Role of Thylakoid Lipids in the Structural Flexibility of Lamellar Aggregates of the Isolated Light-Harvesting Chlorophyll *a*/*b* Complex of Photosystem II[†]

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ABSTRACT: We studied the role of added thylakoid lipids in the light-induced reversible structural changes in isolated macroaggregates of the main light-harvesting chlorophyll *a*/*b* complex of photosystem II (LHCII). Loosely stacked lamellar macroaggregates were earlier shown to undergo light-induced reversible structural changes and changes in the photophysical pathways, which resembled those in thylakoid membranes exposed to excess light [Barzda, V., et al. (1996) *Biochemistry 35*, 8981−8985]. This structural flexibility of LHCII depends critically on the lipid content of the preparations [Simidjiev, I., et al. (1997) *Anal. Biochem. 250*, 169−175]. It is now reported that lamellar aggregates of LHCII are capable of incorporating substantial amounts of different thylakoid lipids. The long-range order of the chromophores is retained, while the ultrastructure of the lipid−protein macroaggregates can be modified significantly. Addition of thylakoid lipids to the preparations significantly enhances the ability of the LHCII macroaggregates to undergo light-induced structural changes. The lipid environment of the LHCII complexes therefore plays a significant role in determining the structural flexibility of the macroaggregates. As concerns the mechanism of these changes, it is proposed that the absorption of light and the dissipation of its energy in the macrodomains induces thermal fluctuations which bring about changes in the shape or in the stacking interactions of the membranes, this in turn affecting the long-range order of the embedded chromophores. In thylakoids, a similar mechanism is likely to explain the light-induced structural changes which are largely independent of the photochemical activity of the membranes.

Biomembranes consist of a bilayer of lipid molecules which embed various membrane proteins. At the molecular level, the structure of these membranes is quite complex; they contain specific mixtures of different lipid molecules and a large number of membrane proteins and their aggregates. Biomembranes also display significant structural flexibility, which is associated with their functional activity and with their ability to respond to changes in the environmental conditions. However, our understanding of the structure and dynamics of membranes is far from complete. Studies on reconstituted systems of purified proteins and lipids may shed light on the basic problem of the self-assembly and structural flexibility of biomembranes (*1*).

LHCII,[1] which accounts for about half of the protein and chlorophyll (Chl) content of the thylakoid membranes, is the most abundant membrane protein in the biosphere. LHCII is one of the few membrane proteins whose structure is known at near atomic resolution (*2*). When isolated from thylakoid membranes with mild, nonionic detergents, LHCII always carries all four major thylakoid lipids (*3*). Half of the polar lipid content of the isolated LHCII macroaggregates is monogalactosyldiacylglycerol (MGDG). This may facilitate the optimal packing of large intrinsic proteins within the bilayer structure (*4*). Digalactosyldiacylglycerol (DGDG) is essential for the formation of 3D and large 2D crystals (*5*). Phosphatidylglycerol (PG) is tightly bound to the polypeptide chain and can participate directly in the formation of trimers and in subunit−subunit interactions (*3*). The role of sulfoquinovosyldiacylglycerol (SQDG) is unclear.

The primary function of LHCII is to absorb light and transfer the excitation energy toward the photosynthetic reaction centers. By mediating the stacking of photosystem II particles, LHCII also plays a significant role in stabilizing the granum ultrastructure (*6*) and in the assembly of chirally organized macrodomains of PSII particles, which is thought to be responsible for the spatial separation of the two photosystems (*7*, *8*). Isolated LHCII trimers readily form large macroaggregates with long-range chiral order, which can be identified by the presence of the intense psi-type circular dichroism (CD) bands (psi, polymerization- or salt-induced) (*9*, *10*).

LHCII participates in various regulatory processes. In long-term regulation, the amounts of these complexes can

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[1] Abbreviations: Chl, chlorophyll; CD, circular dichroism; ΔCD, light-induced reversible CD changes; DGDG, digalactosyldiacylglycerol; LHCII, light-harvesting chlorophyll *a*/*b* protein complex of photosystem II; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; psi, polymer- or salt-induced; SQDG, sulfoquinovosyldiacylglycerol.

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be varied in a broad interval (*11*). Short-term regulatory processes usually involve the phosphorylation of LHCII (*12*). LHCII has also been proposed to be involved in the dissipation of excess radiation (*13*). It has recently been shown that lamellar aggregates of LHCII, closely mimicking the thylakoid membranes, are capable of undergoing light-induced reversible structural changes (ΔCD) which affect the photophysical pathways (*14*). The ultrastructure and structural flexibility of the LHCII macroaggregates depend critically on the lipid content of the preparations (*15*).

In the present work, by means of electron microscopy and CD spectroscopy we studied the ultrastructure and structural flexibility of lipid−protein macroaggregates obtained from purified thylakoid lipids and isolated lamellar LHCII aggregates. The shape of the lipid−protein macroaggregates was found to be determined largely by the amount and type of the lipids that embed the complexes; these lipids can dramatically enhance the light-induced reversible structural changes in the chiral macrodomains of LHCII.

EXPERIMENTAL PROCEDURES

Loosely stacked lamellar aggregates of LHCII were prepared from 2-week-old pea leaves (*Pisum sativum* L.), as described in detail elsewhere (*15*). Briefly, the solubilization step with about 0.7% (v/v) Triton X-100 (Fluka) of thylakoid membranes was critical to obtain lamellar macroaggregates capable of undergoing light-induced reversible changes in their chiral macrostructure. The isolated LHCII was stored in the dark at 4 °C, and was used within 10 days of the isolation.

As revealed by Western blots for D1 protein, the samples contained no contamination of PSII reaction centers. SDS−PAGE demonstrated the presence of 27 and 25 kDa polypeptides (data not shown). The pigment composition in our preparations was 7 Chl-*a*, 6 Chl-*b*, 2 lutein, 1 neoxanthin, and $^1/_4$−$^1/_3$ violaxanthin molecule per LHCII polypeptide (*16*).

Total lipids from thylakoid membranes were isolated by the procedure described in ref *17*. All organic solvents were saturated with N_2 and contained butylated hydroxytoluene (BHT) (1 mg/mL). Lipids were dissolved in hexane−BHT at an approximate concentration of 8 mg/mL and stored at − 20 °C. Lipids were separated on precoated TLC plates (Kieselgel, Merck) as described in ref *18*, and fatty acids were subjected to methanolysis with 5% HCl in methanol at 80 °C for 2 h after the addition of heptadecanoic acid. Fatty acid methyl esters were extracted with hexane and analyzed by capillary gas liquid chromatography in a 30-m SP2330 column (SUPELCO). The amount of each lipid was calculated from the GC data obtained with heptadecanoic acid as internal standard.

For the preparation of different lipid−protein macroaggregates, LHCII was added at a Chl concentration of 40 μg/mL. Lipids, with the exception of MGDG, were dissolved by vortexing in 20 mM Tricine buffer, pH 7.6. MGDG was solubilized according to ref *19*. LHCII was mixed with lipids by 30-s vortexing, then centrifuged in an Eppendorf centrifuge at 1000*g* for 5 min. Lipid concentrations were adjusted according to their highest enhancement of ΔCD. The optimal lipid concentrations were as follows: PG, 2.5 mg/mg of Chl; SQDG, 2.75 mg/mg of Chl; DGDG, 4.5 mg/mg of Chl; MGDG, 15.5 mg/mg of Chl. Under the given conditions, virtually all the lipids were "absorbed" by the preparations. No systematic studies were performed above these concentrations, because of the gradual decrease in ΔCD.

CD spectra were recorded at a Chl concentration of 20 μg/mL in a CD6 dichrograph (Jobin−Yvon) equipped with a side-illumination attachment (*14*). Before measurements the samples were kept for 20 min in the dark. Kinetic traces were recorded during three light/dark cycles; the observed traces were essentially identical.

The sizes and structures of the complexes were determined in a Zeiss 902 electron microscope and laser scanning confocal microscope (Zeiss, LSM 410). For electron microscopy, LHCII aggregates were pelleted in Eppendorf tubes. The pellet was fixed with 2% gluteraldehyde, postfixed with osmium tetroxide, dehydrated with ethanol, and embedded in Araldite. Ultrathin sections were cut out in a Tesla BS 478 ultramicrotome and stained with 2% uranyl acetate and lead citrate.

RESULTS

The incorporation of substantial amounts of PG and SQDG (about 2.5 mg/mg of Chl for both lipids; about 35 lipid molecules per monomer of LHCII) had no noticeable effect on the basic features of the lamellar structure (cf. ref *15*), albeit PG somewhat disorganized the lamellar aggregate stacks (Figure 1a).

The DGDG-enriched lipid−protein macroaggregates formed bilayer vesicles about 1−3 μm in diameter, in close contact with each other (Figure 1b). No granumlike stacks or multilayer sheets were observed.

The most spectacular transformation of the sheets was observed in the presence of high concentrations of MGDG, which at 15 mg lipid/mg of Chl induced the formation of loosely stacked, concentrically arranged, sometimes onion-like multilayers, 2−12 μm in diameter (Figure 1c). We believe that this is the first demonstration that the nonbilayer-forming lipid MGDG is capable of forming lamellae upon association with purified LHCII. It is interesting that these formations often resemble the granum. The incorporation of this lipid in the macroaggregates, corresponding to about 200 lipid molecules per LHCII monomer, was probably partly due to a nonspecific binding of lipids and the formation of lipid aggregates inside the macrostructures, as indicated by the osmiophilic droplets in the electron micrographs. However, when the concentration of the added lipid decreased, the transformation of the sheets into spherical multilayers remained incomplete. At 6 and 11 mg lipid/mg of Chl, the electron micrographs revealed only increased undulations of the membranes, but no indication of the formation of vesicular stacked multilayers (data not shown).

The different lipid−LHCII macroaggregates exhibited almost identical CD spectra, i.e., there was no significant dependence on the ultrastructure of the complexes (Figure 2). Large, anomalous CD bands at (−)493 and (−)682 nm with psi-type CD features were obtained for all samples. This shows that LHCII in all cases retained the long-range chiral order, i.e., the incorporation of added lipids into the aggregates did not disturb the chiral macroorganization of the chromophores. These data also demonstrate that the CD

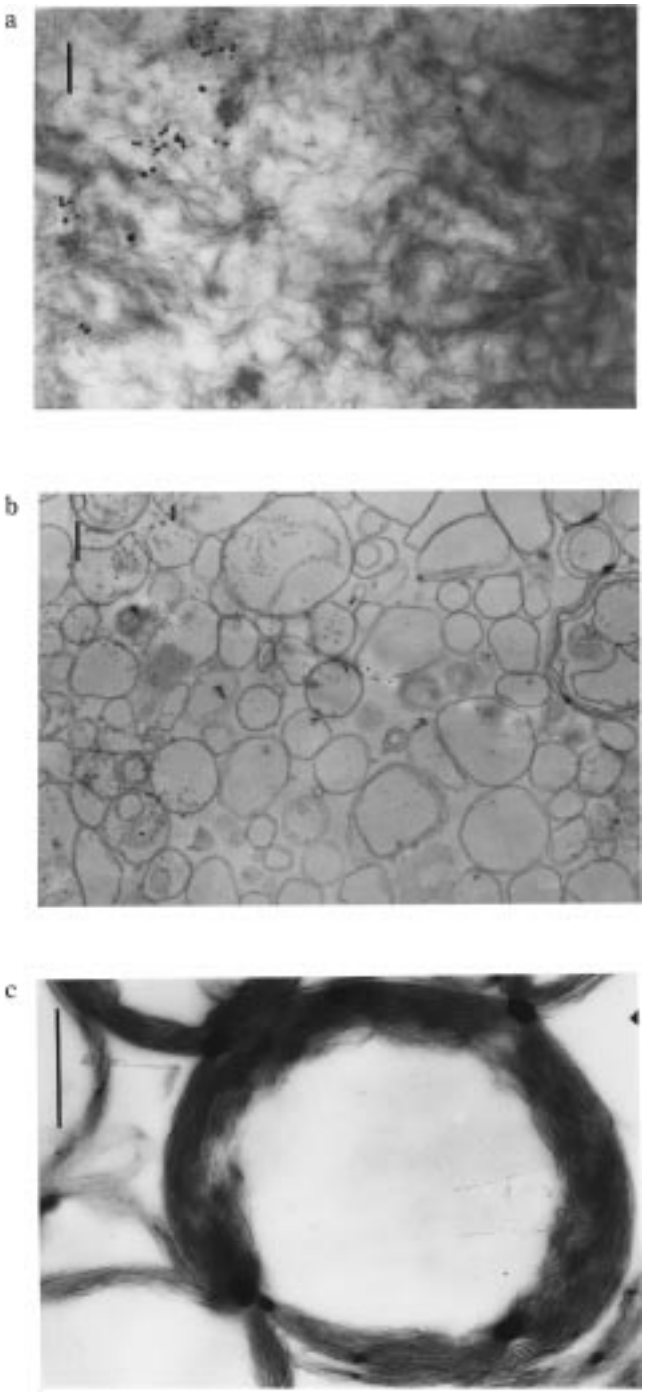

FIGURE 1: Electron microscopic pictures of loosely stacked lamellar aggregates of LHCII enriched with PG (a), DGDG (b), or MGDG (c). Bars represent 1 μm. For further details, see Experimental Procedures.

features are hardly influenced by the overall shape of the lamellae that contain the chiral macrodomains. This means that the macrodomains must be considerably smaller than the vesicles. Estimations of the sizes of the macrodomains from absorbance measurements were typically 200−300 nm, but in some cases up to 1 μm (*20*), whereas the lamellae were considerably larger. Nonetheless, these data are in good agreement with the assignment of the psi-type CD bands to chirally organized macrodomains with sizes commensurate with the wavelength of visible light (*10*).

The different lipid−LHCII macroaggregates were tested for structural flexibility. The addition of thylakoid lipids

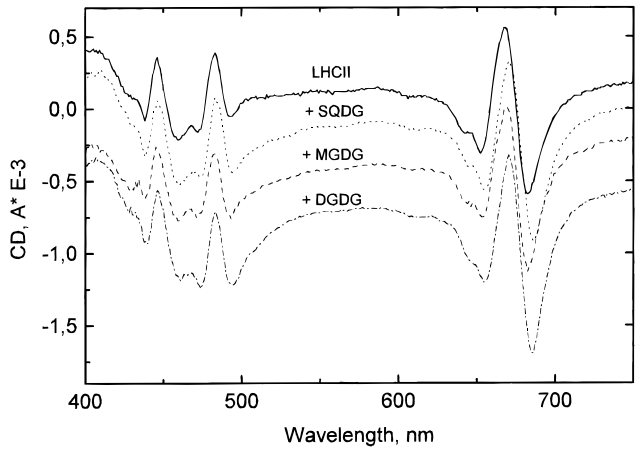

FIGURE 2: CD spectra of loosely stacked lamellar aggregates of LHCII and of different LHCII−lipid macroaggregates. For the structures of the different macroaggregates, see Figure 1.

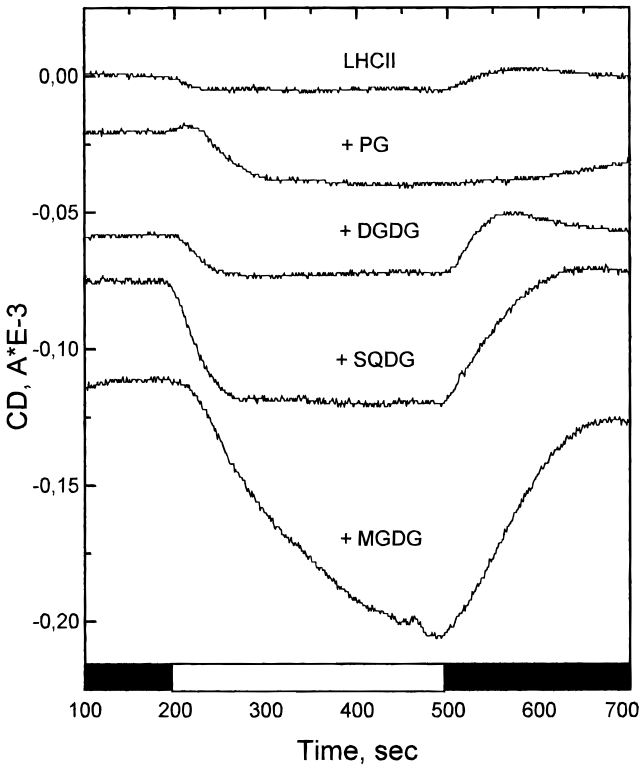

FIGURE 3: Light-induced CD changes for LHCII macroaggregates enriched in different thylakoid lipids. Structures and CD spectra are shown in Figures 1 and 2, respectively. The structure and spectrum of SQDG do not differ noticeably from those of PG−LHCII.

enhanced (to different extents) the ability of LHCII to undergo light-induced changes in its chiral macroorganization (Figure 3).

The smallest enhancement was obtained in PG−LHCII macroaggregates. PG most probably rigidifies the membranes, and the ΔCD enhancement can simply be due to the enhancement of the lamellar structure. Lamellar organization appears to be the basic structural requirement for ΔCD in LHCII (*14*).

In contrast, SQDG, which also had only a marginal effect on the shape of the lamellae, increased ΔCD by a factor of almost 10. The DGDG−LHCII complexes afforded relatively small (3-fold) enhancement of ΔCD, relative to the control. The example of DGDG−LHCII macroaggregates

illustrates that, while stacking of at least two adjacent membranes is likely to play a role in the structural flexibility, the formation of multilayers is not required. It can be inferred from this that structural rearrangements in the granum probably occur in the stacked regions, but do not necessarily involve changes in the overall ultrastructure.

The most significant enhancement in ΔCD was obtained in the presence of the most abundant thylakoid lipid, MGDG.

DISCUSSION

This work has shown that added thylakoid lipids can alter the ultrastructure of lamellar macroaggregates of LHCII and enhance their ability to undergo light-induced reversible structural changes.

As far as the ultrastructure is concerned, the effect of MGDG desires special attention, because formation of lamellae in artificial systems containing high concentrations of this nonbilayer-forming lipid has earlier not been reported. MGDG, with its conical shape, occupies the inner leaflet of the highly curved margins of the thylakoid membrane, and has been proposed to be packed around the hydrophobic parts of the embedded membrane proteins in the form of "lipidic particles" (*21*). This organization of MGDG in the native thylakoids still has no experimental support since inverted micelles have not been reported in freeze-fractured thylakoids, and in fact nonbilayer structures would probably destroy the barrier properties of the membranes. It was recently hypothesized that nonbilayer-forming lipids may exert a lateral packing pressure on the membrane proteins and thereby facilitate the embedding of proteins (*22*). Our findings, and particularly the large multilamellar macroaggregates with long-range chiral order in the presence of a high concentration of MGDG, are in harmony with this proposal.

Our data show that upon the addition of lipids the structural flexibility of the lipid−LHCII macroaggregates increases substantially, albeit the extent of the enhancement of the light-induced structural changes depends strongly on the lipid species used. This dependency is most likely explained by specific interactions between the lipids (probably their headgroups) and the protein or by differences in the bulk properties of the lipids. The view that headgroups play a significant role in the structural flexibility is suggested by the finding that PG−LHCII and SQDG−LHCII complexes, although possessing very similar electron microscopy structures, differ substantially in their abilities to undergo structural changes. This difference is most likely explained by the difference in rigidity of PG and SQDG.

Further, although MGDG and DGDG belong in the same group of galactolipids and have almost the same fatty acid composition, enrichment of LHCII with MGDG or with DGDG led to substantially different flexibilities. However, it can be speculated that the considerable flexibility of the MGDG−LHCII lamellae is at least in part a consequence of a presumed lower stability of the lipid−LHCII macroaggregates with respect to those enriched in bilayer-forming lipids.

The shape of lipid vesicles is known to be governed mainly by the curvature and bending undulations with wide ranges of amplitude and wavelength (*1*). The intensity of these fluctuations depends on the temperature and the bending rigidity. Small temperature changes, of even less than one degree, can lead to large changes in membrane curvature (*1*).

The high sensitivity of membranes to temperature suggests a mechanism that could account for the light-induced reversible structural changes observed in lamellar aggregates of LHCII (*14*), and the nonphotochemically driven light-induced structural changes in thylakoid membranes (*24*); the absorbance of light by the LHCII (-containing) macrodomains in a lipid environment induces local heating. This can lead to fluctuations in the shape of the lamellae and/or change the strength of the membrane stacks or increase the amplitude of the undulations, which result in changes in the long-range order of the chromophores of the proteins in the artificial or natural membranes.

This explanation, based on the deformability of the membranes, is similar to that proposed earlier (*14*), in terms of a thermo-optic effect in liquid crystals (cf. ref *25*). However, the threshold level of thermal fluctuations sufficient to induce noticeable structural changes is likely to be considerably lower for membranes and for thylakoids than for liquid crystals.

Further studies are required to elucidate the exact nature of the structural changes, the extent and duration of the thermal fluctuations, the site of energy dissipation, the propagation of the "heat package" inside the macrodomains and the lamellae, and the physiological significance of such structural rearrangements.

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REFERENCES

1. Lipowsky, R. (1995) *Curr. Opin. Struct. Biol. 5*, 531−540.
2. Kühlbrandt, W., Wang, D. G., and Fujiyoshi, Y. (1994) *Nature 367*, 614−621.
3. Nussberger, S., Dórr, K., Wang, D. G., and Kühlbrandt, W. (1993) *J. Mol. Biol. 234*, 347−356.
4. Israelachvili, J. N., Marcelja, S., and Horn, R. G. (1980) *Q. Rev. Biophys. 13*, 121−200.
5. Butler, P. J. G.; Kühlbrandt, W. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3797−3801.
6. Arntzen, C. J. (1993) *Curr. Top. Bioenerg. 8*, 111−160.
7. Barzda, V., Mustárdy, L., and Garab, G. (1994) *Biochemistry 33*, 10837−10841.
8. Garab, G., Faludi-Dániel, A., Sutherland, J. C., and Hind, G. (1988) *Biochemistry 27*, 2425−2430..
9. Garab, G. (1996) Linear and Circular Dichroism. in *Biophysical Techniques in Photosynthesis* (Amesz, J., and Hoff, A. J., Eds.) pp 11−40, Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. Keller, D., and Bustamante, C. (1986) *J. Chem. Phys. 84*, pp 2972−2979.
11. Anderson, J. M., and Andersson, B. (1988) *Trends Biol. Sci. 13*, 351−355.
12. Allen, J. F. (1995) *Physiol. Plant. 93*, 196−205.
13. Horton, P., Ruban, A., and Walters, G. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 47*, 655−684.
14. Barzda, V., Istokovics, A., Simidjiev, I., and Garab, G. (1996)- *Biochemistry 35*, 8981−8985.
15. Simidjiev, I., Barzda, V., Mustárdy, L., and Garab, G. (1997) *Anal. Biochem. 255*, 167−173.

16. Naqvi, K. R., Melo, T. B., and Bangar Raju, B. (1997) *Spectrochim. Acta, Part A 53*, 2659−2667.
17. Chapman, D. J., and Barber, J. (1987) *Methods Enzymol. 148*, 294−319.
18. Vígh, L., Horváth, I., and Thompson, G. A., Jr. (1988) *Biochim. Biophys. Acta 937*, 42−50.
19. Staehelin, L. A., Sprague, S., and Dunahay, T. G. (1984) *Photosynth. Res. 5*, 293−296.
20. Naqvi, K. R., Melo, T. B., Bangar Raju, B., Jávorfi, T., Simidjiev, I., and Garab, G. (1997) *Spectrochim. Acta, Part A 53*, 1925−1936.
21. Murphy, D. J. (1982) *FEBS Lett. 150*, 19−26.
22. De Kruijff, B. (1997) *Nature 386*, 129−130.
23. Garab, G., Leegood, R. C., Walker, D. A., Sutherland, J. C., and Hind, G. (1988) *Biochemistry 27*, 2430−2434.
24. Istokovics, A., Simidjiev, I., Lajkó, F., and Garab, G. (1997) *Photosynth. Res. 54*, 45−53.
25. Jánossy, I. (1991) *Optical Effects in Liquid Crystals*, Kluwer Academic Publishers, Dordrecht, The Netherlands.

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