaller than the trapping torque (see earlier). Hence, when trapped in laser tweezers, single particles with sufficiently high electric polarizabilities can also be oriented with external electric fields. Chloroplasts, for instance, can be readily aligned with field strengths of about 10 V cm^{-1} (Gagliano et al. 1977). Submicroscopic particles (such as isolated photosystems, or viruses) may require a field 1–2 orders of magnitude higher (van Haeringen et al. 1994; Garab 1996).

It is of interest to calculate the expected torque that is exerted by circularly polarized light. We unsuccessfully attempted to rotate chloroplasts by using circularly polarized light. This observation is in agreement with the findings of Bayoudh et al. (2003) using a 1,064-nm laser beam. This might indeed argue against the idea that the orientation mechanism is due to birefringence, or at least to birefringence alone. Using the same input parameters as for the torque calculations discussed earlier (10 mW, $\Delta\phi = 0.12$), we obtained a torque of 3.8×10^{-20} N m for circularly polarized light (7.6×10^{-20} N m at 20 mW, i.e., at maximum laser power). This is about 16 times weaker than the torque due birefringence in a linearly polarized laser beam of the same intensity (note the different dependencies of the torque in the two cases, in Eqs. 1, 2). It is also considerably smaller than the threshold value, $1.9–2.5\times10^{-19}$ N m, estimated for the torque arising from birefringence at the minimum laser power required to orient the chloroplasts (see earlier). If the contribution from the flat shape in the orientation of chloroplasts with a linearly polarized laser beam is commensurate with the torque arising from the birefringence, free rotation would be expected at even higher laser powers. (This is because with circularly polarized light there is no contribution from the shape factor (see ''Theory'')). In the geometry adopted by Bayoudh et al. (2003), assuming a similar contribution from birefringence, the expected mean rotation speed would probably be too slow for a free rotation. At 30 mW of the linearly polarized beam, the speed was already as low as 0.73 rad s^{-1}; hence, the effect of a more than 10 times weaker torque with a circularly polarized beam could vanish. It can thus be concluded that the observations are in good agreement with the expectations, i.e., with the lack of free rotation in the presence of circularly polarized light. Nonetheless, other factors are also likely to be involved. The complex organization of the multilamellar membrane system should probably be taken into account for a more detailed interpretation and for a better understanding of the unusual properties of chloroplasts in polarized light. This also makes them differ from simpler, nonbiological samples.

It should be realized that it is not entirely appropriate to use the average value of the birefringence of the whole chloroplast to estimate the exerted torque because the laser tweezers do not grab the entire chloroplast. This is partly due to the small beam diameter compared with the size of chloroplasts. Furthermore, it turns out that the tweezers can trap the chloroplasts in several positions. This becomes evident after the chloroplasts are released, the laser beam is moved and the chloroplasts are grabbed again in a different position. However, for all grabbing positions, reorientation could be achieved by rotating the polarization plane of the beam, and in all cases the long axis did align along the polarization direction. Figure 3d illustrates how the observed magnitude of birefringence changes when the laser is scanned along the long axis of the chloroplast (along the line in Fig. 3c). It is evident that significant variations occur and the actual local birefringence may be 3 times as large as the average value. When a chloroplast is grabbed by the laser tweezers at such a highly birefringent part, the actual torque will also be 3 times as large. This would also facilitate the rotation in the presence of circularly polarized light. However, it is unclear, how the large inhomogeneity, on the scale that is commensurate with the wavelength of the laser beam, can be taken into consideration. The granum stacks with a typical diameter of about 500 nm are wound around in a helical fashion by 2–300-nm-wide strips of stroma thylakoids (Mustárdy and Garab 2003). Because of the larger distances between the unstacked stroma thylakoids, there is a large density variation at the granum–stroma junctions.

The observation that the trapping (and the orientation) is (are) driven by the grana, i.e., the stacked regions inside the chloroplasts' thylakoid membrane system, can most clearly be demonstrated by showing the alignment of subchloroplast particles in the linearly polarized beam of the laser tweezers. These fragments, despite their quite irregular overall shape (Fig. 4a, b), could readily be aligned as long as they had at least one or two edge-aligned grana in the tweezers (Fig. 4c, d). Because of their irregular overall shapes and small sizes, compared with those of chloroplasts, it seems unlikely that these particles can be aligned with a mechanism and efficiency valid for large isotropic disk-shaped objects. However, birefringence in these particles, which constitute the chloroplast, can be large. Strong birefringence and dichroism of all photosynthetic membranes originate mainly from the intrinsic, inherently anisotropic molecular architecture of the pigment–protein complexes and supercomplexes, and their ordered arrays in the membrane (Breton and Vermeglio 1982; Garab 1996; Boekema et al. 2000). It has also been well established that in photosynthetic membranes the contribution of form or shape birefringence is small (Sauer and Calvin 1962). The magnitude of textural dichroism, compared with the anisotropic molecular organization, is also negligible in photosynthetic membranes (Breton and Vermeglio 1982).

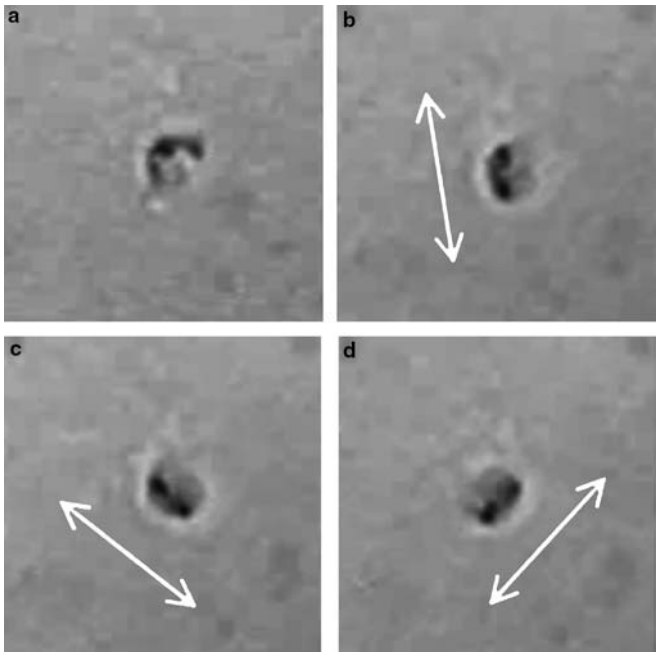

Fig. 4 Microscopic images of subchloroplast particles, obtained by sonicating isolated chloroplasts, before their trapping by the laser tweezers (**a**) and successive frames showing their alignment (**b–d**) with linearly polarized light of different polarization planes (indicated by *arrows*). The particles were continuously realigned parallel to the plane of polarization of the trapping laser beam, with an incident power of 20 mW. A $\lambda/2$ plate was rotated rapidly (about 10 rps), which forced the particle to follow this rotation without noticeable slip

We observed that we were able not only to align these subchloroplast particles of 1–2 μm in diameter but they could be rotated at much higher rates, up to 10 Hz, than chloroplasts. This observation can most likely be accounted for by the substantially reduced drag torque, which is proportional to the third power of the diameter of the particles (Bayoudh et al. 2003). This easy orientability of the particles implied that they might be able to rotate freely in the presence of a circularly polarized beam, which, however, was not observed. These data also show that additional factors must be taken into consideration to understand the interaction of thylakoid membranes with polarized light:

1. The internal structure of thylakoid membranes is known to play a significant role in the light scattering of chloroplasts. A systematic investigation of the anisotropic scattering of linearly polarized light by magnetically oriented *Chlorella* cells led to the conclusion that the anisotropy and wavelength dependence of this scattering could be accounted for by form factors in the Rayleigh–Gans approximation, and the orientation of the chlorophyll molecules in the membrane (Swenberg and Geacintov 1979).
2. The angular dependence of the nonpolarized scattering of a random suspension of chloroplasts appears to originate from particles approximately 500 nm in diameter, i.e., probably from grana, rather than from the whole chloroplast (Garab et al. 1988b).

3. A noteworthy point that also may also influence the ability of granal chloroplasts to rotate in circularly polarized light is that they differentially scatter left- and right-circularly-polarized light with ''anomalous'' intensity and angle dependence, exhibiting intense lobes of alternating signs. This differential scattering originates from the long-range chiral order of the chromophores (Garab et al. 1988b). As pointed out in a theory for the so-called psi-type aggregates, such as chromosomes and other DNA condensates (Keller and Bustamante 1986; Tinoco et al. 1987), valid also for LHCII-containing macrodomains (Garab 1996), the interaction of chromophores with light involves long-range coupling interactions, corresponding to internal scattering and intermediate term interaction. These are known to give rise to ''anomalous'' spectroscopic and very strong local, microscopic circular dichroism features, as indeed observed in chloroplasts (Finzi et al. 1989).

It is worth pointing out that the same orientational mechanism(s) may play a role in the orientation of much smaller particles, e.g., bacteria, viruses, isolated biological membranes and perhaps even individual biological macromolecules. For instance, there is no reason to believe that the lower retardation due to the smaller size of some particles or of macromolecules may not be overcome by increasing the laser power. It might be important that the drag torque for these smaller objects can be small. Furthermore, it is equally possible to superimpose a quasistatic electric field in order to reduce the light intensity. Furthermore, the birefringence of thylakoid membranes, which contain many partly randomly oriented molecules, might be much lower than that of individual macromolecules such as DNA or proteins with high α-helical contents which present regions of long-range order. Such birefringence-based orientational possibilities, together with the purely shape related orientation mechanisms may open up a new exciting field of research on anisotropic and asymmetrically shaped biological structures.

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