Reversible changes in macroorganization of the light-harvesting chlorophyll a/b Pigment-protein complex detected by circular dichroism

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Reversible Changes in Macroorganization of the Light-Harvesting Chlorophyll *a*/*b* Pigment–Protein Complex Detected by Circular Dichroism†

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Received July 9, 1987; Revised Manuscript Received December 3, 1987

ABSTRACT: Light-induced changes in circular dichroism (CD) were studied in thylakoids isolated from spinach. The following features of CD responses occurring in the time range of 10 s to 1–3 min were noted: (i) The kinetics and relative amplitudes of the responses are similar over broad spectral ranges surrounding the major CD bands, i.e., between 670 and 760 nm and between 480 and 550 nm. This applies not only to randomly oriented samples but also to magnetically aligned membranes having markedly different CD spectra in the dark. (ii) Photosystem I is much more effective than photosystem II and can drive a 40–80% decrease in CD signal relative to the dark control level. (iii) Photosystem I driven changes are fully inhibited by nigericin or NH_4Cl but are largely insensitive to gramicidin. CD changes driven by photosystem II, on the other hand, are sensitive to all of these reagents. (iv) The CD responses can be shown to originate in circular differential scattering rather than in circular differential absorbance. They can also be distinguished from light-induced, nonpolarized scattering changes. The data are qualitatively evaluated with respect to the theory of circular differential scattering of large helically organized macroaggregates, the size of which is commensurate with the wavelength of the measuring beam [Bustamante, C., Maestre, M. F., & Keller, D. (1985) *Biopolymers 24*, 1595–1612]. The observed decrease of the large CD signal is ascribed to a partial loss of macrohelicity in the light-harvesting chlorophyll *a*/*b* protein complex, in response to a proton gradient and/or surface electrical field generated most effectively by photosystem I.

Thylakoid membranes and their component complexes undergo conformational changes in response to illumination [see review by Barber (1982)]. Current understanding of these dynamic events is far from complete owing to a limited availability of techniques suited to the study of ultrastructural rearrangements within membranes.

In the preceding paper (Garab et al., 1988), we showed that the circular dichroism (CD)[1] of thylakoids is sensitive to the

† This work was carried out under the auspices of the U.S. Department of Energy, with funding from its Office of Basic Energy Sciences, Division of Biological Energy Research, and from its Office of Health and Environmental Research. Additional funding was received under Research Grant 211.81 from the North Atlantic Treaty Organization.
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[1] Abbreviations: CD, circular dichroism; CDA, circular dichroism of absorbance; CDS, circular differential scattering; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting chlorophyll *a*/*b* pigment–protein complex of photosystem II; PMS, *N*-methylphenazonium methosulfate; FCCP, carbonyl cyanide *p*-(trifluoromethyl)phenylhydrazone.

0006-2960/88/0427-2430$01.50/0

macroorganization of membrane complexes. A close association was found between the "giant" CD of thylakoids and circular differential scattering (CDS). CDS is thought to reflect the existence of large chiral domains in the light-harvesting chlorophyll *a*/*b* pigment–protein complex (LHC).

Here we investigate the dynamic properties of the LHC "lattice" as revealed by the reversible light-induced CD changes first observed by Gregory (1975) and investigated by Faludi-Daniel et al. (1984). These CD changes, observed in the red spectral region, amount to ~10% of the amplitude of the CD signal and were originally attributed to alteration in the interactions between Chl *a* molecules.

In this work, we show (i) that light-induced reversible CD changes have essentially identical kinetics and amplitude in all bands associated with CDS, i.e., between 480 and 560 nm as well as between 670 and 760 nm, (ii) that under optimal conditions, light induces a 40–80% decrease in the CD signal in the above spectral regions, (iii) that these changes can tentatively be assigned to reversible changes in the domain size of the LHC, with domain dimensions in the dark being comparable to the wavelength of the light where CDS is observed [cf. Bustamante et al. (1985)], and (iv) that the force driving these structural rearrangements is a proton gradient and/or surface electrical field generated most effectively by photosystem I.

Experimental Procedures

Spinach thylakoids were prepared as previously described (Garab et al., 1988). Essentially identical results were obtained with intact chloroplasts (not shown) and with mesophyll chloroplasts of maize (not shown) isolated as described by Crowther et al. (1983).

Light-induced CD changes were measured in an apparatus that was equipped with a side illumination attachment and an electronic circuit similar to that described by Olson et al. (1985). Interference from the actinic beam on the CD signal was prevented by the introduction of a mechanical beam chopper (Princeton Applied Research, Model 125) at the entrance slit of the monochromator (Sutherland et al., 1976) and a lock-in amplifier (Princeton Applied Research, Model 186). The actinic light provided by a 500-W projector lamp was filtered through 2 cm of water and (for CD responses under 600 nm) a Corning 2-64 red filter; the photomultiplier was protected by a Corning 4-96 blue-green filter. For measurements above 650 nm, the position of the two filters was interchanged. The intensity of the red or blue actinic light was 45 mW/cm^2. CD spectra could be obtained in the simultaneous presence of actinic light and a magnetic field of 1 T. Kinetics of the light-induced CD changes were recorded on a chart recorder. The CD changes described here could be observed between 4 and 60 μg of Chl/mL with no noticeable difference in characteristics.

Light-induced changes in the intensity of the transmitted light were recorded in the same apparatus by bypassing the 50-kHz demodulation circuit carrying the CD component and recording the anode current at a constant photomultiplier voltage.

Simultaneous O_2 evolution and CD measurements were made in an unstirred CD sample cell adapted to accommodate a modified Clark electrode, which was coupled to a low-drift amplifier. The assay medium was supplemented with 10% Ficoll. O_2 evolution and uptake rates were also measured conventionally with a Hansatech electrode.

Results and Discussion

Kinetics of the Light-Induced CD (CDS) Changes in Different Spectral Regions. Previous studies of chloroplasts (Garab et al., 1986, 1988) established that the principal CD bands, having peaks around 510 and 690 nm and tails extending toward longer wavelengths, are closely associated with CDS. Light-induced CD changes observed in the red (Gregory, 1975; Faludi-Daniel et al., 1984) could conceivably originate, therefore, in structural changes within macrohelical domains rather than in changes of the electronic interaction between Chl *a* molecules. To examine this possibility, we compared data from spectral regions close to, and remote from, the main CD bands and found little variation in the light-induced CD changes; this would be predicted for an origin in CDS. Figure 1 shows typical data for 485, 510, and 690 nm. The similarity in the kinetics is noteworthy since in these spectral regions, absorbance dipoles, whose interaction could theoretically be responsible for the corresponding CD bands, belong to the Chl *b* higher singlet excited state, the carotenoids, and the Chl *a* lowest singlet excited state, respectively. It is highly unlikely that these different states would respond identically to an effect of actinic illumination. It is more reasonable to assume that the light-induced CD changes are of CDS origin.

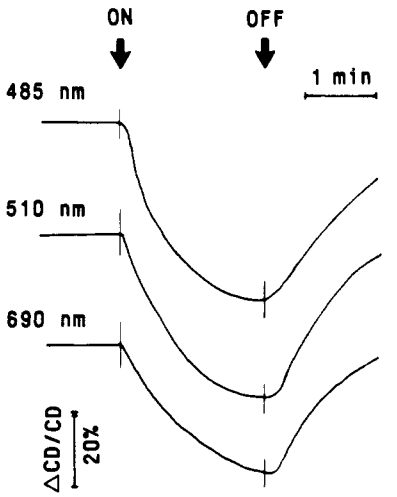

FIGURE 1: Time course of light-induced changes in the CD signal of randomly oriented spinach thylakoids at different wavelengths. Samples contained 120 μM PMS, 1 mM ascorbate, 20 μM DCMU, and 20 μg of chlorophyll/mL. Each trace is the average of three records taken after two conditioning cycles (2 min light/2 min dark). actinic light was turned on and off as shown by the arrows.

Confirmation of this came from a separate set of experiments which showed that the kinetics recorded in the long tails between 710 and 760 nm and between 530 and 560 nm were very similar to each other and to those recorded at the CD peaks (690 and 510 nm; data not presented).

Dark minus Light Difference CD Spectra in Randomly Oriented and Magnetically Aligned Thylakoids. Figure 2 confirms that light-induced CD changes are evident at wavelengths distant from the principal CD spectral bands. This is clearly seen in the dark minus light difference spectra between 530 and 590 nm as well as between 690 and 750 nm. The red maximum of the difference CD spectrum does not coincide with the maximum of the CD spectrum in the dark. Phenomenologically, this "shift" owes its origin to a change in sign of the CD signal around 680 nm during the illumination period. A reasonable explanation for sign inversion within a restricted spectral interval is that a diminishing CDS component in the apparent CD signal increases the significance of a CDA band of opposite sign.

A light-induced decrease in amplitude of the CD signal and in the long tails that are unequivocally attributed to CDS [cf. Keller and Bustamante (1986) and Garab et al. (1988)] was

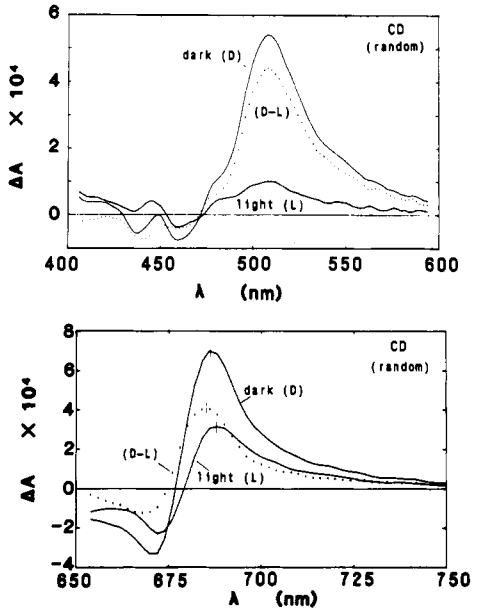

FIGURE 2: CD spectra of randomly oriented thylakoids in the dark (D) and after three conditioning cycles (2 min actinic light/2 min dark) followed by 3 min in actinic light (L). D–L, dark minus light difference spectrum. The ordinate displays the apparent absorbance difference between left and right circularly polarized light; it includes scattering and true absorbance components. Other conditions were as in Figure 1.

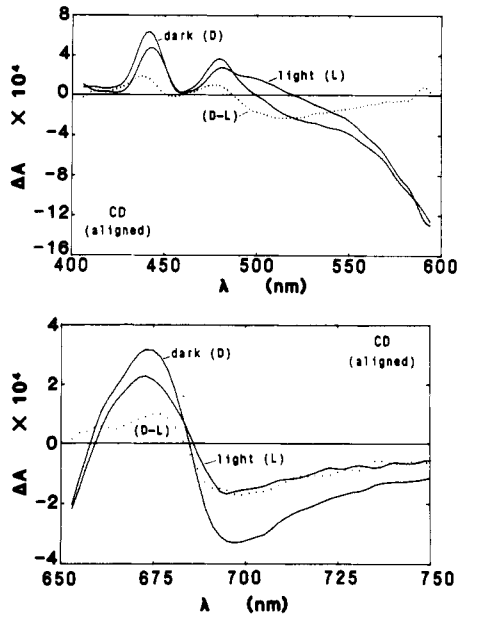

FIGURE 3: CD spectra of thylakoids aligned in a 1.0-T magnetic field and kept in the dark (D) or illuminated (L) as for Figure 2. D–L, dark minus light difference spectrum. Other conditions were as in Figure 1.

also observed in magnetically aligned thylakoids (Figure 3). As in the random suspension, light-induced changes were seen over a broad spectral interval. Theoretical analysis of circular differential scattering (Bustamante et al., 1985) reveals that the magnitude of the CDS signal depends largely on the geometry of the helix, specifically on the radius and the pitch length. Diminishing CDS amplitude thus testifies to a structural rearrangement of the large LHC domains believed to be responsible for the anomalous CD signal in thylakoids.

Comparison of the Kinetics of the Light-Induced CDS and Nonpolarized Scattering Changes. Figure 4 shows the kinetics of the light-induced CD and transmission changes at 730 nm. At this wavelength, no interference from absorbance can occur;

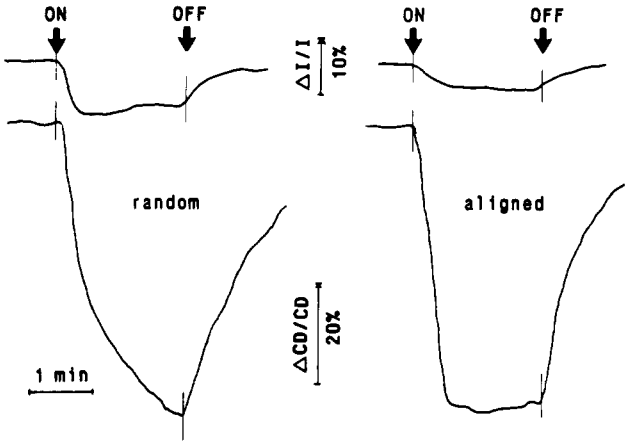

FIGURE 4: Time course of light-induced changes in the CDS signal (lower curves) and in the intensity of transmitted light (upper curves) at 730 nm. Left panel, randomly aligned sample; right panel, magnetically aligned sample. The actinic light intensity was 30 mW/cm², and the chlorophyll content was 30 μg/mL. Sample components and conditioning were as in Figure 1.

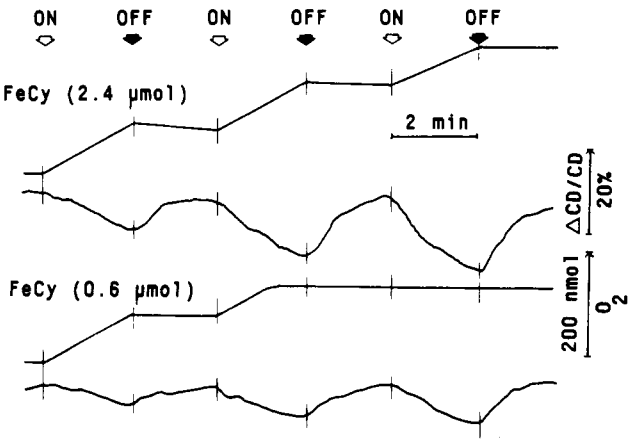

FIGURE 5: Simultaneous time courses of light-induced CD changes at 510 nm (lower traces) and of oxygen evolution. Samples contained 2.4 or 0.6 μmol of potassium ferricyanide as indicated. Actinic red light of 55 mW/cm² intensity was turned on and off as shown by the arrows.

hence, the changes in the CD signal, which resemble those shown in Figure 1, have to be attributed to CDS changes. The CDS changes in the randomly oriented sample are kinetically quite distinct from the transmission changes. It is also noteworthy that the relative CDS changes are more intense than the scattering-related relative transmission changes.

Although the relative amplitudes of the CDS changes were similar for randomly oriented and magnetically aligned thylakoids, the two differed markedly in kinetics (Figure 4). This observation may account for the biphasic kinetics of CD changes observed under some experimental conditions [cf. Faludi-Daniel et al. (1984)] and may indicate some heterogeneity among the chiral scatterers.

Optimization of the CD Changes. The amplitude and maximal rate of the light-induced CD change increased with the intensity of actinic illumination up to 64 mW/cm², with apparent half-saturation at 20 mW/cm² (data not shown, conditions as for Figure 1). The response also increased during successive cycles of 1–2 min of illumination followed by a similar dark interval (Figure 5). This induction effect was prolonged at low light intensities and was somewhat irreproducible in terms of the number of cycles required to attain the maximal light-induced response. As an example, Figure 6 shows records lacking pronounced induction phases. Figure 5 illustrates the surprising relation between light-driven CD

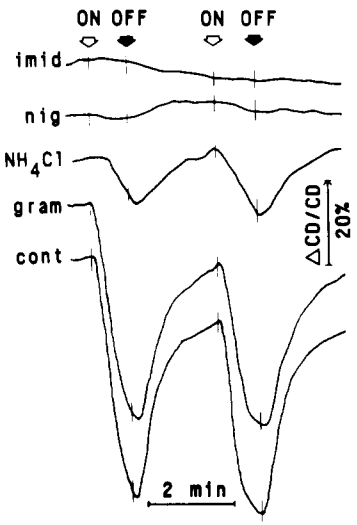

FIGURE 6: Effect of uncouplers on light-induced CD changes at 510 nm. Samples were prepared as for Figure 1. Where indicated, 0.5 mM imidazole, 2 μM gramicidin, 2 μM nigericin, or 4 mM NH_4Cl was added. Low-intensity (6 mW/cm^2) red light was turned on and off as indicated by the arrows.

responses and ferricyanide-catalyzed O_2 evolution. The upper pair of simultaneous records shows a correlation between the CD change and electron flow, with a marked induction effect in evidence; however, in the lower traces, the consumption of all the ferricyanide and cessation of O_2 evolution are seen to have little effect on the induction of the CD change. Unreported data have confirmed the demonstration (Faludi-Daniel et al., 1984) that without a terminal electron acceptor such as ferricyanide, light-induced CD changes are not seen. Thus, an electron acceptor is required principally in the early induction phase.

Induction is assumed to reflect a gradual decrease in membrane rigidity, resulting in increased mobility of the complexes participating in the structural rearrangement. Structural rearrangement of the LHC array, for instance, could be more or less constrained through its association with photosystem II. Variability in this association, arising from sample or leaf history, might account for differences in the observed responses to light.

Partial Electron Transport Reactions and the Effect of Uncouplers. The light-induced CD changes could be fully inhibited by DCMU, confirming earlier observations (Gregory, 1975; Faludi-Daniel et al., 1984). Studies of partial electron transport reactions established that cyclic flow around photosystem I, catalyzed by PMS, supports large light-induced CD changes. This system was adopted for the study of uncoupler effects.

Figure 6 confirms that photosystem I driven CD changes are inhibited by nigericin (Faludi-Daniel et al., 1984) and by NH_4Cl. FCCP and the permeant buffer imidazole were also inhibitory, whereas valinomycin and nonactin were ineffectual (data not shown). These findings tend to implicate the light-generated, transmembrane proton gradient as the driving force for the CD changes.

In contrast to prediction, however, gramicidin (2–10 μM) did not affect the kinetics or extent of the CD changes (Figure 6). By monitoring the electrochromic absorbance change and the rate of ferricyanide-dependent O_2 evolution, it was possible to verify that the applied gramicidin concentrations were sufficient to collapse the transmembrane electrical field and to uncouple linear electron transport. No significant difference could be observed in the rate of linear electron transport fully uncoupled with nigericin, NH_4Cl, or gramicidin. Gramicidin

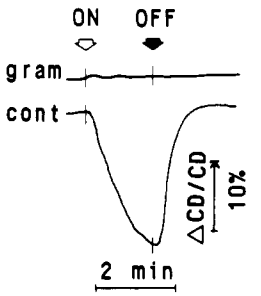

FIGURE 7: Time course of the light-induced CD changes at 510 nm in thylakoids in the presence of 2 mM diaminodurene and 5 mM ferricyanide, in the absence and presence of 200 nM gramicidin (lower and upper curves, respectively). The actinic red light (15 mW/cm^2) was turned on and off as indicated by the arrows. The same chloroplast isolate was used as in Figure 6.

insensitivity of the light-induced CD changes was not confined to the artificial cycle maintained by PMS but was also observed with ferricyanide as electron acceptor, and with the partial electron transport reaction from 2,3,5,6-tetramethyl-*p*-phenylenediamine to methylviologen in the presence of DCMU. Indeed, in this latter case, the addition of gramicidin, by increasing the rate of electron transport, slightly enhanced the light-induced CD changes (data not shown).

Partial reactions of photosystem II were also able to support light-induced CD changes, though at lesser rates and to lesser extents than were observed in the presence of PMS (cf. Figures 6 and 7, noting the higher light intensity in the latter). This difference cannot be attributed simply to a lower electron transport rate, since electron flow from water to 2,5-dimethylquinone, or to oxidized 2,3,5,6-tetramethyl-*p*-phenylenediamine, gave a high uncoupled rate of electron transport. In sharp contrast to what was observed when CD changes were driven by photosystem I, nanomolar gramicidin concentrations completely abolished the light-induced CD changes (Figure 7). Gramicidin has been shown to affect differently the ability of neutral red to detect protons released in the thylakoid lumen by the two photosystems (Theg & Junge, 1983); internal proton deposition through photosystem I activity was only marginally affected even by micromolar gramicidin concentrations while nanomolar concentrations were sufficient to divert protons from water oxidation into a saturable buffering compartment. Similar findings were reported in a study of proton uptake and phosphorylation by Opanasenko et al. (1985) and Pick et al. (1987).

These results and other lines of evidence [e.g., see Sigalat et al. (1985) and Beard and Dilley (1986)] support the notion of microcompartmentation of protons within the thylakoid membrane. The CD changes described here seem to be preferentially driven by photosystem I and would thus be attributed to an effect of the bulk, delocalized transmembrane proton gradient.

An alternative interpretation rests on a model calculation (Zimanyi & Garab, 1982; unpublished results) suggesting that ion gradients may be formed around charges generated by electron transport and localized near the dielectric/electrolyte interface. Taking into account the lateral separation of the two photosystems (Anderson, 1986), a strong lateral ion gradient is presumed to exist around the junction of the granal and stromal membranes, which are enriched in LHC associated with photosystem II and photosystem I, respectively. Lateral gradients of this type could be insensitive to a channel-forming ionophore such as gramicidin but sensitive to nigericin which is able to shuttle ions not only in the transmembrane direction but also in the surface plane. It is noteworthy that the LHC aggregate, as judged from its ori-

entation at very low (<10 V/cm) field strengths (J. G. Kiss, G. I. Garab, and A. Faludi-Daniel, unpublished results), can be readily mobilized in an electrical field.

Relation of CDS Changes to Nonpolarized Light Scattering. The scattering of nonpolarized light by thylakoids is known to increase upon establishment of a transmembrane proton gradient (Packer & Crofts, 1967; Dilley, 1971). These scattering changes reflect structural reorganization and are kinetically slower than the ion fluxes which drive them (Hind & Jagendorf, 1965). A similar relationship may be presumed between proton uptake and CDS changes. In an unreported study, nigericin was added immediately after turning off the light and was found to have no effect on the relaxation kinetics of the CDS change; it follows that this response, too, is kinetically limited by structural factors rather than by ion gradients. The same conclusion was reached from a study of the effect on kinetics of partially inhibitory concentrations of nigericin; the dark recovery rate of the CD changes was not accelerated despite the increased proton permeability of the membrane (data not shown). Hence, these results are in harmony with assignment of the observed CDS changes to structural rearrangements involving the thylakoid complexes.

The transmittance component of nonpolarized light-scattering changes has been related (Murakami & Packer, 1970) to closer appression of the grana membranes and overall flattening of the grana stacks. Promotion of this response by phenazine methosulfate and the relative insensitivity of light-induced transmittance changes to DCMU in infiltrated leaves (Miller & Nobel, 1972) point to efficient energization by photosystem I and suggest a relationship to CDS changes. The CDS response shown in Figure 4 is, however, even slower than the transmittance change in nonaligned membranes and is kinetically influenced by alignment. Furthermore, the 90° component of nonpolarized scattering (not studied here) bears even less kinetic resemblance to CDS changes, being faster than the transmittance component (Coughlan & Schreiber, 1984a,b). CDS responses thus seem to have no direct counterpart among known components of nonpolarized light scattering. The theory of circular intensity differential scattering predicts that circularly polarized light scattering will yield more specific information about the organization of chiral macrocomplexes than will nonpolarized scattering (Bustamante et al., 1980). CDS is thus a specific and perhaps unique tool for studying structural rearrangements associated with the photosynthetic activity of thylakoids.

The following working hypothesis is based on data presented in this and the preceding paper (Garab et al., 1988). LHC exists in large macrohelical arrays in the grana membranes. This "lattice", which includes photosystem II, becomes partially disorganized under the influence of the radial field developed around the granal margins by photosystem I, and/or the delocalized transmembrane proton gradient. In subsequent darkness, or when the membrane is otherwise deenergized, the macrohelical structure regenerates owing to an intrinsic capacity for self-organization.

ACKNOWLEDGMENTS

We thank Dr. C. Bustamante for communication of results and helpful discussion. We also thank Drs. Pick, Weiss, and Rottenberg for advance communication of their observations on gramicidin uncoupling. The assistance of John Trunk in setting up the instrumentation was invaluable.

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